1973

Conformational induction in block copolypetides

Thomas Leo Klug

Iowa State University

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Conformational induction in block copolypeptides

by

Thomas Leo Klug

A Dissertation Submitted to the
Graduate Faculty in Partial Fulfillment of
The Requirements for the Degree of
DOCTOR OF PHILOSOPHY
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\(n_c = 10, t = 0,\) and \(\Delta F = 0.23\) kcal/mole,
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\[ n = 10, \ t = 0, \ \Delta F = 0.14 \ \text{kcal/residue-mole} \]

\[ n = 8, \ t = 3 \text{ or } 4, \ \Delta F = 0.37-0.42 \ \text{kcal/residue-mole} \]

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\[ n = 18, \ t = 0, \ \Delta F = \text{kcal/mole} \]

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CHAPTER 1. INTRODUCTION

Synthetic polypeptides have been found to exist in many conformations characteristic of those found in proteins. The helical structure was first assigned to a poly-$\alpha$-amino acid by X-ray diffraction studies (1,2). The structures have been assigned to several polypeptides, chiefly by infrared spectroscopy and X-ray diffraction studies (3,4). Other helical forms have been reported; the poly-L-proline and poly-L-hydroxyproline structure typical of the collagen fold (5), and more recently the $\omega$ form, which is a distorted $\alpha$ helix, and has been suggested to be the $4_{13}$ helix (6). Thus, synthetic polypeptides form an important class of substances which have become the model for the general study of the physical-chemical properties of proteins, such studies aiding in the interpretation of protein structures and activities (7-10).

Of all the conformations responsible for maintaining the proteins in their unique folded states, that of the $\alpha$ helix is perhaps the best understood. The concept of the $\alpha$ helix has evolved from the original Pauling hypothesis (11) in which hydrogen bonds were considered to be the primary stabilizing force, to the present theories in which the net stability of the helix is believed to be the result of hydrogen bonds, hydrophobic bonds, van der Waals forces, and electrostatic forces (12). A helical conformation consisting of L- or D- amino acids can exist in two spiral forms of opposite handedness. The left- and right-handed helices are non-superimposable and in addition are not mirror images (except for glycine). The screw sense of the $\alpha$ helix has long been a controversial question. Huggins predicted a right-handed sense for the
α helix based upon stability differences between the two senses due to steric arguments (13). Moffit and Yang (14) first predicted a right-handed helix based upon the sign of the experimental $b_o$ value (negative for a right-handed helix and positive for a left-handed helix) (14). Subsequently, X-ray studies upon myoglobin, hemoglobin, and lysozyme have revealed that all the helical sequences in these proteins are right-handed (15,16). Therefore, it is reasonable to conclude that proteins and poly-L-amino acids do favor the formation of a right-hand helix, and therefore that their optical rotatory dispersion properties will be similar, in so far as these depend only on the helix backbone. The helix sense may be assigned on the basis of the circular dichroism (CD) of the polypeptide as well as from the optical rotatory dispersion (ORD) (9,10,17). The ORD and CD properties of simple helical polypeptides are well understood and unequivocal assignments of the helix sense may be made in these cases. Simple polypeptides are those which have alkyl side chains, side chains with very weak chromophores (esters, amino, and carboxyl groups), or aromatic groups beyond the γ-carbon. In polypeptides with aromatic rings attached to the β-carbon of the side chain (poly-L-tyrosine, poly-L-phenylalanine, poly-L-tryptophan, etc.), the proximity of the strong chromophore drastically alters the optical rotatory properties, and the helix sense in such cases remains very difficult to determine.

The usual experimental approach (18) to the determination of the helix sense of polypeptides with unusual optical rotatory properties is to determine the optical rotatory properties of a series of copolymers of the unusual and the simple amino acids. If the two amino acid
polypeptides prefer the same helix sense the ORD (\(b_0\)) and CD ([\(\Theta\)]) behaviors of these copolymers should change in a linear or a gradual manner with the mole fraction of the amino acid residues. Conversely, if the two poly-\(\alpha\)-amino acid helices prefer the opposite helix senses, the ORD and CD behavior of the copolymers will show a marked change over a relatively narrow range of the molar compositions of the two amino acids. This latter difference in the observed behavior in these cases arises because the residues of the poly-\(\alpha\)-amino acid whose helix is more stable at a given molar composition presumably constrains or induces the residues of the other amino acid into a similar helical conformation, that is, until the molar composition ratio of the copolymer is such that the former helix is destabilized, whereupon the helix sense changes abruptly to that preferred by the other poly-\(\alpha\)-amino acid. This experimental approach has been used in the conformational studies of poly-\(\beta\)-benzyl-L-aspartate (18), poly-\(\beta\)-p-nitrobenzyl-L-aspartate (19), poly-L-tyrosine (20), poly-L-tryptophane (21), and poly-L-phenylalanine (22). These studies led to the assignment of the right-handed helix in all of the above L-polypeptides except poly-\(\beta\)-benzyl-L-aspartate (18). However, the amount of information that may be gotten from the dependence of the ORD and CD upon the copolymer composition is limited by the fact that it is nearly impossible to prepare a series of copolymers in which the distribution of the residues of both amino acids is uniform over the entire chain length for each polymer, that is, one in which the two amino acid residues are interspersed in a truly random manner (23). Therefore, barring some demonstration that the residues are indeed distributed in a
random manner (no long blocks of either residue), the determination of the helix sense by this method is only suggestive. One would prefer a method which retains the simplicity and elegance of the above method, but eliminates the problems inherent in this method, specifically as regards those cited above.

Lundberg and Doty have shown by optical studies upon block polymers of L- and D-poly-7-benzylglutamate that when a preformed L-\(\alpha\)-helix is used to initiate the polymerization of a D-N-carboxyanhydride (NCA) the D-residues add to the initiator in the same helix sense as the initiator, and continue to do so for at least four residues, after which the helix of the D-residues begins to reverse its sense to that preferred for the long D-helix (24, 25). Their kinetic data indicated that the two-step propagation of the polymerization observed in these cases might have been due to this same phenomenon of continuance or induction of the initially grafted D-residues into the same helix sense as the L-polypeptide initiator, and then reversal of the induced helix as the chain grows longer (25).

Experiments of the above kind would seem to provide a tenable method by which to determine the helix sense and stability of a polypeptide in a manner analogous to the random copolymer method and yet avoid the problems associated with that method. In such experiments both helix senses would be generated, and from the induction-reversion phenomenon one might assign a helix sense relative to that of the initiator. The problem of the distribution of the residues in the block copolymers is solved as each copolymer may be uniquely determined in this regard by
synthesis. The purpose of this thesis is the examination and development of this method of helix sense determination, a method which would be applicable to both simple polypeptides and polypeptides with unusual optical rotatory properties. This method has been applied here to a variety of poly-α-amino acids with particular efforts being made to determine the helix sense of poly-L-tyrosine.

The optical rotatory dispersion of poly-L-tyrosine was observed to be unlike that of the simple helical polypeptides (26,27). Fasman showed that random copolymers of poly-L-tyrosine (PLT) and poly-L-glutamic acid (PGA) of varied composition showed a linear change in the Moffit-Yang $b_0$ parameter. He interpreted this to mean that the L-tyrosine residues fit into the right-handed poly-L-glutamic acid helix and that poly-L-tyrosine forms a right-handed helix (20). Subsequently, Fasman et al. extended the optical rotatory dispersion measurements on PLT to 227 nm, and Beychok and Fasman reported the circular dichroism in the region of 214 to 300 nm (28,29). The negative cotton effect observed at 224 nm was assigned to the $n \rightarrow \pi^*$ transition, and since its sign coincided with that of the $n \rightarrow \pi^*$ cotton effects in simple polypeptides in the right-handed helical conformation, a right-handed helix sense was assigned to poly-L-tyrosine. But it is possible that coupling of the amide electronic transitions with those of the phenolic side chains may alter the magnitude or even the sign of the $n \rightarrow \pi^*$ cotton effect in poly-L-tyrosine. Chen and Woody have carried out a theoretical treatment of the optical rotatory properties of poly-L-tyrosine with regard to the helix sense and side chain conformation (30). From a comparison of theoretical and
experimental data, they assigned a right-handed $\alpha$ helical conformation to poly-L-tyrosine. Applequist and Mahr measured the dipole moment per residue of PLT and its brominated side chain derivative in quinoline by dielectric dispersion studies (31). From the change in the dipole moment upon bromine substitution, assuming no conformational change upon substitution, they assigned a left-handed helix sense to poly-L-tyrosine. Conformational energy calculations have been made by Ooi et al. (32), and by Yan et al. (33). These energy calculations indicated that a right-handed poly-L-tyrosine $\alpha$ helix has a lower potential energy than the left-handed helix by $0.4 - 1.8$ kcal/residue-mole.
CHAPTER 2. MATERIALS AND METHODS

N-Carboxy-alpha-amino Acid Anhydrides

All N-carboxy-alpha-amino acid anhydrides (NCAs), with the single exception of 6-p-nitrobenzyl-L-aspartate, were purchased from the New England Nuclear Corporation, Pilot Chemicals Division, Watertown, Mass. The purchased NCAs were used without further purification, as the chemical and optical purities of these compounds, as determined by their chloride contents and specific rotations respectively, were satisfactory as received (Table 1). The values of the specific rotations were in agreement with the literature values where available, and the relative rotations of the D and L enantiomers of the same NCA were opposite, with the possible exception of N-carbobenzoxy-D-lysine NCA. The chloride content was determined gravimetrically as AgCl (Ilse Beetz, Laboratory for Microanalysis, 8640 Kronach, West Germany).

6-p-Nitrobenzyl-L-aspartate NCA was prepared as follows: 6-p-nitrobenzyl-L-aspartate was obtained by the reaction of the lithium salt of the L-aspartic acid-copper complex with p-nitrophenacyl bromide (34); 6-p-nitrobenzyl-L-aspartate NCA was prepared by the reaction of phosgene with 6-p-nitrobenzyl-L-aspartate in anhydrous dioxane (35).

The copper-aspartic acid complex was prepared with cupric oxide (5 grams, "Baker Analyzed" reagent grade) and L-aspartic acid (5 grams, Aldrich Chemical Co.) by addition of the cupric oxide to L-aspartic acid dissolved in hot water, and stirring for one hour at 80-90°C. The filtrate was then allowed to cool, and light-blue amorphous crystals of the cupric L-aspartate appeared. The solution was concentrated and cooled repeatedly
Table 1. Specific rotations and chloride contents of N-carboxy-α-amino acid anhydrides

<table>
<thead>
<tr>
<th>N-Carboxy Anhydride</th>
<th>$^{[2]}_{577}$</th>
<th>conc., solvent</th>
<th>Chloride</th>
</tr>
</thead>
<tbody>
<tr>
<td>8-benzyl-L-aspartate</td>
<td>-61.8</td>
<td>2.5% dioxane</td>
<td>0.05%</td>
</tr>
<tr>
<td>8-p-nitrobenzyl-L-aspartate</td>
<td>-47.5</td>
<td>1% dioxane</td>
<td>0.02%</td>
</tr>
<tr>
<td>γ-benzyl-L-glutamate</td>
<td>-15.0</td>
<td>1% dioxane</td>
<td>0.03%</td>
</tr>
<tr>
<td>γ-benzyl-D-glutamate</td>
<td>15.0</td>
<td>1% dioxane</td>
<td>0.03%</td>
</tr>
<tr>
<td>N-carbobenzoxy-L-lysine</td>
<td>-30.7</td>
<td>2.5% dioxane</td>
<td>--</td>
</tr>
<tr>
<td>N-carbobenzoxy-D-lysine</td>
<td>28.4</td>
<td>2.5% dioxane</td>
<td>--</td>
</tr>
<tr>
<td>O-carbobenzoxy-L-tyrosine</td>
<td>-92.0</td>
<td>1.0% dioxane</td>
<td>0.03%</td>
</tr>
<tr>
<td>O-carbobenzoxy-D-tyrosine</td>
<td>92.0</td>
<td>1.0% dioxane</td>
<td>0.03%</td>
</tr>
</tbody>
</table>
until all of the cupric L-aspartate possible was recovered. The cupric L-aspartate was dried in vacuo for 4 hours at 60°C. Yield, 4.2 grams of cupric L-aspartate (36).

The lithium cupric aspartate salt was prepared by treatment of the cupric aspartate with a twice molar excess of aspartic acid in 0.1N lithium hydroxide (reagent grade, Matheson, Coleman and Bell) (34). Cupric aspartate (4.18 grams) was added to a solution of aspartic acid (2.35 grams) in 320 ml's. of 0.1N LiOH. The solution was stirred vigorously for 4 hours at room temperature, concentrated to 50 ml's. at 50°C under reduced pressure, and precipitated into 400 ml's. of absolute ethanol. The precipitate was dried in vacuo at 60°C for 3 hours. Yield, 4.96 grams of lithium cupric aspartate.

Lithium cupric aspartate (4.96 grams) was dissolved in 25 ml's. of water in a 100 ml. flask, and p-nitrophenacyl bromide (5.5 grams, Matheson, Coleman and Bell) dissolved in 25 ml's. of N,N-dimethylformamide was then added. The resulting suspension was stirred at 42°C for 24 hours. Acetone (25 ml's.) was added to the suspension and the solution filtered, the product being collected as a solid from the suspension. The product, cupric 8-p-nitrobenzyl-L-aspartate, was washed repeatedly with acetone and cold water. Yield, 3.9 grams of cupric 8-p-nitrobenzyl-L-aspartate.

8-p-Nitrobenzyl-L-aspartate was isolated by boiling the cupric 8-p-nitrobenzyl-L-aspartate (3.9 grams) in 100 ml's. of a 0.1N disodium (ethylenedinitrilo) tetraacetate ("Baker Analyzed" reagent grade) solution, filtering, and allowing the solution to cool at 5°C for 12
hours, whereupon flat, white crystals formed. The crystals were washed with ice-cold water, and dried. Yield, 2.5 grams of β-p-nitrobenzyl-L-aspartate. The β-p-nitrobenzyl-L-aspartate was recrystallized several times from hot water, m.p. 191°C, 193°C reported (35). Elemental analysis: calculated, C 49.25%, N 10.4% H 4.48% O 35.82%; found, C 46.92%, N 9.52% H 5.05% O 38.81%. \([\alpha]_D^{25} = +15.13^\circ\) (C=1.0, glacial acetic acid).

β-p-Nitrobenzyl-L-aspartate (3.6 grams) was suspended in 200 ml. of anhydrous dioxane and phosgene was passed through the suspension until it became clear. The phosgene (Matheson Gas Products) was dried by bubbling through concentrated sulphuric acid, and after passing through the dioxane was destroyed by reaction with vapor from an ammonium hydroxide solution. The physical apparatus for the phosgenation procedure is described elsewhere (35). The reaction mixture was maintained at 55°C until all of the β-p-nitrobenzyl-L-aspartate passed into solution. The reaction was complete after approximately one hour. Dry nitrogen was bubbled through the reaction solution to remove the excess phosgene. The N-carboxyanhydride was isolated by evaporating off the dioxane at 45°C under reduced pressure, the crude NCA remaining as a yellow-white solid. The crude NCA was purified by crystallization from dioxane with n-hexane, dissolving the NCA in a minimum volume of dioxane at room temperature, adding n-hexane to the point of opalescence, and storing the solution overnight at -20°C. A white crystalline NCA was obtained after many such recrystallizations, and contained 0.02% chloride as hydrogen chloride (Ilse Beetz). M.p. 181°C, 180-181.5°C reported (35).
infrared spectrum showed the characteristic nitro bands at 1350 and 1530 cm\(^{-1}\), as well as the 1760 and 1860 cm\(^{-1}\) N-carboxy-anhydride bands (35).

Elemental analysis: calculated, C 48.98% N 9.52% H 3.40% O 38.10%; found, C 49.43% N 8.47% H 3.99% O 38.10%.

The N-carboxy-α-amino acid anhydrides were stored at -30°C to prevent thermal degradation. Storage at such temperatures also prevented contamination by moisture if the sample bottles were kept tightly sealed. Before use, the NCAs were brought to room temperature in a sealed desiccator. When possible, the NCAs were used in quantities as contained in a single bottle, or divided into smaller portions to avoid the possibility of contamination by repeated exposures to heat and moisture.

Solvents

The solvents were prepared by the following procedures. Reagent grade 1,4-dioxane (J. T. Baker) was refluxed over sodium metal for at least 24 hours, distilled into a second flask containing sodium metal, a broad middle fraction being collected. The dioxane was refluxed for several hours and fractionally distilled through a 0.7 meter column (containing 1/4" nichrome helices, and insulated with asbestos wrapping) immediately before use, b.p. 101-101.5°C (37).

N,N-Dimethylformamide (DMF) and N,N-dimethylacetamide (DMAc) (Aldrich Chemical Co., Spectrophotometric Grade) were purified by stirring with sodium bicarbonate for 24 hours, decanting, and fractionally distilling from o-phthalic acid at reduced pressure. A broad middle fraction was collected. These treatments were intended to remove the acidic and basic impurities respectively (25).
Trimethyl phosphate (TMP) (Aldrich Chemical Co., 97% pure) was purified by fractional distillation through a 0.7 meter column (containing 1/4" nichrome helices and insulated with asbestos wrapping) at reduced pressure, (b.p. 54°C/1.5 mm Hg). A 30% end fraction was collected, as the front fractions showed a high optical density in the ultra-violet. The fraction collected had an optical density of 0.08 (with respect to air) at 200 nm in a 1 mm fused quartz cell (38,39).

The solvents acetone, benzene, chloroform, ethylenediamine, methanol, and pyridine were all "Baker Analyzed" reagent grade and were used as received without further purification or special treatment.

Nomenclature of Polymers

The abbreviated nomenclature of the polymers discussed follows as closely as possible the recommendations of the IUPAC-IUB commission of biochemical nomenclature for synthetic polypeptides (40,41). Thus a block polymer of γ-benzyl-L-glutamate of 20 residues combined through the α-NH₂ terminus to the α-COOH terminus of an O-carbobenzoxy-D-tyrosine block of 10 residues is written, (D-Tyr-Z)₁₀-(L-Glu-OBzl)₂₀ (40). Designations of the amino acid derivatives studied were abbreviated as follows: (L-Glu-OBzl), γ-benzyl-L-glutamate; (D-Tyr-Z), O-carbobenzoxy-D-tyrosine; (D-Lys-Z), N-carbobenzoxy-D-lysine; (L-Glu-OMe), γ-methyl-L-glutamate; (L-Asp-OBzl), β-benzyl-L-aspartate; (L-Asp-OBzlN₃), β-p-nitrobenzyl-L-aspartate (41).
Polymerization Initiators

The initiators for polymerization of the N-carboxy α-amino acid anhydrides were prepared as follows: reagent grade n-hexylamine (Eastman Organic Chemicals) was refluxed over and fractionally distilled from calcium hydride under reduced pressure (b.p. 35°C/20 mm Hg). The middle fraction was collected and stored in a dessicator at room temperature.

Preformed polymer initiators were prepared by the n-hexylamine initiated polymerization of the corresponding α-amino acid anhydrides in N,N-dimethylacetamide, using a known anhydride to initiator ratio to determine the degree of polymerization of the polymer (25). All polymerizations were done at an initial anhydride concentration of 4% (w:v). Completion of the polymerization was demonstrated by the complete absence of the 1790 cm\(^{-1}\) anhydride band in the infrared spectrum of a vacuum cast thin film. The 1790 cm\(^{-1}\) band is the strongest NCA infrared band and any decrease in the NCA concentration could be readily followed until less than 1% of the original NCA remained in the polymerization solution.

Thin films were cast upon a calcium fluoride window of a demountable infrared cell (Barnes Engineering). The polymer was collected by precipitation into a large volume of anhydrous reagent grade isopropyl ether (1:20, v:v). The polymer was washed repeatedly with anhydrous ethyl ether and dried in vacuo at room temperature for 6-12 hours. The polymers prepared and isolated in this manner contained no detectable NCA by the above infrared spectral method. All of the polymerizations were allowed to proceed for at least 9 half-reaction (NCA + polymer) times and thus it is unlikely that any NCA would remain at concentrations greater than
0.1% of the original NCA concentration. The half-reaction time was determined by the cast film infrared spectral method. The polymer was stored in a brown screw-top bottle at -30°C until use.

**Titration of Preformed Polymers**

Before the preformed polymer was used as an initiator, the amount of "free-amine", i.e., the amount of amine present as polymer terminal amino groups, was determined exactly by conductimetric titration of the polymer (25,42). This was done because chemical processes during and after polymerization cause the amount of titratable end groups to decrease. During polymerization cyclic polypeptides may be formed or carboxyl groups may be formed as a termination step (43,44). After isolation, end group cyclization to form 2,5 pyrrolidones in polypeptides with esterified side chains, or production of carboxyl groups by de-esterification of blocked carboxyl groups, decrease the effective amount of "free amine" (45). Storage at a temperature of -30°C largely eliminated the latter two reactions, and under such storage conditions the "free amine" concentration in (L-Glu-OBzl)$_20$ decreased by approximately 2% per month.

The polymer samples (40-80 mgs) were dissolved in approximately 5 mls. of a phenol-ethanol solution (87:13), and equivalent amounts of water and absolute ethanol were added, usually 1 or 2 mls. The amount of each solvent used depended upon the amount of polymer used, while the total volume was kept less than 10 mls. Sodium chloride was added to raise the starting conductivity when necessary, usually to near $3 \times 10^{-5}$ mho. The solutions were titrated with a 0.01267 N perchloric acid solution in methanol delivered from a 2 ml. microburette. A dipping type conductivity
cell with a cell constant of \( k = 0.80 \text{ cm}^{-1} \) was used with a galvanometer (Leeds and Northrup) for resistance measurements. The cell was calibrated using a 0.0200 N potassium chloride solution, where the specific conductance is equal to 0.002768 ohm\(^{-1}\) cm\(^{-1}\) at 25°C (46). The cell used consisted of 2 electrodes of 4 cm of #18 B and S platinum wire wound in helices of 4 mm diameter, separated by 2.5 cm. The titration procedure was calibrated by the titration of known amounts of n-hexylamine, and was found to be accurate and reproducible to within \( \pm 2\% \). Titrations of polymer solutions were found reproducible to within \( \pm 4\% \). In such a titration the conductance was plotted against perchloric acid volume, the intersection of the initial and final slopes giving the equivalence point (free amine was expressed in meqs/mg.) (Table 2).

Phenol-ethanol solutions were prepared by dissolving 83 grams of purified phenol (42) in 17 grams of absolute ethanol and were stored in a glass volumetric flask in the dark. Perchloric acid solutions were made by mixing 2 ml. of 70% perchloric acid with 2 liters of anhydrous methanol. Standardization of the perchloric acid was carried out conductimetrically against a standardized sodium methoxide solution in a phenol-ethanol-water solution. The sodium methoxide (0.01958 N) was prepared by dissolving 1 gram of sodium metal in 1800 ml. of anhydrous methanol. The sodium methoxide was standardized by titration against the primary standard, potassium acid phthalate (0.0100 N) using phenolphthalein as the indicator.

Since carboxyl groups may be formed as a termination step in the synthesis of the preformed polymer initiator, or by de-esterification of
blocked carboxylic acid groups, the amount of acid present as titratable carboxylic acid groups, or other acidic constituents in the preformed initiators was determined (47). The polymers were dissolved in a chloroform/absolute ethanol solvent mixture (2:1), and titrated with 0.0098 N sodium methoxide using 0.1% phenolphthalein in absolute ethanol as the indicator. The sharpness of the end-point transition could be increased by using the stronger base, potassium methoxide. Titrations of the preformed polymers (usually (L-Glu-OBzl)$_2$) revealed that the carboxyl groups were present in amounts too small to interfere with the function of the preformed polymer as a primary amine initiator (Table 2).

Block Copolymers

Block polymers were prepared by dissolving a known quantity of preformed polymer initiator in the appropriate volume of solvent and then adding the exact amount of N-carboxy α-amino acid anhydride to give the required anhydride to initiator ratio (A/I). For polymerization kinetics and studies of the optical rotation during polymerization, the solution of preformed initiator was thermostated at the desired temperature prior to the addition of the NCA. Polymer blocks of the required length were obtained by taking aliquots from the polymerizing solution at the time calculated from block growth kinetics. Thus from a solution with an A/I of 20, blocks of 6 to 20 residues per block were prepared. To obtain polymer blocks of a more accurate and reproducible length, the A/I ratio was initially adjusted to that of the desired block length. Thus from a solution with an A/I of 10, a block of 10 residues was grafted to the preformed polymer, e.g., (D-Tyr-Z)$_{10}$-(L-Glu-OBzl)$_{20}$ from the initiation of
Table 2. Primary amine and carboxylic acid concentration found for pre­
formed polymer initiators

<table>
<thead>
<tr>
<th>Preformed Polymers</th>
<th>[NH₂] (meq/mg)</th>
<th>[COOH] (meq/mg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>poly(L-Glu-OBzl) I</td>
<td>1.53 x 10⁻⁴</td>
<td>3.00 x 10⁻⁵</td>
</tr>
<tr>
<td>poly(L-Glu-OBzl) II</td>
<td>1.92 x 10⁻⁴</td>
<td>2.94 x 10⁻⁵</td>
</tr>
<tr>
<td>poly(L-Glu-OBzl) III</td>
<td>1.32 x 10⁻⁴</td>
<td>--</td>
</tr>
<tr>
<td>poly(L-Glu-OBzl) IV</td>
<td>1.00 x 10⁻⁴</td>
<td>--</td>
</tr>
<tr>
<td>poly(L-Glu-OBzl) V</td>
<td>1.54 x 10⁻⁴</td>
<td>2.00 x 10⁻⁵</td>
</tr>
<tr>
<td>poly(L-Glu-OBzl) VI</td>
<td>1.85 x 10⁻⁴</td>
<td>3.00 x 10⁻⁵</td>
</tr>
<tr>
<td>poly(L-Glu-OBzl) VII</td>
<td>1.65 x 10⁻⁴</td>
<td>--</td>
</tr>
<tr>
<td>poly(L-Glu-OBzl) VIII</td>
<td>1.47 x 10⁻⁴</td>
<td>--</td>
</tr>
<tr>
<td>poly(L-Glu-OBzl) IX</td>
<td>1.82 x 10⁻⁴</td>
<td>--</td>
</tr>
<tr>
<td>poly(D-Glu-OBzl) III</td>
<td>1.10 x 10⁻⁴</td>
<td>2.30 x 10⁻⁵</td>
</tr>
</tbody>
</table>
O-carbobenzoxy-D-tyrosine NCA with \((L\text{-Glu-OBzl})_{20}\) with an A/I ratio of 10.

The block polymers of \(\gamma\)-benzyl-L-glutamate and \(\gamma\)-benzyl-D-glutamate were prepared by dissolving the preformed initiator \((L\text{-Glu-OBzl})_{20}\) in N,N-dimethylacetamide and adding the \(\gamma\)-benzyl-(D or L)-glutamate NCA to give the required A/I ratio and hence block length. After completion of polymerization the polymer solutions were precipitated into anhydrous isopropyl ether, washed several times with anhydrous ethyl ether, dried in vacuo over \(P_2O_5\) at room temperature for several hours, and stored in a desiccator until use.

The block polymers \((L\text{-Tyr-Z})_{n}(L\text{-Glu-OBzl})_{20}\) and \((D\text{-Tyr-Z})_{n}(L\text{-Glu-OBzl})_{20}\) were prepared by taking aliquots from a 2.5% solution of O-carbobenzoxy-(D or L)-tyrosine NCA initiated by \((L\text{-Glu-OBzl})_{20}\) in dioxane \((A/I=20)\), the aliquots being taken at times ascertained from kinetic studies to yield block lengths for \(L\text{-Tyr-Z}\) of \(n=3, 6, 10, 15,\) and 20. These aliquots were precipitated into anhydrous absolute ethanol, washed several times with absolute ethanol and anhydrous ethyl ether, dried in vacuo at room temperature over \(P_2O_5\) for several hours, and stored in a desiccator until use.

The block polymer \((L\text{-Glu-\check{B}zl})_{20}^{-}(\text{D-Tyr})_{8}\) was prepared by dissolving O-carbobenzoxy-D-tyrosine NCA in N,N-dimethylacetamide to make a 3% solution and adding \(n\)-hexylamine in an amount sufficient to give an anhydride to initiator ratio of 8. After completion of the O-carbobenzoxy-D-tyrosine NCA polymerization as ascertained from the complete disappearance of the NCA IR bands, \(\gamma\)-benzyl-L-glutamate NCA was added to the \((D\text{-Tyr-Z})_{8}\) solution in an amount to yield an A/I of 20. After completion
of the polymerization of the 7-benzyl-L-glutamate NCA, the block copolymer was precipitated into anhydrous isopropyl ether, washed several times with anhydrous ethyl ether, dried in vacuo over P₂O₅ for several hours, and stored in a desiccator.

Poly-L-tyrosine

The poly-L-tyrosine polymers were prepared in a manner exactly similar to that used in the preparation of the preformed polymer initiators as described above (Polymerization Initiators). The poly-O-carbobenzyoxy-L-tyrosine polymers were unblocked by bubbling hydrogen bromide through a 1% solution of the blocked polymer in trifluoroacetic acid-methylene chloride (1:1). The unblocked polymers were precipitated by addition of the removal solution to a large volume of anhydrous isopropyl ether with vigorous stirring being necessary to initiate precipitation of the poly-L-tyrosine. The poly-L-tyrosine was purified by several precipitations of 1% solutions of the polymer in absolute ethanol into anhydrous isopropyl ether. The final polymer obtained was washed in ethyl ether and dried at 60°C over P₂O₅ for several hours. The poly-L-tyrosine was a white powder which was readily soluble in trimethyl phosphate and DMAc.

Selective Removal of O-Carbobenzyoxy Groups

The block polymers of (L or D)-tyrosine and 7-OBzl-(L or D)-glutamate were prepared by the selective removal of the O-carbobenzyoxy group (Z) from the (D or L-Tyr-Z)n block while leaving the benzyl ester groups intact. The two principal methods of removal of the O-carbobenzyoxy moiety are (a) with hydrogen bromide (48-52) and (b) by catalytic hydrogenation (45).
Catalytic hydrogenation was explored as a removal method with partial success using 10% palladium on carbon powder (Baker and Co., Catalysts) and hydrogen pressures slightly above atmospheric pressure in a variety of solvents and solvents systems: dioxane, chloroform, chloroform-ethanol, chloroform-ethanol-acetic acid, dioxane-acetic acid. The results obtained were independent of the solvent and amount of catalyst used, i.e., the removal of the O-carbobenzoxy was very slow, complete removal requiring many hours or days. In addition, after the reaction was complete, part of the carbon powder could not be separated from the solution by physical methods. As apparatus for hydrogenation at higher pressures was not available, this method was not pursued to its full extent. A modification of the hydrogen bromide method was used to selectively remove the O-carbobenzoxy groups of the above polymers. In this method, advantage was taken of the fact that the O-carbobenzoxy group is much more susceptible to removal by hydrogen bromide, at a given hydrogen bromide (HBr) concentration, than the γ-benzyl ester. The procedure used was as follows: hydrogen bromide (Matheson Gas Products) purified by bubbling through 1, 2, 3, 4-tetrahydronapthalene, passing over light copper turnings and anhydrous calcium sulphate, was bubbled through 150 ml. of anhydrous dioxane in a 500 ml. carefully dried solvent flask at a rapid rate for one-half hour. Previous to the addition of the hydrogen bromide, the entire purification system was purged with dry nitrogen for one-half hour to remove any moisture on tubing, etc. The rate of bubbling was controlled to the extent that the flask of dioxane was allowed to become only slightly warm to the touch, not hot as might occur with too rapid an
addition of hydrogen bromide. Under the above conditions a colorless, clear solution of hydrogen bromide in dioxane of approximately 2-3 N in hydrogen bromide was obtained (a saturated solution was approximately 4.5 N). Aliquots of the dioxane/HBr solution were titrated with 0.1768 N sodium methoxide in a chloroform/absolute ethanol solution (2:1). The dioxane/HBr solution was used for only three days at most, as with increasing time the solution became a stronger γ-benzyl ester removing agent, the latter reaction probably facilitated through the slow degradation of the dioxane by hydrogen bromide with the production of water (cleavage of an alkyl ether by hydrogen bromide).

To an approximately 1% solution of the block copolymers of O-carbobenzyloxy-tyrosine and γ-benzyl-glutamate in dioxane, a sufficient volume of dioxane/HBr solution was added to make the resulting solution 1N in hydrogen bromide. Under such conditions the removal time for 95% of the O-carbobenzyloxy groups from a copolymer of O-carbobenzyloxy-D-tyrosine and γ-benzyl-L-glutamate of an equimolar composition was about 28 hours (Figure 1). The rate of O-carbobenzyloxy removal was found to be directly proportional to the concentration of hydrogen bromide and O-carbobenzyloxy groups, but varied with molecular weight, decreasing with increasing molecular weight. But for any series of polymers of approximately equal molecular weights, the time required for a specific extent of O-carbobenzyloxy removal could be predicted from the concentrations of the HBr and polymer and the composition of the copolymer. Care was taken to exclude moisture from this solution by drying all samples, glassware, and storing the reaction mixture in a tightly stoppered solvent flask in a desiccator.
Figure 1. The percent removal of O-carbobenzoxy and benzyl groups from \((\text{L-Tyr-Z})_{20}-(\text{L-Glu-OBzl})_{20}\) as a function of the removal time. (1% copolymer in 1.0 N HBr in dioxane), O-carbobenzoxy (o), benzyl (●).
The O-carbobenzoxy removal was monitored periodically by precipitating a small amount of the solution into isopropyl ether with stirring, dissolving the polymer in dioxane or N,N-dimethylacetamide and casting a thin film for infrared examination of the O-carbobenzoxy band at 1765 cm\(^{-1}\) and/or the \(\gamma\)-benzyl ester band at 1738 cm\(^{-1}\). The removal was allowed to proceed until less than 5\% of the original O-carbobenzoxy groups remained for the tyrosine-\(\gamma\)-benzyl-glutamate block copolymers.

Under the above conditions less than 10\% of the \(\gamma\)-benzyl ester groups were removed if precautions of absolute dryness of all materials were taken, as the removal is almost completely selective in the absence of moisture and bromine (Figure 1). The extent of the \(\gamma\)-benzyl ester actually removed could be crudely estimated from the relative magnitude of the \(\gamma\)-benzyl ester infrared band before and after the unblocking procedure, but as the unionized carboxyl band of glutamic acid (1710 cm\(^{-1}\)) strongly overlaps the \(\gamma\)-benzyl ester band (1738 cm\(^{-1}\)), this method could only detect extensive \(\gamma\)-benzyl ester removal. An accurate determination of the \(\gamma\)-benzyl ester removed was made by the titration of the copolymers of tyrosine and \(\gamma\)-benzyl-glutamate (53). The total amount of acid, both carboxylic acid and weakly acidic phenol, was determined by titration in the basic solvent ethylenediamine with sodium methoxide using 0-nitroaniline as indicator (procedure B, 53). The amount of unblocked ester groups alone was determined by titration in acetone with potassium methoxide using p-hydroxyazobenzene as indicator (procedure C, 53). As the copolymers were not readily soluble in either ethylenediamine or acetone alone they were first dissolved in a minimum amount of trimethyl
phosphate before the titration solvents were added. The titration method was checked using poly(L-Tyr) and poly(L-Glu) and was accurate within $\pm 2\%$. The D-L copolymers with less than 25% of the O-carbobenzoxy groups removed were insoluble in trimethyl phosphate, and thus no titrations were done upon these polymers.

Before the polymers could be titrated, adsorbed hydrogen bromide present from the O-carbobenzoxy removal procedure was removed. The copolymers were dissolved in pyridine, a small amount of absolute ethanol added to assist solution if necessary, and an equal volume of absolute ethanol and chloroform were added with stirring. The solution was precipitated into ethyl ether, washed repeatedly with ether, and dried. This procedure was repeated until a constant value of acidic content was found.

Since the titration procedure required relatively large amounts of sample, not all of the samples studied were titrated, but at least one sample in each series of polymers was thus titrated, and since the same dioxane/HBr solution was used for each sample in a series, checks upon each sample were deemed neither necessary nor economical. Certain of the polymer samples crucial in their implications to any subsequent theoretical inference were titrated in addition to the single sample in each series.

After removal of the O-carbobenzoxy group was complete, the dioxane/HBr/polymer solution was precipitated into anhydrous isopropyl ether with vigorous stirring being necessary to initiate precipitation. The polymer was washed several times with anhydrous ethyl ether until the eluate was colorless (initially faintly yellow). The ethyl ether was intended to
remove the products of the unblocking procedure, chiefly benzyl bromide. The resulting white to yellow-white polymer was dried in vacuo for several hours over P_2O_5 at room temperature. Highly colored samples were purified by dissolving the polymer in a suitable solvent, usually dioxane or DMAc and reprecipitation into ethyl ether. The polymers were stored in a desiccator until use. All block copolymers of tyrosine and \(\gamma\)-benzyl-glutamate are soluble in dioxane, trimethyl phosphate, N,N-dimethylacetamide, N,N-dimethylformamide, quinoline, pyridine, absolute ethanol, and an ethanol/chloroform mixture. Polymers of the poly(D-)–poly(L-) type frequently exhibited instances of partially insoluble aggregation, especially in the intermediate stages of O-carbobenzoxy unblocking.

Removal of O-Carbobenzoxy and \(\gamma\)-Benzyl Ester Groups

The completely unblocked block polymers of tyrosine and glutamic acid were prepared from the corresponding blocked polymers using the hydrogen bromide method with the hydrogen bromide purified as above. The hydrogen bromide was bubbled directly into a 2\% solution of the blocked polymers until a white fluffy precipitate formed. The solution was then set aside for several hours, with care taken to exclude moisture. The precipitate and solution were then transferred to a round bottomed flask and the hydrogen bromide and dioxane were removed using a rotary evaporator and gentle heating. The resultant yellow oil was treated with anhydrous ethyl ether whereupon a precipitate formed. The precipitate was collected by centrifugation, washed several times with ethyl ether and dried in vacuo for several hours at room temperature. The unblocked polymer was
further purified by dissolving the precipitate in a small amount of sodium hydroxide solution, pH 12, placing this solution in a dialysis bag and dialyzing against a large volume of 0.01N hydrochloric acid. The 0.01N hydrochloric acid solution was replaced with a 0.001N hydrochloric acid solution after precipitation of the polymer, and dialysis was continued for several hours. The dialysis bags were boiled in a 10% sodium bicarbonate solution for several minutes prior to use. The polymer was collected by lyophilization of the contents of the dialysis bag, dried at 100°C in vacuo over P₂O₅ for several hours, and stored in a desiccator.

Determination of Polymer Composition

The determination of the exact compositions of the block polymers of 0-carbobenzoxy-(L or D)-tyrosine and 7-benzyl-(L or D)-glutamate was attempted by several methods. Standard amino acid analysis upon the totally unblocked polymers (Beckmann 120 C Amino Acid Analyzer) gave relative compositions much different than expected. This was most probably due to the destruction of the tyrosine under the hydrolytic conditions prior to the analysis (6N HCl, 110°C for 20 hours) (54-58). A standard tyrosine:glutamic acid sample of a molar ratio of 1:1.29 gave, upon analysis following this hydrolytic procedure, a tyrosine:glutamic acid molar ratio of 1:1.87. Several analyses upon the standard varying the hydrolysis time demonstrated that the destruction of the tyrosine increased with time, for after 40 hours the tyrosine:glutamic acid molar ratio was 1:2.08. But this destruction pattern was not sufficiently reproducible to use as an analytical method.

The compositions of the block copolymers of 0-carbobenzoxy-(L or D)-
glutamate were ultimately determined from the optical density of the tyrosine phenolic ultraviolet absorption band(s) in a sample of known concentration. The polymers were dissolved in an aqueous sodium hydroxide solution, pH 12, at concentrations of from 3 to $5 \times 10^{-5}$ gm/ml, and from the optical density at 294 nm (pH 12, NaOH) the molar ratios of tyrosine and glutamic acid were found.

The extinction coefficient of poly(L-Tyr) was determined from a polymer prepared by n-hexylamine initiation of O-carbobenzoxy-L-tyrosine NCA in dioxane with an anhydride to initiator ratio of 30. Removal of the blocking groups and purification was exactly the same as the method used upon the O-carbobenzoxy-(L or D)-tyrosine, 7-benzyl-(L or D)-glutamate block polymers. Elemental analysis after purification: calculated N 8.54%; found N 8.46%; or 98.5% tyrosine. The extinction coefficient ($c$) of (L-Tyr)$_{30}$ in aqueous NaOH, pH 12, at 294 nm was $1960 \pm 40$ liter/mole cm. The extinction coefficient of poly(L-Glu) (Schwarz Bioresearch, Inc.) under exactly the same conditions was 20 liter/mole cm. The method was checked against a standard mixture of poly(D-Tyr-Z) and poly(L-Glu-OBzl); calculated for standard, tyr:glu::0.57:0.43; found by analysis after removal procedure, tyr:glu::0.54:0.46. Thus the compositions were determined to within ±3%, and were reproducible to within ±1% (Table 3). Note that in Table 3 the block lengths of the respective polypeptides obtained from the kinetics do not agree precisely with those obtained from the UV analysis. This is due to the fact that a certain amount of the initiator, (L-Glu-OBzl)$_{20}$, is present as "dead" polymer, that is, it possesses no free amine terminal group and hence may
Table 3. Composition and (L-Tyr) or (D-Tyr) block lengths, n, for the block copolypeptides \((\text{L or D-Tyr})_n^\text{-(L-Glu-OBzl)}_m\) obtained by several methods (\(m\) equals 20 in all polymers)

<table>
<thead>
<tr>
<th>Block Copolymer</th>
<th>Mole % Tyr(^a)</th>
<th>(n) Kinetics</th>
<th>(n) UV Abs.</th>
<th>(n) Corrected(^b)</th>
</tr>
</thead>
<tbody>
<tr>
<td>((L\text{-Tyr})_n^-)</td>
<td>21 ± 1</td>
<td>6.0</td>
<td>5.3</td>
<td>6.9</td>
</tr>
<tr>
<td>((L\text{-Tyr})_n^-)</td>
<td>28 ± 1</td>
<td>10.0</td>
<td>7.8</td>
<td>9.8</td>
</tr>
<tr>
<td>((L\text{-Tyr})_n^-)</td>
<td>44 ± 1</td>
<td>18.0</td>
<td>15.8</td>
<td>17.0</td>
</tr>
<tr>
<td>((L\text{-Tyr})_n^-)</td>
<td>47 ± 1</td>
<td>20.0</td>
<td>17.8</td>
<td>19.4</td>
</tr>
<tr>
<td>((D\text{-Tyr})_n^-)</td>
<td>18 ± 1</td>
<td>6.0</td>
<td>4.4</td>
<td>5.4</td>
</tr>
<tr>
<td>((D\text{-Tyr})_n^-)</td>
<td>27 ± 1</td>
<td>10.0</td>
<td>7.4</td>
<td>9.2</td>
</tr>
<tr>
<td>((D\text{-Tyr})_n^-)</td>
<td>39 ± 1</td>
<td>14.0</td>
<td>12.8</td>
<td>15.6</td>
</tr>
<tr>
<td>((D\text{-Tyr})_n^-)</td>
<td>47 ± 1</td>
<td>20.0</td>
<td>17.8</td>
<td>19.4</td>
</tr>
</tbody>
</table>

\(^a\)Mole % Tyr calculated from the UV absorption.

\(^b\)Block length corrected by accounting for the \((\text{L-Glu-OBzl})_m\) present as initially inactive (no free amine) initiator.
not initiate polymerization. The block lengths for these copolymers are henceforth expressed as those obtained from the kinetics as these block lengths agree well with the corrected block lengths.

Ultraviolet Absorption Measurements

Ultraviolet absorption measurements were obtained at ambient temperatures using a Cary 15 recording spectrophotometer, with matched fused-quartz 1 cm cells.

Infrared Kinetics

All infrared measurements were performed on a Beckmann IR-4 Double-Beam Spectrophotometer using a sodium chloride prism. The sample and reference cells were demountable calcium fluoride cells with the sample cell jacketed for thermostatic control. Experiments in the kinetics of polymerization were carried out with 0.1 mm cell spacers, the exact cell thickness calculated for both the sample and reference cells by using benzene (neat) as the standard absorber (thickness in mm = 0.1 x absorbance at 1960 cm⁻¹) (59). The temperature of the polymerizing solution was held constant at 30 ± 0.1°C by thermostating the jacketed sample cell with a Haake circulator, Series F, Brinkmann Instruments. Fluctuations in the sample cell temperature, if any did occur, were necessarily slow due to the large heat capacity of the jacketed sample cell. The ambient temperature around the cells was 40 ± 1°C.

The infrared kinetic method was used to determine the kinetics of N-carboxy α-amino acid anhydride (NCA) polymerizations (60). Preformed polymer initiator was dissolved in the appropriate solvent and warmed
to 30°C before the NCA was added to give an initial NCA concentration of 2.5 or 4% (w:v). A portion of this solution was transferred to the infrared absorption cell (warmed to 30°C) by a 1.0 ml interchangeable glass syringe (Luer-Lok). Measurements were begun 5 minutes after the addition of the NCA, the infrared absorption of the NCA bands in the polymerizing solution being recorded at 5-15 minute intervals by monitoring the absorbance at 1860 and/or 1790 cm⁻¹ until the reaction was complete. Since the 1790 cm⁻¹ NCA band was far more intense than the 1860 cm⁻¹ NCA band, initial absorption measurements were made at 1860 cm⁻¹, switching to the 1790 cm⁻¹ band after the polymerization was approximately two-thirds complete. Both bands gave comparable kinetic rates during any single polymerization period, and several points were measured for both bands simultaneously to allow complete continuity of the kinetic data. A baseline of solvent versus solvent was done prior to the kinetics study with the cells to be used in order to establish a reference or zero of optical density. Pure solvent was used in the reference cell during all kinetic measurements.

The plot of the log of absorbance (or optical density) at 1860 and/or 1790 cm⁻¹ versus time gave the rate curve directly. The rate constants derived from the anhydride consumption, K₁, K₂, etc., were calculated from Equations (1), (2), and (3), where K₁ represents the initial second-order rate constant for anhydride consumption, K₂ the rate constant observed in the subsequent stage, etc.

\[- \frac{d[A]}{dt} = KA[I] \]  (1)
\[ K = \frac{\ln(OD_1/OD_2)}{(t_2-t_1)[I]} \]  

Equation (2)

\[ \frac{\ln \left[ \frac{[A_o]}{[A_2]} \right] - \ln \left[ \frac{[A_o]}{[A_1]} \right]}{(t_2-t_1)[I]} \]

Equation (3)

\[ K = \frac{\ln(OD_1/OD_2)}{(t_2-t_1)[I]} \]

\[ K = \frac{\ln \left[ \frac{[A_o]}{[A_2]} \right] - \ln \left[ \frac{[A_o]}{[A_1]} \right]}{(t_2-t_1)[I]} \]

\[ [A] \] and \([I]\) are the concentrations of NCA and initiator, respectively, \(OD\) is the optical density of the NCA infrared absorption band, and \(t\) is the time. Equation (1) is valid assuming that during the polymerization the concentration of growing chains remains constant and is equal to the initiator concentration, \([I]\). The integrated form of the rate equation gives Equation (2), the equation for a pseudo-first-order reaction, where \(OD_1\) is the optical density recorded at time \(t_1\), etc. Since the optical density is directly proportional to \([A]\), the rate data may be expressed solely in terms of \([A]\) and \([A_o]\), as in Equation (3). The kinetic rates were calculated from Equation (2), and graphically expressed in terms of \([A_o]/[A]\) (log scale) versus time. The initiator concentration, \([I]\), was calculated from the concentration of preformed polymer in the polymerizing solution, as known from the initial amount added as initiator, and the equivalence of N-terminal amine in that amount of polymer. Thus the initiator concentration (in moles or equivalents per liter) was taken as the concentration of the preformed polymer (grams/liter) divided by the equivalent weight of the N-terminal amine (grams/equivalent) in that polymer.
Infrared Spectra

Infrared (IR) spectra were recorded at ambient temperatures (40°C) using the Beckmann IR4 recording spectrophotometer with sodium chloride prisms. The infrared spectra of polypeptide solutions were determined using two demountable liquid cells with calcium fluoride windows and pathlengths of 1 mm (Barnes Engineering Co., Stamford, Conn.). Polymer concentrations of 2 to 4% (w:v) were used, and a solvent to solvent baseline was done prior to running the spectra of the solutions in the double beam mode. The IR spectra of thin films were obtained by casting a portion of a 2% (w:v) polypeptide solution upon a calcium fluoride IR window. Evaporation of the solvent was hastened by heating the sample and window with an IR heat lamp (the temperature of the sample was not allowed to exceed 60°C). The thin film spectra were run in the double beam mode with air as the reference.

Optical Rotatory Studies

Optical rotatory measurements were made on a modified Rudolph manual spectropolarimeter, Model 200S/340/80 AQ6 (61). A 2 decimeter jacketed polarimeter tube with glass end-windows was used for the rotation measurements (Type 2J-3.0-200-1.4, O. C. Rudolph and Sons). The temperature was controlled with a Haake circulator (Type F, Brinkmann Instruments), all measurements being regulated at 30 ± 0.1°C. Rotations were measured at 577 nm using a high intensity mercury vapor arc lamp as the light source, as other sources did not provide light of sufficient intensity for accurate measurements with the solutions and long cell pathlengths used in rotation studies.
The optical rotation studies of the polymerizing solutions were done as follows: preformed polymer initiator was dissolved in the polymerizing solvent and warmed to 30°C before the NCA was added in an amount to give a 2.5 or 4% (w:v) initial NCA concentration with an anhydride to initiator ratio of 10-40. A portion of this solution was transferred to the 2 decimeter polarimeter tube using a 9-inch Pasteur pipette, the polarimeter tube having been previously warmed to 30°C. Rotation measurements were started after the solution in the polarimeter tube had come to equilibrium, and taken at convenient intervals until the polymerization was complete.

The results were calculated in terms of $\Delta \alpha$, $[\alpha]_p$, and $[m']_p$, as a function of the polymerization time or chain length (Equations 4-8), where $\Delta \alpha$ is the observed change in the angle of rotation (deg), $[\alpha]_p$ the specific rotation of the polymer (deg cm$^3$/gm dm), and $[m']_p$ the effective residue rotation (deg cm$^2$/decimole). Contributions to the change in rotation, $\Delta \alpha$, arise from two sources, the growing polymer, $\Delta \alpha_p$, and the vanishing NCA, $\Delta \alpha_{NCA}$ (Equation 5). The contribution of the growing polymer to the optical rotation may be calculated if the specific rotation of the NCA, $[\alpha]_{NCA}$, and the concentration of the NCA are known (Equation 6). The specific rotation of the polymer, $[\alpha]_p$, may be converted to the effective residue rotation, $[m']_p$, from the relationship in Equation (8) where $M_o$ is the residue weight in grams per mole, $n$ is the refractive index of the solvent at a specified wavelength, and $l$ is the pathlength in decimeters.

$$\Delta \alpha = \alpha_{obs} - \alpha_{initial}$$ (4)
Circular Dichroism

Circular dichroism spectra were obtained on a Jasco ORD/CD/UV-5 spectropolarimeter with a modified circular dichroism mode (Sproul Scientific SS-20-2 CD modification) which allowed very accurate circular dichroism measurements from 183-700 nm. Measurements were also made upon a Jasco CD/SP spectropolarimeter at the University of Minnesota, Minneapolis, through the courtesy of Dr. Donald Wetlaufer. Spectra obtained from the latter instrument were largely supplanted by those obtained from the former renovated instrument, due to their greater accuracy, especially in the region of 183-210 nm. Spectral determinations were done primarily with a 1 mm fused quartz cell, 0.5 mm and 0.2 mm pathlength fused quartz cells being used to check spectra at wavelengths below 195 nm when necessary, as in highly absorbing samples. Circular dichroism results were reported in terms of molar ellipticity, [θ] (deg cm²/dmole), and were not corrected for the refractive index of the solvent (62). The

\[ \Delta \alpha = \Delta \alpha_p + \Delta \alpha_{NCA} \]  

\[ \Delta \alpha_p = \Delta \alpha - [\alpha]_{NCA} \Delta C_{NCA} \]  

\[ [\alpha]_p = \frac{\Delta \alpha_p}{\Delta C_p \, \lambda} \]  

\[ [m']_p = \frac{3M_o [\alpha]_p}{100(n^2+2)} \]
molar ellipticity \([\theta]\) is related to \(\Delta\varepsilon\), the difference between the residue-molar extinction coefficients (liter/mole cm) for left and right circularly polarized light by

\[
[\theta] = 3298 \Delta\varepsilon. \tag{9}
\]
CHAPTER 3. KINETICS OF POLYMERIZATION

The primary amine polymerization of N-carboxy-α-amino acid anhydrides presumably occurs by the following mechanism (25,63):  

Initiation;  
\[
\text{R-CH-CO} \quad \text{NH-CO} + R'-\text{NH}_2 \xrightarrow{k_1} \text{R}-\text{NH-CO-CH-NH}_2 + \text{CO}_2
\]

Propagation;  
\[
\text{R-CH-CO} \quad \text{NH-CO} + R'-\text{NH-CO-CH-NH}_2 \xrightarrow{k_p} \text{R}-\text{NH-(CO-CH-NH)}_{n-1}\text{H} + \text{CO}_2 \quad p=1,2,\ldots
\]

Termination;  
\[
\text{R-CH-CO} \quad \text{NH-CO} + R'-\text{NH-(CO-CH-NH)}_n\text{H} \xrightarrow{k_t} \text{R}-(\text{CO-CH-NH})_n\text{-CO-NH-CH-COOH}
\]

The termination reaction(s) may proceed by different mechanisms than that show above, depending in part upon the nature of the polymer, as in the case of the cyclization of the end groups in poly-γ-benzyl-L-glutamate (64). In the absence of a significant extent of the termination reaction, a polypeptide having a degree of polymerization (DP) equal to the mole ratio of anhydride to initiator will result. If the initiation rate is fast relative to the propagation rate(s), and if an anhydride to initiator ratio of less than 100 is utilized, only those rates due to propagation will be seen. Unless the termination rate is fast relative to the propagation rate(s), the termination rate will be largely masked by the faster propagation rate(s). Under the above
conditions, the resulting polypeptide will have a narrow molecular weight distribution (63,65).

According to the reaction scheme given above, the consumption of anhydride can be represented as follows using \([A], [I]\), and \([P]\) for the molar concentrations of anhydride, initiator, and polymer respectively.

\[- \frac{d[A]}{dt} = k_i[A][I] + k_p[A][P] + k_t[A][P] \]  

(10)

However, if \(k_i \geq k_p \gg k_t\) and \([P] = [I]\), this can be represented by a pseudo-first-order rate equation

\[- \frac{d[A]}{dt} = k_p[A][P] = k'[A] \]  

(11)

The above relationships among the rate constants will hold if the initiation rate is much faster than the subsequent polymerization rates and also assuming that termination reactions are negligible; that is, the concentration of all the growing chains is constant and equal to the original initiator concentration, \([I]\). Consequently, the plot of the logarithm of the ratio of the initial anhydride concentration to the anhydride concentration at time \(t\) \((A_0/A_t)\) versus the polymerization time can be expected to result in a straight line. Our data are represented in this manner where the rate constants are always given in the units of liter/mole sec.

The results of the kinetic experiments obtained from the initiation of polymerization with preformed polymer initiator using \(\alpha\)-amino acid N-carboxyanhydrides in dioxane are shown in Figures 2-5. These
correspond to the polymerizations at a constant anhydride to initiator ratio of twenty \([A]/[I] = 20\). The figures on the curves are the rate constants expressed in units of liter/mole sec.

In the experiments in which the anhydride and preformed initiator were of the same configuration (L enantiomer), inspection suggests that the initiation reaction generally occurred faster than, or at the same rate as the subsequent propagation reactions. Thus the kinetics may be expressed by a single rate constant for the growth of the polymer, \(k_1\). The exception to this behavior was found for the \((L\text{-Glu-OBzl})_{20}\) initiation of the \(L\text{-Asp-OBzl}\) N-carboxyanhydride, where the kinetic behavior was unlike that found in all of the other kinetic studies; that is, the kinetics of the \(L\)-initiator with the \(L\)-anhydride demonstrated three successive kinetic rates in the latter case (Table 4).

For the experiments in which the configurations of the initiator and anhydride were opposite, a complex kinetic behavior was observed (Figures 2-5). The initiation rate is faster than, or approximately equal to the subsequent propagation rates, of which there were two or three distinctly observable from the first-order rate plots. If in the cases where three rates are seen, the first rate constant is identified with the initiation reaction, then all of the propagation reactions in which the initiator and anhydride are of the opposite configuration fit a two-rate kinetic scheme. Again, as above, the \((D\text{-Glu-OBzl})_{20}\) initiation of \(L\text{-Asp-OBzl}\) anhydride formed an exception to the general behavior, a single propagation rate being observed (Table 4). Therefore,
these kinetic results may be expressed by two successive rate constants for chain propagation, $k_1$ and $k_2$, and the initiation reaction rate, $k_i$ (the problems associated with the identification of the first rate constant as that due to the initiation reaction where three distinct rates are seen are discussed in Chapter 6, page 98).
<table>
<thead>
<tr>
<th>Anhydride</th>
<th>Initiator</th>
<th>$k_i \times 10^3$</th>
<th>$k_1 \times 10^3$</th>
<th>$k_2 \times 10^3$</th>
</tr>
</thead>
<tbody>
<tr>
<td>L-Tyr-Z</td>
<td>(L-Glu-OBzl)$_{20}$</td>
<td>---</td>
<td>---</td>
<td>18.4</td>
</tr>
<tr>
<td>D-Tyr-Z</td>
<td>(L-Glu-OBzl)$_{20}$</td>
<td>---</td>
<td>1.7</td>
<td>7.3</td>
</tr>
<tr>
<td>L-Lys-Z</td>
<td>(L-Glu-OBzl)$_{20}$</td>
<td>---</td>
<td>---</td>
<td>192.0</td>
</tr>
<tr>
<td>D-Lys-Z</td>
<td>(L-Glu-OBzl)$_{20}$</td>
<td>---</td>
<td>24.5</td>
<td>116.0</td>
</tr>
<tr>
<td>L-Asp-OBzl</td>
<td>(L-Glu-OBzl)$_{20}$</td>
<td>4.3</td>
<td>1.2</td>
<td>2.1</td>
</tr>
<tr>
<td>L-Tyr-Z</td>
<td>(L-Glu-OMe)$_{20}$</td>
<td>---</td>
<td>---</td>
<td>12.4</td>
</tr>
<tr>
<td>D-Tyr-Z</td>
<td>(L-Glu-OMe)$_{20}$</td>
<td>11.0</td>
<td>1.3</td>
<td>6.7</td>
</tr>
<tr>
<td>L-Glu-OBzl</td>
<td>(L-Glu-OBzl)$_{20}$</td>
<td>---</td>
<td>---</td>
<td>36.0</td>
</tr>
<tr>
<td>D-Glu-OBzl</td>
<td>(L-Glu-OBzl)$_{20}$</td>
<td>---</td>
<td>9.5</td>
<td>24.0</td>
</tr>
<tr>
<td>L-Asp-OBzl</td>
<td>(D-Glu-OBzl)$_{20}$</td>
<td>---</td>
<td>---</td>
<td>5.0</td>
</tr>
</tbody>
</table>
Figure 2. The kinetics of the \( (\text{L-Glu-OBzI})_{20} \) initiated polymerization of D-Tyr-Z (●,▲) and L-Tyr-Z (○,△) NCA in dioxane at 30°C. For \( A/I = 20 \) (●,○), \([I] = 0.0074 \text{ moles/liter}, [A] = 0.143 \text{ moles/liter}\). For \( A/I = 40 \) (▲,△), \([I] = 0.0074 \text{ moles/liter}, [A] = 0.290 \text{ moles/liter}\). The figures on the graph are the kinetic rates in liters/mole sec.
Figure 3. The kinetics of the (L-Glu-OBzl)_{20} initiated polymerization of D-Lys-Z (●) and L-Lys-Z (○) NCA in dioxane at 30°C, A/I = 20. [I] = 0.0072 moles/liter, [A] = 0.138 moles/liter. The figures on the graph are the kinetic rates in liters/mole sec.
\( \frac{A_0}{A_t} \) vs Time (Minutes)

- Time (Minutes): 10, 20, 30, 40, 50, 60, 70
- Values: 1.94, 1.16, 0.24
Figure 4. The kinetics of the \((L-\text{Glu-OMe})_{20}\) initiated polymerization of D-Tyr-Z (●) and L-Tyr-Z (○) NCA in dioxane at 30°C, A/I = 20. \([I] = 0.0069 \text{ moles/liter}, \quad [A] = 0.120 \text{ moles/liter.}\) The figures on the graph are the kinetic rates in liters/mole sec.
Figure 5. The kinetics of \((\text{L-Glu-OBzl})_2\) \text{O} initiated polymerization of L-Asp-OBzl NCA, (●), and \((\text{D-Glu-OBzl})_2\) \text{O} initiated polymerization of L-Asp-OBzl NCA, (○), in dioxane at 30°C, A/I = 20. \([I] = 0.0062 \text{ moles/liter, } [A] = 0.115 \text{ moles/liter.} \) The figures on the graph are the kinetic rates in liters/mole sec.
CHAPTER 4. OPTICAL ROTATION STUDIES

A possible insight into the origins of the kinetic behavior of the polymerizations may be obtained from following the optical rotation of the polymerization solution during the polymerization reaction, noting the effects of the solvent and initiator upon the optical rotation when the various α-amino acid N-carboxyanhydrides are initiated with the pre­formed polymers. Such studies hopefully may provide information about the stereochemical and structural features of the initiator-anhydride system which give rise to the kinetic rate differences. The results of such studies for a variety of solvents, initiators, and anhydrides are shown in Figures 6 to 14 (the anhydride to initiator is twenty unless otherwise specified).

The work of Lundberg and Doty (25), specifically the study of the optical behavior for the initiation of L-Glu-OBzl and D-Glu-OBzl NCA by poly (L-Glu-OBzl) in dioxane (along with other studies done by these authors) first suggested the possibilities of similar studies for the elucidation of helical sense and stability, as contained in this work.

The addition of the L-anhydride to the L-preformed initiating polymer in dioxane resulted in a monotonic increase in the observed change in the optical rotation, $\Delta \alpha$, throughout the course of the polymerization reaction in all cases, with the exception of the poly (L-Glu-OBzl) initiation of L-Asp-OBzl NCA (Figure 8).

The addition of the D-anhydride to the L-initiator in dioxane resulted in an initial increase in the observed optical rotation, which
after reaching a maximum, decreased to some final negative value. The parallel experiment was not done with D-Asp-OBzl NCA, but instead L-Asp-OBzl NCA was initiated by poly(D-Glu-OBzl) in dioxane. As was the experience in the kinetic studies, poly(L-Asp-OBzl) formed an exception to the general optical behavior of the other initiator-anhydride systems studied. The growth of the L-Asp-OBzl block upon the initiator, (L-Glu-OBzl)\textsubscript{20}, in dioxane resulted in an initial increase in the (change in) optical rotation, which was followed by a small decrease. The growth of the L-Asp-OBzl block upon the poly(D-Glu-OBzl) initiator in dioxane resulted in a monotonic decrease in the change in the optical rotation observed. Thus, the optical rotation behavior of poly (L-Asp-OBzl) is unlike that for the other L-polypeptides formed by initiation of their NCAs with preformed polypeptides in dioxane.

The affect of the nature of the initiating polymer upon the optical behavior during polymerization is shown in the Figures 6 and 11, where the comparison of the poly(L-Glu-OMe) with poly(L-Glu-OBzl) in the initiation of D-Tyr-Z and L-Tyr-Z NCAs in dioxane demonstrates a possible quantitative difference between these two cases. This comparison is even more striking in the instance of the initiation of D-Lys-Z and L-Lys-Z NCA. The initiation of polymerization by poly(L-Glu-OMe) of the D-Lys-Z and L-Lys-Z NCAs in dioxane resulted in a monotonic decrease or increase in the optical rotation, respectively. This behavior contrasts sharply with the poly(L-Glu-OBzl) initiation of these NCAs (Figures 12 and 7).
The affect of the solvent upon the optical behavior during the course of the polymerization reaction is clearly demonstrated in Figures 13 and 14, where N,N-dimethylacetamide is the polymerization solvent. The poly(L-Glu-OBzI) initiation of L-Tyr-Z and D-Tyr-Z NCAs showed only a quantitative difference from its behavior in dioxane, but the preformed polymer initiation of L-Lys-Z and D-Lys-Z NCAs showed a qualitative difference, in that, the growth of the D-Lys-Z upon the poly(L-Glu-OBzI) initiator showed no initial increase in the change in the optical rotation as was observed for the same experiment in dioxane.

The changes in the optical rotation during the polymerization reaction are due to the changes in the contributions to the optical rotation by the optically active NCA and the optically active growing polypeptide. The knowledge of the kinetic rates and the specific rotations of the NCAs allows the calculation of the contribution to the observed rotation by the NCA and polypeptide at any time (Equations 4-7, Chapter 2). The results of such a calculation demonstrate that $\Delta \alpha_p$ is significantly different from $\Delta \alpha_{obs}$. The effective residue rotations of several homopolypeptides in a variety of solvents are given in Table 5. The effective residue rotations are those found for the long isolated $\alpha$ helical polypeptides ($[m']'$) and for the same polypeptides presumably of the opposite helix sense ($[m']''$). These latter effective residue rotations were obtained from rotation measurements upon the block copolymers where it was assumed that for short grafted blocks the helix sense of the block was the same as that of the initiating block. As an example, the value of $[m']''$ for poly(L-Glu-OBzI) was obtained from the rotation
Table 5. Effective residue rotations of several α helical homopolypeptides for the preferred helix sense (\([m']^1\)) and the opposite sense (\([m']^2\))

<table>
<thead>
<tr>
<th>Polypeptide</th>
<th>Solvent</th>
<th>([m']^1) 577 (deg cm(^2/dm))</th>
<th>([m']^2) 577 (deg cm(^2/dm))</th>
</tr>
</thead>
<tbody>
<tr>
<td>Poly-γ-benzyl-L-glutamate</td>
<td>dioxane</td>
<td>14.3</td>
<td>-62.0</td>
</tr>
<tr>
<td>Poly-γ-benzyl-L-glutamate</td>
<td>TMP</td>
<td>7.3</td>
<td>-32.0</td>
</tr>
<tr>
<td>Poly-γ-benzyl-L-glutamate</td>
<td>quinol.</td>
<td>33.0</td>
<td>---</td>
</tr>
<tr>
<td>Poly-γ-methyl-L-glutamate</td>
<td>dioxane</td>
<td>15.8</td>
<td>---</td>
</tr>
<tr>
<td>Poly-β-benzyl-L-aspartate</td>
<td>dioxane</td>
<td>-205.0</td>
<td>-45.0</td>
</tr>
<tr>
<td>Poly-ε-N-carbobenzoxy-L-lysine</td>
<td>dioxane</td>
<td>4.0</td>
<td>---</td>
</tr>
<tr>
<td>Poly-0-carbobenzoxy-L-tyrosine</td>
<td>dioxane</td>
<td>350.0</td>
<td>-240.0</td>
</tr>
<tr>
<td>Poly-L-tyrosine</td>
<td>dioxane</td>
<td>300.0</td>
<td>-220.0</td>
</tr>
<tr>
<td>Poly-L-tyrosine</td>
<td>TMP</td>
<td>190.0</td>
<td>-165.0</td>
</tr>
<tr>
<td>Poly-L-tyrosine</td>
<td>quinol.</td>
<td>192.0</td>
<td>-165.0</td>
</tr>
<tr>
<td>Poly-L-tyrosine</td>
<td>DMAc</td>
<td>194.0</td>
<td>---</td>
</tr>
</tbody>
</table>
measurements upon the copolymer \((D\text{-}Gl u\text{-}O B z l)\text{4}\)\(+(L\text{-}Gl u\text{-}O B z l)\text{20}\) assuming that the difference in the effective residue rotation of this copolymer as compared to \((L\text{-}Gl u\text{-}O B z l)\text{20}\) was due to the grafted \((D\text{-}Gl u\text{-}O B z l)\text{4}\) block. The value of \([m']\text{}'\) for poly\((L\text{-}Gl u\text{-}O B z l)\) will then be just the negative of the value found for the grafted \(D\text{-}Gl u\text{-}O B z l\) block.
Figure 6. The optical rotation change observed as a function of time for the \((L-\text{Glu-OBzl})_{20}\) initiated polymerization of D-Tyr-Z and L-Tyr-Z NCA in dioxane at 30°C, \(A/I = 20\), D-NCA (●), L-NCA (○), and the optical rotation change due to the growing block copolymer only (---- and --○--). \([I] = 0.0036\) moles/liter, and \([A] = 0.073\) moles/liter; pathlength = 2 decimeters.
The graph illustrates the change in angle ($\Delta \alpha$) as a function of time (hours). Different curves represent various conditions or data sets, with each curve showing a distinct behavior over time. The x-axis represents time in hours, ranging from 0 to 80, and the y-axis represents the change in angle, ranging from 0° to -14°.
Figure 7. The optical rotation change observed as a function of time for the (L-Glu-OBzl)$_{20}$ initiated polymerization of D-Lys-Z and L-Lys-Z NCA in dioxane at 30°C, [A]/[I] = 20, D-NCA (●), L-NCA (○), and the optical rotation change due to the growing block copolymer only (---●--- and --○--). [I] = 0.0034 moles/liter, and [A] = 0.069 moles/liter; pathlength = 2 decimeters.
Figure 8. The optical rotation change observed as a function of time for the (L-Glu-OBzI)$_{20}$, (o), and (D-Glu-OBzI)$_{20}$, (●), initiated polymerization of (L-Asp-OBzI) NCA in dioxane at 30°C, A/I = 36, and the optical rotation change due to the growing block copolymer only (--- and --●--). [I] = 0.0033 moles/liter, and [A] = 0.116 moles/liter; pathlength = 2 decimeters.
Figure 9. The optical rotation change observed as a function of time for the (L-Glu-OBz1)$_2$, (o), and (D-Glu-OBz1)$_2$, (●), initiated polymerization of (L-Asp-OBz1Np) NCA in dioxane at 30°C, A/I = 20. [I] = 0.0073 moles/liter, and [A] = 0.146 moles/liter; pathlength = 2 decimeters.
Figure 10. The change in the specific rotation observed as a function of time of polymerization for the (D- or L-Tyr)\textsubscript{n}-(L-Glu-OBzI)\textsubscript{20} block copolymers in dioxane, (●, o), and trimethyl phosphate, (▲, △), A/I = 20; (D-Tyr)\textsuperscript{n} (● and ▲), (L-Tyr)\textsuperscript{n} (o and △). The degree of polymerization (n) of the (D- or L-Tyr)\textsubscript{n} as marked on the graph.
Figure 11. The change in the observed optical rotation as a function of time for the (L-Glu-O-Me)$_n$ initiated polymerization of D-Tyr-Z and L-Tyr-Z NCA in dioxane at 30°C, A/I = 20, D-NCA (●), L-NCA (○), and the optical rotation change due to the growing block copolymer only (---●--- and ---○---). [I] = 0.0029 moles/liter and [A] = 0.057 moles/liter; pathlength = 2 decimeters.
Figure 12. The optical rotation change observed as a function of the polymerization time for the \((\text{L-Glu-OMe})_{20}\) initiation of D-Lys-Z and L-Lys-Z NCA in dioxane at 30°C, \(A/I = 20\), D-NCA (●), L-NCA (○). \([I] = 0.0036\) mole/liter and \([A] = 0.072\) moles/liter; pathlength = 2 decimeters.
Figure 13. The optical rotation change observed as a function of the polymerization time for the \((\text{L-Glu-OBzl})_2\) \(_2\) initiation of D-Lys-Z and L-Lys-Z NCA in N,N-dimethylacetamide at 30°C, \(A/I = 30\), D-NCA (●), L-NCA (○). \([I] = 0.0048\) moles/liter and \([A] = 0.140\) moles/liter; pathlength = 2 decimeters.
Figure 14. The optical rotation change observed as a function of the polymerization time for the (L-Glu-OBzl)\textsubscript{20} initiation of D-Tyr-Z and L-Tyr-Z NCA in N,N-dimethylacetamide at 30°C, A/I = 20, D-NCA (●), L-NCA (○). [I] = 0.0037 moles/liter and [A] = 0.070 moles/liter; pathlength = 2 decimeters.
CHAPTER 5. CIRCULAR DICHROISM OF BLOCK POLYMERS

The optical studies of block polymers at a single wavelength provide information as to the magnitude of the rotations and their changes as a function of composition, etc., but the interpretation that these rotation changes ultimately originate as a consequence of specific conformational changes is a debatable question, since the dichroic absorption bands giving rise to the optical rotation are far removed from the visible wavelength region. Therefore, it is necessary to study the dichroic absorption bands characteristic of specific conformational states; that is, to study the circular dichroism of the block polymers and correlate these data with those obtained from the single wavelength studies, thereby determining whether such studies yield information directly applicable to a specific conformation or conformational change.

Figure 15 shows the circular dichroism (CD) patterns for a series of poly(D-Glu-OBzl)-poly(L-Glu-OBzl) block copolymers of various compositions in trimethyl phosphate (TMP), the circular dichroism of the poly(D-Glu-OBzl) blocks alone being shown. The circular dichroism of the added D-blocks was obtained from the following equation:

\[
[\theta]_D = \frac{[\theta]_{DL} - X_L [\theta]_L}{X_D}
\]

where \([\theta]_{DL}\) is the molar ellipticity of the block copolymer, \([\theta]_D\) and \([\theta]_L\) the molar ellipticities of the D- and L-blocks respectively, and \(X_D\) and \(X_L\) the mole fraction of the D- and L-blocks in the copolymer. The compositions of the block polymers were determined from the net
amounts of initiator and NCA, assuming that all of the NCA reacted to yield the polymer block. The circular dichroism of the \((\text{D-Glu-OBzI})_{10}\) block manifests a CD pattern qualitatively similar to that of the initiating \((\text{L-Glu-OBzI})_{20}\) block, while the CD of the \((\text{D-Glu-OBzI})_{20}\) block is qualitatively opposite to that of the initiating block. The analogous experiment of adding blocks of poly(\(\text{L-Glu-OBzI}\)) to the \((\text{L-Glu-OBzI})_{20}\) initiator gave CD patterns for the added L-blocks similar to that of the initiating block (Figure 16).

Similar CD studies for the block copolymers of poly(L- and D-Tyr) and poly(L-Glu-OBzI) in trimethyl phosphate were done. The results of such experiments are shown in Figures 17 and 18, and Table 6. The CD of the \((\text{D-Tyr})_n\) block on \((\text{L-Glu-OBzI})_{20}\) shows a distinct dependence of the sign of the CD upon the chain length, the sign of the largest CD band at 200-203 nm passing from a positive band for short chains, to a negative band for long chains. The CD of the \((\text{L-Tyr})_n\) blocks on \((\text{L-Glu-OBzI})_{20}\) shows a dependence of the magnitude of the circular dichroism upon the chain length.

The circular dichroism for the \((\text{L-Glu-OBzI})_{20}(\text{D-Tyr})_8\) block copolymer in trimethyl phosphate (Table 6) demonstrates a CD pattern qualitatively similar to that for the very long poly-D-tyrosine but is smaller in magnitude. Note that in this case, the \((\text{L-Glu-OBzI})_{20}\) block lies to the N-terminus side of the junction region between the two blocks, and further that the CD of the \((\text{D-Tyr})_8\) block in this copolymer is much different from that in the \((\text{D-Tyr})_{10}(\text{L-Glu-OBzI})_{20}\) block copolymer (Figure 18).
The molar ellipticities of the above block copolymers in trimethyl phosphate are given in Table 6.

The circular dichroism of poly-L-tryosine in trimethyl phosphate is shown in Figure 19, and the molar ellipticity and molar residue rotation of poly-L-tyrosine as a function of the chain length in trimethyl phosphate is given in Table 7.
Figure 15. The circular dichroism in trimethyl phosphate of $(D$-Glu-OBzl)$_{10}$ and $(D$-Glu-OBzl)$_{20}$ polypeptide blocks grafted to $(L$-Glu-OBzl)$_{20}$. The circular dichroism curves are for the D-blocks and L-block independently.

$(D$-Glu-OBzl)$_{10}$: ----
$(D$-Glu-OBzl)$_{20}$: -----
$(L$-Glu-OBzl)$_{20}$: ———
Figure 16. The circular dichroism of (L-Glu-OBzl)$_{20}$, (L-Glu-OBzl)$_{30}$, and (L-Glu-OBzl)$_{40}$, in trimethyl phosphate.
Figure 17. The circular dichroism of the \( (D\text{-Tyr})_n \) block of the block copolymer \( (D\text{-Tyr})_n-(L\text{-Glu-OBzl})_{20} \) in trimethyl phosphate, where \( n \) equals \( 6,\ldots, 10,\ldots, \) and \( 20,\ldots \).
Figure 18. The circular dichroism of the \((L\text{-Tyr})_n\) block of the block copolymer \((L\text{-Tyr})_n-(L\text{-Glu-OBzl})_{20}\) in trimethyl phosphate, where \(n\) equals 10, 20, and 25.
Figure 19. The circular dichroism of (L-Tyr)$_n$ in trimethyl phosphate for a variety of chain lengths or degrees of polymerization (DP), where DP (number average) equals 10, 20, 40, and approximately 200.
Table 6. Molar ellipticities of grafted block polymers in trimethyl phosphate

<table>
<thead>
<tr>
<th>Polypeptide(^a)</th>
<th>([\theta] \times 10^{-4}) (deg cm(^2)/dmole)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>193 nm</td>
</tr>
<tr>
<td>(L-Glu-OBzl)(_{20})</td>
<td>8.30</td>
</tr>
<tr>
<td>(D-Glu-OBzl)(<em>{10}) - (L-Glu-OBzl)(</em>{20})</td>
<td>2.30</td>
</tr>
<tr>
<td>(D-Glu-OBzl)(<em>{20}) - (L-Glu-OBzl)(</em>{20})</td>
<td>-5.20</td>
</tr>
<tr>
<td>(D-Tyr)(<em>6) - (L-Glu-OBzl)(</em>{20})</td>
<td>4.50</td>
</tr>
<tr>
<td>(D-Tyr)(<em>10) - (L-Glu-OBzl)(</em>{20})</td>
<td>0.05</td>
</tr>
<tr>
<td>(D-Tyr)(<em>{20}) - (L-Glu-OBzl)(</em>{20})</td>
<td>-5.20</td>
</tr>
<tr>
<td>(L-Glu-OBzl)(_{20}) - (D-Tyr)(_8)</td>
<td>-5.00</td>
</tr>
<tr>
<td>(L-Tyr)(<em>{10}) - (L-Glu-OBzl)(</em>{20})</td>
<td>6.20</td>
</tr>
<tr>
<td>(L-Tyr)(<em>{20}) - (L-Glu-OBzl)(</em>{20})</td>
<td>10.40</td>
</tr>
</tbody>
</table>

\(^a\)The molar ellipticities are for the blocks marked with an asterisk(\(*\)).
Table 7. Dependence of the molar ellipticity and effective residue rotation of poly-L-tyrosine upon the degree of polymerization in trimethyl phosphate

<table>
<thead>
<tr>
<th>DP_n</th>
<th>$[\eta]^{30}_{577}$</th>
<th>$[\theta] \times 10^{-4}$ (deg cm$^2$/dmole)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>202 nm</td>
</tr>
<tr>
<td>10</td>
<td>20.0</td>
<td>1.22</td>
</tr>
<tr>
<td>20</td>
<td>45.0</td>
<td>1.50</td>
</tr>
<tr>
<td>40</td>
<td>66.0</td>
<td>1.90</td>
</tr>
<tr>
<td>200</td>
<td>190.0</td>
<td>11.00</td>
</tr>
</tbody>
</table>
Lundberg and Doty (24, 25) have observed the kinetic and optical behavior of the poly-γ-benzyl-L-glutamate initiated polymerization of L- and D-γ-benzyl-glutamate N-carboxyanhydride in dioxane. They observed kinetic and optical behavior very similar to that found here for the mixed initiator-anhydride systems. Lundberg and Doty explained the kinetic rate and optical rotation change observed as having their origins in a conformational change of the growing polymer (25). In the case of the addition of the D-anhydride to the L-preformed polypeptide initiator, the observed kinetic behavior was described by an initial nearly linear rate curve followed by a faster propagation step. The behavior which would be anticipated from observations of the non-polymeric primary amine initiation of N-carboxyanhydrides is that after a relatively slow initiation reaction (presumably due to configurational selectivity) a single linear propagation rate would be observed, approximately equal to the L-L growth rate (66). Therefore, in the experiments of Lundberg and Doty, the effect of the opposite enantiomorph upon the reaction rate was seen to be greater than expected if it was assumed that only the last residue of the growing polymer exerted configurational selectivity. The most probable explanation of such an effect is based upon the steric influences of residues further removed from the growing terminal residue, these being relatively enhanced if the conformation of the polymer were α helical. Other studies tend to support this view (67). For the addition of the D-anhydride to the L-preformed polymer
initiator, the observed change in the optical rotation as a function of the polymerization time increased initially until a block length of approximately 4 residues was added whereupon the optical rotation decreased continuously to a final negative value (25). Lundberg and Doty interpreted this to mean that the initial D-residues added to the L-polymer initiator in the same helix sense as the initiator and subsequently this "induced" helix reverted to its preferred sense at longer block lengths (25).

The kinetic and optical studies of the initiation by preformed polypeptides of a variety of amino acid N-carboxyanhydrides (NCAs) and studies upon the block copolymers thus formed gives additional support to and expands upon the previous explanations for the kinetic and optical phenomenon observed. From these studies it is possible to develop explanations for many of the experimental facts in terms of a single effect. This effect, whereby the conformation of one polypeptide block of a block copolymer is dependent upon the conformation of the adjacent polypeptide block will be called "conformational induction".

Optical Rotation Studies

The optical rotation studies lend strong support to the theory of conformational induction, and demonstrate a dependence of the phenomenon upon the polymerization solvent and the nature of the preformed polypeptide initiator.

In the case of the addition of the D-NCA, the residues which add to the initiating L-polypeptide may do so initially in the same helix
sense as the initiator. If it is assumed that the optical rotation due to such a short induced helix of D-peptides residues is positive, then conformational induction would explain the initial increase in the optical rotation observed. The subsequent decrease in the rotation after a maximum value has been reached may indicate that after sufficient D-residues have been grown upon the preformed initiator, the helix sense of the grafted block reverts to that sense characteristic of the long isolated D-polypeptide whose rotation is equal to and opposite that of the long L-polypeptide.

In the case of the addition of the L-NCA to the initiating L-polypeptide (with the exception of L-Asp-OBzl) the residues may do so in the same helix sense as the L-initiator and continue in that helix sense for the growth of the entire polypeptide. Since the optical rotation of such an L-α-helix is found to be positive at the wavelength at which the studies were done (Table 5), the observed rotation would be expected to increase in a monotonic fashion, and asymptotically approach a final positive value, as indeed was the observed behavior in most cases.

The behavior of poly(L-Asp-OBzl) might hopefully be explained in a manner analogous to the D-L block copolymer situation, where the fact that this polymer prefers a helix sense opposite that of the initiator would lead to an induction-reversion phenomenon (18,68-70). In such a case the L-Asp-OBzl residues would initially add to the L-initiator in the same helix sense as the initiator, and then revert to the opposite sense at some longer chain length. An optical behavior similar to the D-L copolymer experiment would, under these conditions, be anticipated.
The optical behavior actually found for such experiments differs considerably from that for the D-L copolymer experiments, for the initiation of L-Asp-OBzl NCA with poly(L-Glu-OBzl) in dioxane resulted in an initial small decrease in the optical rotation observed for the grafted L-Asp-OBzl residues (Figure 8). Therefore, induction could only be assumed to occur in this case if the optical rotation of the induced helix were negative. Previously we have assumed (at least tacitly) that the optical rotation due to an \( \alpha \) helix of a single sense has the same sign regardless of the configuration of the constituent residues. Therefore, in the above case one would expect that the change in the rotation upon the induction of the L-Asp-OBzl residues into the same helix sense as the L-initiator would be positive, not negative. But this may prove to be a totally erroneous assumption. Previous studies upon the random copolymers of L-Glu-OBzl and (L or D)-Asp-OBzl of various compositions demonstrated that the rotational contribution of the L-Asp-OBzl residues in the helix sense preferred by poly(L-Glu-OBzl) may indeed be negative (18,68). In these studies it was found that the poly(L-Asp-OBzl, L-Glu-OBzl) random copolymers would persist in a single helix sense, as estimated from the Moffit-Yang \( b_0 \) parameter, over a wide range of the mole-fraction of L-Asp-OBzl residues. Since the change in the specific rotation was nearly linear over a fairly broad range of composition, it is possible to calculate the optical rotation due to the L-Asp-OBzl from any copolymer whose composition is known, or by extrapolation (Table 5) (18). The optical behavior of the poly(D-Glu-OBzl) initiation of the L-Asp-OBzl NCA in dioxane may indicate that the residues initially add
to the D-initiator in the helical sense of the initiator and continue in that same sense during the entire block growth. Therefore, it appears that the optical behavior for the initiation of the L-Asp-OBzl NCA with D- or L-initiator is not inconsistent with conformational induction.

For reasons still not understood, the introduction of a nitro group into the para position of the aromatic ring in the side chain can reverse the helix sense of poly(L-Asp-OBzl) (19, 35, 71). Optical rotatory dispersion studies on copolymers of these two amino acids revealed that the transition from the left-hand helical sense of poly(L-Asp-OBzl) occurred at 26 to 32 mole per cent nitro residue content. Thus it appears that the right-handed poly(L-Asp-OBzlNp) helix is the more stable one (19, 71). The poly(L-Glu-OBzl) initiation of L-Asp-OBzlNpNCA in N,N-dimethylacetamide shows an optical behavior similar to that of poly-(D-Glu-OBzl) initiation of L-Asp-OBzl NCA in dioxane (Figures 8 and 9). This result is in keeping with the interpretation that the poly(L-Asp-OBzlNp) and poly(L-Asp-OBzl) prefer opposite helix senses.

While the poly(L-Glu-OBzl) initiation of D-Lys-Z and D-Tyr-Z NCAs both show optical behavior characteristic of conformational induction in dioxane (Figures 6 and 7), when N,N-dimethylacetamide was the polymerization solvent this effect was qualitatively different for the D-Tyr-Z (Figure 14) and did not occur at all for the D-Lys-Z case (Figure 13). Since it is known that helical conformations are generally stable in DMAc, the explanation of this behavior is not immediately evident (64). However, the distinctive properties of this solvent in solvating polypeptide chains and preventing intermolecular association by the
forming of hydrogen bonds without disrupting the helical conformation may provide a tenable interpretation. If due to these distinctive properties the growing end of the polypeptide initiator chain is highly solvated, it is reasonable that these terminal residues may not be in the helical conformation, and thus no induction would take place. The grafted polypeptide chain would form its preferred helix after a sufficient number of residues had been added to form a stable helix. Further, the competition between the solvent and growing polypeptide for the initiator's hydrogen bonding groups may mitigate against induction in certain instances, as hydrogen bonding is bound to play an important role in as energetically marginal a structure as the induced helix.

The difference between the optical behavior of the poly(L-Glu-OBzl) and poly(L-Glu-OMe) initiated polymerization of D- and L-Lys-Z and D- and L-Tyr-Z NCAs in dioxane evinces the character of the interactions of the growing polypeptide with the preformed initiator as it depends upon the nature of the initiator (Figures 6 and 11). The initiation of the D- and L-Tyr-Z NCAs demonstrated an optical behavior similar for both initiators, although the poly(D-Tyr-Z) showed no β structure when grafted to the poly(L-Glu-OMe) block. The initiation of the D-Lys-Z NCA by poly(L-Glu-OMe) in dioxane showed no signs of the conformational induction phenomenon. These differences in behavior may have been due to the relative stabilities of the α helical initiators, as the poly(L-Glu-OBzl) is known to form a more stable α helix than poly(L-Glu-OMe) for similar solvents (72,73). Thus, the terminal residues of the poly(L-Glu-OMe) may be more highly solvated and therefore less helical
in character. Such a circumstance may largely preclude conformational induction, an effect much like that of the solvent DMAc as discussed above. Yet, as conformational induction occurs for the poly(L-Glu-OMe) initiation of D-Tyr-Z NCA in dioxane, the source of the stability or instability of the induced helix must be partially a result of the side chain interactions between the preformed polypeptide initiator and the grafted polypeptide block, the exact nature of which remains unclear.

Circular Dichroism

The circular dichroism (CD) of the block polymers provides qualitative evidence in support of the conformational induction hypothesis, as the CD establishes in an unambiguous manner that the conformation of the induced polypeptide block is indeed substantially α helical for all of the cases studied, and further supports all of the other general features ascribed to the phenomenon, such as helix reversion.

The paradigm case for conformational induction from the circular dichroism studies was seen for the poly(D-Glu-OBzl)-poly(L-Glu-OBzl) copolymers in trimethyl phosphate (Figure 15). The CD of the (D-Glu-OBzl)$_{10}$ block of the (D-Glu-OBzl)$_{10}$-(L-Glu-OBzl)$_{20}$ copolymer in trimethyl phosphate was qualitatively similar in every respect to that of the (L-Glu-OBzl)$_{20}$ initiating block (Table 6 and Figure 16). This circular dichroic pattern for a polypeptide is that for a right-handed α helix (17). This observation supports the previous assumption that the optical rotation for a short induced helix of D-residues induced by an α helical L-initiator is positive (in the visible wavelength region), as the
optical rotation for the (L-Glu-OBzl)\textsubscript{20} is known to be positive (Table 5). The CD of the longer (D-Glu-OBzl)\textsubscript{20} block of the (D-Glu-OBzl)\textsubscript{20}-(L-Glu-OBzl)\textsubscript{20} copolymer had a CD in trimethyl phosphate that was nearly the mirror-image of the (L-Glu-OBzl)\textsubscript{20} initiator, exactly as expected for the long D block reverted to its preferred helix sense (Table 6). The analogous experiment of grafting blocks of (L-Glu-OBzl)\textsubscript{n} to the (L-Glu-OBzl)\textsubscript{20} initiator, where n equals 10 or 20 residues, showed the small chain length dependence of the CD of poly(L-Glu-OBzl) in trimethyl phosphate (Figure 16), further disposing of the possibility that the similarity between the CD of the (D-Glu-OBzl)\textsubscript{10} grafted block and the (L-Glu-OBzl)\textsubscript{20} initiator may have been due to the failure to account for the elimination of an "end-effect", whereby the addition of the D-block, regardless of its conformation, would increase the apparent CD of the (L-Glu-OBzl)\textsubscript{20} block (74,75).

The above circular dichroism studies, where the two blocks consist of the same polypeptide, demonstrate more unambiguously the occurrence of conformational induction than would have been the case had the two polypeptide blocks consisted of different amino acid residues, since one may deduce from first principles that the long L- and D-polypeptides will prefer the opposite helix sense. If the two blocks consist of different amino acids, the demonstration that conformational induction is occurring in the case of one configuration of the grafted polypeptide and not for the other becomes more difficult.

The circular dichroism of the (D-Tyr)\textsubscript{n} block of the (D-Tyr)\textsubscript{n}-(L-Glu-OBzl)\textsubscript{20} block copolymer is best explained by the phenomenon of
conformational induction for the short (D-Tyr)_n chains, and reversion for longer chain lengths in trimethyl phosphate (Figure 17 and Table 6). The (D-Tyr)_6 grafted block had a CD somewhat similar to that of the long poly(L-Tyr) block, but the exact features of the CD spectra for the (D-Tyr)_6 block in the induced conformation were difficult to obtain, as so short a block contributed relatively little to the CD of the total block copolymer. The (D-Tyr)_{10} block had a CD that was on the average nearly zero. This result is in keeping with the conformational induction hypothesis if we assume that at this chain length one-half of the induced (D-Tyr)_{10} blocks have reverted to their preferred helix sense. The (D-Tyr)_{20} block had a CD qualitatively exactly similar to that for a long poly(D-Tyr) chain, but was smaller in magnitude. The CD band at 280 nm, which is due to the side chain phenolic chromophore, appeared to be largely insensitive to both the helix sense and chain length of the polypeptide backbone, although a small shift in the band position and shape may have taken place (Table 6).

The analogous study for L-Tyr suggests that the (L-Tyr)_n block grew upon the initiator in the same helix sense as the (L-Glu-OBzI)_{20} initiating block, and demonstrated a considerable chain length dependence (Figure 18). However, these CD spectra may also be interpreted as indicative of conformational induction where the smaller molar ellipticity of the (L-Tyr)_{10} block was due to the presence of both induced and reverted helices, the observed molar ellipticity being an average of the induced and reverted molar ellipticities. For the longer L-Tyr chain lengths,
the CD of the grafted block becomes increasingly that of the totally reverted helix. This latter case would require that the CD of the induced helix be a near mirror image of the reverted helix (in a qualitative sense) since the band positions and relative intensities of the \((\text{L-Tyr})_{10}\) and \((\text{L-Tyr})_{20}\) blocks were observed to be exactly the same. Even if it were discovered that the CD of \((\text{L-Tyr})_n\) on \((\text{L-Glu-OBzl})_{20}\) showed a chain length dependence it would be possible to argue that the \((\text{L-Tyr})_n\) block is completely induced for \(n\) less than 20, and would have reverted to the preferred helix sense for some larger value of \(n\). However, the molar rotations of these block copolymers have been determined for values of \(n\) (chain length) greater than 40 residues and no optical behavior characteristic of reversion was observed. Therefore, the CD behavior of both the \((\text{D-Tyr})_n\) and \((\text{L-Tyr})_n-(\text{L-Glu-OBzl})_{20}\) copolymers may be explained by the conformational induction hypothesis and hence the determination of the helix sense in this case may be somewhat ambiguous. However, it seems from the above evidence that the \((\text{D-Tyr})_n-(\text{L-Glu-OBzl})_{20}\) case would be more easily rationalized by the occurrence of conformational induction than the \((\text{L-Tyr})_n-(\text{L-Glu-OBzl})_{20}\) case, and since conformational induction clearly can not occur in both instances, it is not unreasonable to conclude that poly(L-Tyr) forms an \(\alpha\) helix of the same sense as poly(L-Glu-OBzl) in trimethyl phosphate, that is, a right-handed helix. The CD of the phenolic side chain band(s) at 280 nm appeared to be split into two bands for the \((\text{L-Tyr})_{20}\) grafted block in trimethyl phosphate. This may be due to electronic band splitting (exciton bands) due to an interaction between the phenolic groups in a
helical array (38), but this interpretation has been disputed (39). If there is coupling between the tyrosine side chain electronic transitions, it is possible that such an interaction is to some extent responsible for the aromatic groups acquiring rotatory strength, a mechanism which would be expected to show a chain length dependence of the molar ellipticity (76). Electronic coupling between side chain groups has been reported for poly-L-tyrosine (77) and poly-L-tryptophane (21).

The circular dichroism of \((L-\text{Glu-OBzl})_{20}-(D-\text{Tyr})_8\) is very similar to that found for the long D-Tyr block, suggesting that no conformational induction occurs in this copolymer (Table 6). The difference in the behavior of the D-Tyr block in this copolymer as compared to that in the \((D-\text{Tyr})_n-(L-\text{Glu-OBzl})_{20}\) copolymer may be due to the interaction of the D-Tyr and L-Glu-OBzl residues near the block junction region.

Polymerization Kinetics

The kinetics of the polymerization of D-\(\alpha\)-amino acid N-carboxy-anhydrides (NCAs) with L-polypeptide initiators in dioxane all exhibited multi-rate phenomenon. Poly(L-Glu-OBzl) initiation of L-Asp-OBzl NCA in dioxane, and the poly(L-Glu-OMe) initiation of D-Tyr-Z NCA in dioxane exhibited an apparent three-rate kinetic scheme. For an anhydride to initiator ratio of twenty \((A/I = 20)\), as in these experiments, the initiation reaction would theoretically consume \(1/20\) th of the anhydride. In the above two cases, a significantly greater amount than this was consumed during the course of the initial kinetic rate \((1/10\) th or more of the anhydride). Thus, in these cases the initially observed rate may not be
that due to the initiation reaction, and while this does not necessarily place these cases beyond interpretation by a mechanism similar to that in the two-rate kinetics, it makes such an interpretation more complex. Since the initial step in these cases was of short duration with respect to the total reaction time, it may be that these rates were not real but arose from inadequate thermostating during the initial phase of the kinetic studies, or from some impurity in the system. But as the kinetics were reproducible and great care was taken to insure accurate thermostating, the three rates are believed to be distinctly real. One may perhaps rationalize these multi-rate phenomenon within the framework of the conformational induction hypothesis.

Models show that the complexing of the NCAs with the non-hydrogen bonded amide moiety (-NH) of the terminal residue of an α-helix is greatly reduced if the terminal residue at the growing end of the polypeptide has the configuration opposite to that of the previous residues, since the side chain of this terminal residue makes unfavorable contacts with the body of the helix. By analogy, the side chains of the initially induced D-residues may make bad contacts with the helical L-initiating block. This interaction may be minimized by rotation about the C'-C= bond of the terminal residue, but in consequence sterically favorable contacts between the NCA and the terminal residue become less likely. Under such conditions the rate of polymerization might be relatively reduced (decrease in the entropy of activation). After sufficient residues have been added to the induced helical chain, sterically favorable contacts between the adsorbed NCA and the terminal amine of the helix
may arise from an increased degree of torsional freedom, the absence of 
bad contacts of the terminal residue side chain with the more remote 
configurational junction, and/or the reversion of the induced helix to 
its preferred sense in which the sterically unfavorable contacts would 
be minimized. These latter situations might then lead to an increased 
rate of polymerization.

The kinetics of polymerization of L-anhydrides with L-initiators 
in dioxane all fit the one-rate kinetic scheme, as a single rate of 
propagation was found in all cases except for L-Asp-OBzl NCA initiated 
with (L-Glu-OBzl)\(_{20}\) in dioxane where more than one propagation rate was 
found (Figure 5). For the instance of the L-polypeptide initiation of 
L-NCA no sterically unfavorable situations are anticipated by the above 
model for the addition of the initial residues. Since it is reasonable 
to assume that the same reaction mechanism is operating regardless of 
the respective configurations of the initiator and anhydride, by analogy, 
sterically unfavorable contacts may occur since the initiator and growing 
polypeptide chains are not identical and may prefer very different rela-
tive conformations. This may be particularly true for the L-Asp-OBzl 
residue where the side chain is large and relatively close to the poly-
peptide backbone. Therefore, the kinetic behavior might be explained by 
the fact that unlike the other L-homopolypeptides, poly(L-Asp-OBzl) pre-
fers a helix sense opposite to that of the L-polypeptide initiators (18, 
68-70). The origin of the multi-rate reaction kinetics in this latter 
case could then be ascribed to the fact that the initiator and growing 
polypeptide block preferred opposite helical senses, but the argument
in this case is not completely analogous to the other cases where the multi-rate reaction kinetics were observed, since in those cases the initiator and growing block were of the opposite configuration.

The interpretation of the kinetic behavior solely upon the basis of the conformational induction effect, while certainly probable in many instances, may not be essential to the explanation of the kinetic behavior, or even possible in some cases. The origin of the initial rate step observed in the cases where three rates are observed remains obscure, perhaps residing in some as yet unelucidated steric or structural feature of the anhydride-initiator system. Perhaps of greater consequence to the interpretation of the kinetics solely through steric or other features related to conformational induction was the case of the \((L-\text{Glu-OBzl})_{20}\) initiation of D-Tyr-Z NCA in dioxane. The kinetics exhibited a two-step kinetic scheme (Figure 2), but the infrared spectrum demonstrated that at all stages of the D-Tyr-Z block growth, the D-Tyr-Z Z block existed largely in an extended or \(\beta\) structure (Table 8). Initially, all of the D-Tyr-Z block existed in the \(\beta\) structure which at longer chain lengths partially changed to a random coil or helical structure. The theory of conformational induction clearly doesn't apply in this case as this theory only treats helical states. In addition, studies with poly(D-Glu-OBzl)-poly(L-Glu-OBzl) block polymers showed that conformational induction may occur (as evidenced from optical studies) where only one kinetic rate was observed, as in the case of the poly \((L-\text{Glu-OBzl})\) initiation of D-Glu-OBzl NCA in \(N,N\)-dimethylacetamide (78). This may be due to the solvation of the terminal residues of the polymer.
by this solvent, thereby eliminating the steric influences responsible for the kinetic behavior.

Infrared Spectra

In the model for conformational induction, only helical states are considered to be present in the copolymer. Since the amide I and II infrared absorption bands are helpful in assigning specific polypeptide conformations, infrared spectroscopy should aid in establishing whether the conformation of the block copolymer is consistent with the model for conformational induction. Although the absorption frequencies of the helical amide I and II bands have been found to depend upon the helix sense for certain polypeptides (79), the magnitude of this effect is small and therefore would be difficult to observe in the block copolymers. As an example, the characteristic absorptions for the left-handed poly(L-Asp-OBzI) are observed at 1666 ± 2 cm⁻¹ (amide I) and 1560 ± 2 cm⁻¹ (amide II), whereas the characteristic absorptions for the right-handed poly(L-Glu-OBzI) are observed at 1654 ± 1 cm⁻¹ (amide I) and 1546 ± 2 cm⁻¹ (amide II) (79 and Table 8). In addition, where spectral shifts in the absorption bands of the block copolymers are seen, it would be difficult to assign these to the presence of an induced helix, as a similar shift would be observed for the presence of a random coil conformation (1659 cm⁻¹ and 1549 cm⁻¹ for poly(L-Glu-OBzI)). Therefore, the infrared spectra are useful to the extent that they may allow or disallow certain block polymers from consideration for conformational induction.
The infrared (IR) spectra of the block copolymers of poly(D-Glu-OBzl) with poly(L-Glu-OBzl) in trimethyl phosphate suggested that these copolymers were in an essentially helical state in this solvent (Table 8). However, the small shift in the position of the amide I band towards higher frequencies with respect to the homopolypeptide may have indicated the presence of some random structure.

The IR spectra of the block copolymers of D-Tyr with L-Glu-OBzl in trimethyl phosphate showed a shift toward higher frequencies with the increasing molar ratio of D-Tyr (Table 8). This result is consistent with the relatively higher amide I band frequency for helical polytyrosine (1661 cm\(^{-1}\)) (38). The IR spectra of the poly(L-Tyr) and poly(L-Glu-OBzl) block copolymers showed a similar behavior. Therefore, it appears that these block copolymers were helical.

The IR spectra of block polymers of D-Tyr-Z with L-Glu-OBzl in dioxane demonstrated a rather complex behavior. For short chains the D-Tyr-Z block formed an extended or \(\beta\) structure. For longer D-Tyr-Z block lengths, a mixture of \(\beta\) structure and random coil existed. Differential IR spectroscopy confirmed that the \(\beta\) structure and random coil arose from interactions in the D-Tyr-Z block. The differential spectroscopy was done by observing the IR spectra of \((\text{L-Glu-OBzl})_{20}\) initiated polymerization of D-Tyr-Z NCA in dioxane with equivalent concentrations of \((\text{L-Glu-OBzl})_{20}\) in both the sample cell (containing NCA) and the reference cell, and thereby only the IR spectra of the D-Tyr-Z NCA and poly(D-Tyr-Z) were seen. The block copolymers of L-Tyr-Z and L-Glu-OBzl
showed spectra that were consistent with the interpretation that these polymers were in an \( \alpha \) helical conformation.

Therefore, the IR spectra of the above block copolymers were not inconsistent with the occurrence of conformational induction, with the exception of \((\text{D-Tyr-Z})_n-(\text{L-Glu-OBzl})_m\), where a definite \( \beta \) structure was found.
Table 8. The infrared amide I and II absorption bands for poly(Tyr-Z), poly(Tyr), poly(Glu-OBzl), and their block copolymers in dioxane and trimethyl phosphate (TMP).

<table>
<thead>
<tr>
<th>Polymer</th>
<th>Solvent</th>
<th>Ref.</th>
<th>State of Sample</th>
<th>Amide I cm(^{-1})</th>
<th>Amide II cm(^{-1})</th>
</tr>
</thead>
<tbody>
<tr>
<td>(L-Glu-OBzl)(_{20})</td>
<td>TMP</td>
<td>TMP</td>
<td>soln.</td>
<td>1654 ± 1</td>
<td>1542 ± 2</td>
</tr>
<tr>
<td>(D-Glu-OBzl)(<em>{10})-(L-Glu-OBzl)(</em>{20})</td>
<td>TMP</td>
<td>TMP</td>
<td>soln.</td>
<td>1655</td>
<td>1550</td>
</tr>
<tr>
<td>(D-Glu-OBzl)(<em>{20})-(L-Glu-OBzl)(</em>{20})</td>
<td>TMP</td>
<td>TMP</td>
<td>soln.</td>
<td>1656</td>
<td>1548</td>
</tr>
<tr>
<td>(L-Tyr)(_{600})</td>
<td>dioxane</td>
<td>dioxane</td>
<td>soln.</td>
<td>1661</td>
<td>1544</td>
</tr>
<tr>
<td>(L-Tyr-Z)(_{10})</td>
<td>dioxane</td>
<td>dioxane</td>
<td>soln.</td>
<td>1672</td>
<td>1542</td>
</tr>
<tr>
<td>(L-Tyr-Z)(_{20})</td>
<td>dioxane</td>
<td>dioxane</td>
<td>soln.</td>
<td>1672, 1628</td>
<td>1542</td>
</tr>
<tr>
<td>(D-Tyr-Z)(<em>{10})-(L-Glu-OBzl)(</em>{20})</td>
<td>dioxane</td>
<td>dioxane</td>
<td>soln.</td>
<td>1654, 1628</td>
<td>1546</td>
</tr>
<tr>
<td>(D-Tyr-Z)(<em>{20})-(L-Glu-OBzl)(</em>{20})</td>
<td>dioxane</td>
<td>dioxane</td>
<td>soln.</td>
<td>1654, 1628, 1670</td>
<td>1546</td>
</tr>
<tr>
<td>(L-Tyr-Z)(<em>{10})-(L-Glu-OBzl)(</em>{20})</td>
<td>dioxane</td>
<td>dioxane</td>
<td>soln.</td>
<td>1655</td>
<td>1546</td>
</tr>
<tr>
<td>(L-Tyr-Z)(<em>{20})-(L-Glu-OBzl)(</em>{20})</td>
<td>dioxane</td>
<td>dioxane</td>
<td>soln.</td>
<td>1655</td>
<td>1546</td>
</tr>
<tr>
<td>(D-Tyr)(<em>{10})-(L-Glu-OBzl)(</em>{20})</td>
<td>TMP</td>
<td>TMP</td>
<td>soln.</td>
<td>1656</td>
<td>1546</td>
</tr>
<tr>
<td>(D-Tyr)(<em>{20})-(L-Glu-OBzl)(</em>{20})</td>
<td>TMP</td>
<td>TMP</td>
<td>soln.</td>
<td>1658</td>
<td>1546</td>
</tr>
<tr>
<td>(L-Tyr)(<em>{10})-(L-Glu-OBzl)(</em>{20})</td>
<td>TMP</td>
<td>TMP</td>
<td>soln.</td>
<td>1655</td>
<td>1546</td>
</tr>
<tr>
<td>(L-Tyr)(<em>{20})-(L-Glu-OBzl)(</em>{20})</td>
<td>TMP</td>
<td>TMP</td>
<td>soln.</td>
<td>1655</td>
<td>1546</td>
</tr>
<tr>
<td>(D-Tyr)(<em>{20})-(L-Glu-OBzl)(</em>{20})</td>
<td>TMP</td>
<td>Air</td>
<td>film</td>
<td>1656</td>
<td>1544</td>
</tr>
<tr>
<td>(L-Tyr)(<em>{10})-(L-Glu-OBzl)(</em>{20})</td>
<td>TMP</td>
<td>Air</td>
<td>film</td>
<td>1657</td>
<td>1545</td>
</tr>
<tr>
<td>(L-Tyr)(<em>{20})-(L-Glu-OBzl)(</em>{20})</td>
<td>TMP</td>
<td>Air</td>
<td>film</td>
<td>1657</td>
<td>1545</td>
</tr>
</tbody>
</table>
The purpose of this chapter is to show how the behavior of a simple model for conformational induction in helical polypeptides may be related to certain thermodynamic parameters governing helix stability.

The polypeptide chain has the sequence $B_nA_m$ where $A$ and $B$ are different amino acids and the carboxyl end of the chain is at the right. A long polymer of $A$ is assumed to form a stable $\alpha$ helix of a particular sense, designated $\alpha'$, and a long polymer of $B$ is assumed to form a stable $\alpha$ helix of the opposite sense, designated $\alpha''$. The following diagramatic conventions will be used:

\[ \alpha' \quad \alpha'' \]

A reversal of the helix sense is thus represented by the following:

\[ \alpha'' \]

The diagrams are drawn with the carboxyl end at the right, so that the $A$ block is also at the right. When helix reversal occurs, the ends of the two helices at the point of reversal are assumed to be essentially equivalent to the ends of the corresponding helices isolated from each other. That is, if all possible hydrogen bonds within a given helix are intact, then there are at least three broken hydrogen bonds at the
point of helix reversal and one amino acid residue with an essentially random conformation about its N-C$^\alpha$ and C$^\alpha$-C$^i$ bonds.

Conformational induction occurs when all or part of one block takes on the helix sense of the other block. The number of residues in each helix sense will be designated as follows:

- $m' = \text{no.}\ A\text{ residues in helix } \alpha'$
- $m'' = \text{no.}\ A\text{ residues in helix } \alpha''$
- $n' = \text{no.}\ B\text{ residues in helix } \alpha'$
- $n'' = \text{no.}\ B\text{ residues in helix } \alpha''$

We will assume that $m = m' + m''$ and $n = n' + n''$, although this would require that all residues be in one helix sense or the other, and this is not necessarily the case at the ends of the helices.

The free energy of formation $\Delta G$ of a given conformation from the randomly coiled chain in the same solvent is the sum of the free energy changes of the residues for this process. The interactions between residues are taken into account by assigning to a given residue the free energies of its interactions with other residues removed from it towards the carboxyl end of the polypeptide chain. Thus $\Delta G$ is composed of the following terms: $F$ for each residue, where $F$ is the free energy of formation of a very long helix from the random coil in energy units per residue-mole; $E$ for each carboxyl end of a helical region, in energy units per mole of carboxyl ends ($E$ serves as a correction factor for the lack of intrachain interactions of residues near the carboxyl end); and $J$ for each BA junction when this junction occurs within a single helical region, in energy units per mole of junctions ($J$ serves as a correction
factor for the interaction of B residues with A residues near the junction region). The helix sense to which F, E, or J applies is denoted by ′ or ″, and the amino acid or polypeptide to which F or E applies is denoted by the subscript A or B.

Since long helices are stable we have

\[ F'_A < 0 \]  \hspace{1cm} (13) \\
\[ F'_B < 0 \]  \hspace{1cm} (14)

Also, from the definition of the preferred helix senses we have

\[ \Delta F_A = F'_A - F_A > 0 \]  \hspace{1cm} (15) \\
\[ \Delta F_B = F'_B - F_B > 0 \]  \hspace{1cm} (16)

Since the E terms compensate for the absence of interactions whose net effect is to stabilize the helical conformation, we may also expect that, usually,

\[ E > 0 \]  \hspace{1cm} (17)

To illustrate the calculation of \( \Delta G \) consider the conformation in Figure 20, where block A is indicated by a solid line and block B is indicated by a dashed line.

![Figure 20. Calculation of \( \Delta G \)](image-url)
Thus we have

\[ \Delta G = mF_A^I + nF_B^I + n^I F_B^I + E_A^I + E_B^I + J^I \]

\[ = mF_A^I + nF_B^I + n^I \Delta F_B + E_A^I + E_B^I + J^I. \] (18)

In the case illustrated, \( n^I \) residues of block B have been "induced" by block A. From Equations 16 and 18 it is seen that \( \Delta G \) becomes more positive as \( n^I \) increases. This means that \( n^I \) will tend to remain small if this form of induction occurs at all, and we will henceforth assume that \( n^I = t \), where \( t \) is a sufficient number of residues (three or four) to establish a BA junction between two turns of one of the helical regions. Thus we may write

\[ \Delta G = U + t\Delta F_B + E_A^I + E_B^I + J^I \] (19)

where we have set

\[ U := mF_A^I + nF_B^I. \] (20)

Figure 21 illustrates the five types of helical conformations of \( B_n^A m \) which seem likely to be significant. The terms given under each diagram give the value of \( \Delta G - U \) for that conformation. The most stable conformation is that for which \( \Delta G - U \) is a minimum. In conformation (c) there is no induction, while (a) and (e) each have one block completely induced and (b) and (d) have partial induction in one block, as discussed in the previous paragraph.

It will be noted that only (a) and (e) have free energy terms which depend upon the block lengths. Moreover, these terms are positive,
Figure 21. The five types of helical conformations of the block copolypeptide $B^m_A^n$ which are likely to be significant, and the values of $\Delta G - U$ for these conformations.
Figure 21
according to Equations 15 and 16. Therefore, (a) is unstable when \( n \) is large and (e) is unstable when \( m \) is large. If both blocks are long, only (b), (c), and (d) need be considered. The distinction among these three states is not very great, since \( t \) is small; if the \( E \) terms are of similar magnitude, the equilibrium would be determined by the balance between the \( t\Delta F \) terms (positive) and the \( J \) terms (positive, negative, or unpredictable).

Consider the case in which the length \( n \) of block B is increasing while the length \( m \) of block A is fixed at some large value. Then state (e) is eliminated from the start. On the other hand, (a) has the advantage over the middle row of states in Figure 21 in having only one of the positive \( E \) terms; thus if \( n \) is not too large and \( J' \) is not too large, state (a) will predominate. That is, induction of block B will be complete when \( n \) is small. As noted above, (a) becomes unstable for large \( n \), so that at some critical value of \( n \), block B reverts mostly to its preferred helix sense, and the predominant state is (b), (c), or (d). Further growth of the chain has no effect on the equilibrium. The critical length for reversion from (a) to (b), for example, can be calculated from the fact that \( \Delta G \) for the process (a) to (b) must be negative if the process is to occur spontaneously. From the free energy terms in Figure 21 one thus obtains the following conditions for helical reversion.

\[
(a) \rightarrow (b) \quad n > \frac{E_B'}{\Delta F_B} + t \tag{21}
\]

\[
(a) \rightarrow (c) \quad n > \frac{E_B' - J'}{\Delta F_B} \tag{22}
\]
The state to which reversion takes place will be that for which the critical length is the smallest. It will be noted that all of the symbols in Equations 21-23 represent quantities that are known to be or expected to be positive with the exception of $J'$ and $J''$, which are of unknown sign and magnitude. It is therefore conceivable that the critical length, as defined, could be negative, given the appropriate values for $J'$ and $J''$, in which case state (a) would never be stable and reversion of the helix would not occur.

It has been noted above that state (e) is unstable if $m$ is large. As previously, the critical length for conversion to other states can be determined from the free energy criterion. The results are as follows.

\[
(e) \rightarrow (a): \quad m > \frac{E_A' - E_A'' + n\Delta F_B + J' - J''}{\Delta F_A} \quad (24)
\]

\[
(e) \rightarrow (b): \quad m > \frac{E_B' + E_A' - E_A'' + t\Delta F_B + J' - J''}{\Delta F_A} \quad (25)
\]

\[
(e) \rightarrow (c): \quad m > \frac{E_A' + E_B' - E_A'' - J''}{\Delta F_A} \quad (26)
\]

\[
(e) \rightarrow (d): \quad m > \frac{E_A'}{\Delta F_A} + t \quad (27)
\]

If $m$ is greater than the smallest of these critical lengths, the state (e) will be unstable. In the case of the (e) to (a) conversion, it is clear that the critical $m$ is small only if $n$ is also small.
In an experiment in which the growth of block B is being studied, it is possible that \( m \) will be smaller than the smallest critical length (Equations 21 - 23). Then (e) is stable throughout the growth of block B, and no reversion occurs. However, it could be that (e) is stable with respect to (b), (c) and (d), but unstable with respect to (a), by virtue of Equation 24, where \( n \) is small. In this case, when \( n \) exceeds the value satisfying Equation 24 there is a conversion from (a) to (e); that is to say, the entire molecule reverses helix sense from \( \alpha' \) to \( \alpha'' \). This form of reversion may resemble experimentally that described for the growth and induction of block B on block A.

It has been assumed in the above that the helical regions were stable with respect to the random coil. We will now examine the conditions under which this is actually true. We require that the contribution of the helical region to \( \Delta G \) be negative in order for that helix to be stable. For example, for the \( \alpha' \) helix of state (b), illustrated in Figure 20, we can write that

\[
mE'_A + tF'_B + E'_A + J' < 0 \quad (28)
\]

is the condition to be satisfied for helix stability. This can be re-arranged, taking into account Equation 13, into the form

\[
m > - \frac{E'_A + tF'_B + J'}{\frac{F'_A}{E'_A}} \quad (29)
\]

The corresponding conditions for each helical region in each of the states in Figure 21 are derived in a similar manner, and are given in
Table 9. Where possible, the conditions are stated in terms of a critical length, as in Equation 29.

Consider the case of the growth of block B on a long block A. In the early stages of the growth, where state (a) is the most stable of those shown in Figure 21, let us assume that the condition for stability of (a) with respect to the random coil cited in Table 9 is also satisfied. (If it is not, the experiment is likely to be of little interest.) We now ask, when reversion occurs, is the \( \alpha'' \) helical region in the resulting state stable with respect to the random coil? The answer will be affirmative if the critical length for reversion is greater than the critical length or the critical helix size for the stability of the \( \alpha'' \) helix. (The related question of whether the \( \alpha' \) helical region is stable is also of interest, but this stability can be assumed by making \( m \) large enough, according to the criterion in Table 9.) We may therefore compare the critical lengths given by Equations 21-23 with those for the \( \alpha'' \) helix in Table 9 and obtain the following conditions for the stability of the \( \alpha'' \) helix at the chain length at which helix reversion takes place.

\[
\begin{align*}
(a) \rightarrow (b): \quad & -F_{B}^{m} > \Delta F_{B} & (30) \\
(a) \rightarrow (c): \quad & -F_{B}^{m} (1 - \frac{J_{A}}{F_{B}^{m}}) > F_{B} & (31) \\
(a) \rightarrow (d): \quad & -F_{B}^{m} \left( \frac{E_{A}^{m} + tA_{A}^{m} + J_{A}^{m} - J_{B}}{E_{B}^{m} + tA_{B}^{m} + J_{B}^{m}} \right) > \Delta F_{B}. & (32)
\end{align*}
\]

Thus Equation 30 requires simply that the stability of a long \( \alpha' \) helix with respect to the random coil be greater than its stability with
respect to the \( \alpha' \) helix. This situation would also aid in satisfying Equations 31 and 32; however, a close examination of the additional factors in these cases shows that there is one situation in which these inequalities would not be satisfied, and that is when \( J' \) is strongly positive. One may therefore reasonably expect to find that reversion to a stable helix indeed occurs, although there are circumstances under which reversion to a random coil in block B would be favored.
### Table 9. Conditions for helix stability

<table>
<thead>
<tr>
<th>State</th>
<th>Helix $\alpha'$</th>
<th>Helix $\alpha''$</th>
</tr>
</thead>
<tbody>
<tr>
<td>(a)</td>
<td>$mF_{A}^I + nF_{B}^I + E_{A}^I + J_{A}^I &gt; 0$</td>
<td></td>
</tr>
<tr>
<td>(b)</td>
<td>$m &gt; \frac{E_{A}^I + tF_{B}^I + J_{A}^I}{F_{B}^I}$</td>
<td>$n &gt; t - \frac{E_{B}''}{F_{B}''}$</td>
</tr>
<tr>
<td>(c)</td>
<td>$m &gt; -\frac{E_{A}^I}{F_{A}^I}$</td>
<td>$n &gt; -\frac{E_{B}''}{F_{B}''}$</td>
</tr>
<tr>
<td>(d)</td>
<td>$m &gt; t - \frac{E_{A}^I}{F_{A}^I}$</td>
<td>$n &gt; -\frac{E_{A}'' + tF_{A}'' + J_{A}''}{F_{B}''}$</td>
</tr>
<tr>
<td>(e)</td>
<td>$mF_{A}^I + nF_{B}^I + E_{A}'' + J_{A}'' &lt; 0$</td>
<td></td>
</tr>
</tbody>
</table>
CHAPTER 8. PHYSICAL INTERPRETATION OF CONFORMATIONAL INDUCTION PARAMETERS

In the previous chapter a simple model for conformational induction was developed in terms of several thermodynamic parameters. The purpose of this chapter is to enlarge upon the nature of these parameters and develop a more conceptual framework in which ideas and experiments in conformational induction may be readily expressed.

The correction factor for the lack of intrachain interactions of residues near the carboxyl end of the polypeptide, $E$, is a conceptually difficult term to deal with. It may perhaps be better to restate this term in a more positive manner. This correction factor accounts for the free energy difference of the terminal residue(s) with respect to the free energy of the non-terminal residues, the free energies being the free energies of formation with respect to the random coil. Therefore, the free energy of formation of the terminal residue may be more positive than the free energy of the non-terminal residues, due to its greater enthalpy, since the terminal residue is involved in fewer hydrogen bonds. Since the hydrogen bonds are the main stabilizing interaction in the $\alpha$ helix, the value of $E$ is most likely to be positive. In addition, there is considerable experimental evidence that $E > 0$ in that there is a critical helix size for helix stability (24,25,64).

Unlike the previous parameter, the correction term for the interaction of $B$ residues with $A$ residues near the junction of the $A_m$ and $B_n$ polypeptide blocks, $J$, more readily lends itself to a physical interpretation on the basis of steric interactions between the polypeptide
side chains near the junction region. In order to discuss the nature of these interactions, it is first necessary to develop a concise manner in which to express the possible side chain orientations relative to the residue configuration and the helix sense. The side chain orientations are described in an abbreviated form after that used by Yan et al. (33).

There are two general classes of side chain orientations relative to the helix axis: axial, a, where the side chains lie or extend along the helix axis, either towards the C-terminus, a(+), or towards the N-terminus of the polypeptide chain, a(-); and transverse, t, where the side chains are wrapped tangentially around the helix axis, either clockwise as viewed from the N-terminus in the direction of the C-terminus, t(+), or counterclockwise when viewed in the same manner, t(-). Two helical senses of the polypeptide backbone are possible; a right-handed sense, designated r, and a left-handed sense, designated l. With the addition of the description of the configuration of the $C^\alpha$ asymmetry as D or L, the "complete" character of the orientation of the side chain may be described in a concise form. As an example, a right-handed helix in which the side chains extend axially towards the carboxyl end of the polypeptide may be expressed as Lra(+) or Dra(+) depending upon the configuration of the constituent residues, L or D respectively. This explicit expression may in reality be somewhat difficult to assign, particularly for side chains of an intermediate length ($C_4$ to $C_2$). Even in the clearest cases no side chain will be completely axial or transverse,
clockwise or counterclockwise, but may be considered as being predominantly of a single character in a practical sense.

As a specific instance from which to develop generalities, consider the side chain interactions at a D-Tyr-L-Tyr junction in the hypothetical block copolypeptide (D-Tyr)$_n$-(L-Tyr)$_m$ where the polypeptide exists entirely in a right-handed $\alpha$ helix. The tyrosine side chain has two possible conformers which we will designate as conformer I and conformer II (these two conformers correspond to the potential energy minima for the rotation of the tyrosine side chain about the $C^\alpha$-$C^\beta$ bond (31,80), and are described by the dihedral angle $\chi_1$ using the standard convention (81); for conformer I, $\chi_1 = 172^\circ$, for conformer II, $\chi_1 = 303^\circ$). Therefore, in this instance there exists four possible orientations when considering both the D and L configurations. These orientations are shown in their Newman projections of the $C^\alpha$-$C^\beta$ bond and also with respect to the direction of the right-handed helix axis in Figure 22.

Figures 23 and 24 show the relative spatial relationships of the tyrosine residues about the D-L junction, depicting the $i$th and $(i+3)$ tyrosine residues since these residues have their side chains in closest proximity in the right-handed $\alpha$ helical conformation. The figures also include the abbreviated conformational designations and a schematic representation of the structural relationships in a less complex manner which is in keeping with the diagramatic conventions of the previous chapter. From the figures we may see that the steric interactions between the tyrosines near the junction depend upon the side chain configuration and conformation. As an example, if the D-tyrosine residue has the side
Figure 22. Newman projections of the $C^\alpha - C^\beta$ bond for D- and L-tyrosine, showing the allowed relative orientations of the side chain and helix axis in a right-handed $\alpha$ helix.
Figure 22

Conformer I, $\chi_1 = 171.5^\circ$

L-configuration

Conformer II, $\chi_1 = 302.9^\circ$

L-configuration

Conformer I, $\chi_1 = 171.5^\circ$

D-configuration

Conformer II, $\chi_1 = 302.9^\circ$

D-configuration
Figure 23. The spatial relationship of D-tyrosine (i) and L-tyrosine
(i + 3) residues near a D-L B A junction region where the
conformations of the residues are Drt(+) (1) and Lra(+) (1).
Figure 23
Figure 24. The spatial relationship of D-tyrosine (i) and L-tyrosine (i+3) residues near a D-L junction region where the conformations of the residues are Drt(-) (II) and Lra(+) (I).
Figure 24
chain conformation II, it is seen that spatial coincidences occur between this residue and the L-tyrosine residue at the junction regardless of the conformation of the L-tyrosine side chain as in this case the D-tyrosine side chain will interact with the \( \text{C}^\beta \) group of the L-tyrosine residue (Figure 24). The figures depict interactions at a junction region where the D-residues lie to the N-terminus of the D-L block copolypeptide (type \( B_n A_m \)), but if we make similar inquiries for the L-D block copolypeptide where the D-residues lie to the C-terminus of the junction (type \( A_m B_n \)), the interactions are found to be much different, in fact, no spatial coincidences will occur near the junction region for any combination of D- and L-tyrosine side chain conformations in this case. Thus, the steric interactions near the junction region will depend upon the 'polarity' of the junction with respect to the direction of the helix axis. The above considerations were for the block copolymer in the right-handed helix, and as might be expected, models (Dreiding-Stereomodel, Swissco Instruments, Greenville, Illinois) show that the interactions near the junction region depend upon the helix sense as well as the previously described factors. While there are no strictly symmetrical relationships between them, the interactions near the junction region of the D-L type \( B_n A_m \) polypeptide of the left-handed sense are similar to those of the L-D type \( A_m B_n \) polypeptide of the right-handed sense, and likewise for the left-handed L-D \( A_m B_n \) and right-handed D-L \( B_n A_m \) block polypeptides. In certain situations, the hydrogens of the \( \beta \) carbon of the \( i \)th D-residue and the \((i+3)\) L-residue will be in close proximity near the junction region. Structural models show that
these carbon hydrogen atoms are separated by an internuclear distance of approximately 2 Å, or slightly less than twice the accepted van der Waals radius for hydrogen (1.20 Å) (82). All steric interactions will occur for three D-L residue pairs, that is, for about one turn of the helix. The steric interactions near the junction region are tabulated in Table 10 as a function of the helix sense, junction polarity, and side chain conformation, where $\Sigma$ designates a severe spatial coincidence between side chains, $\beta$ an interaction between the side chain $\beta$ carbon hydrogens, and $\theta$ the lack of any steric interaction. It is important to note that where any steric overlap or spatial coincidence is found, it is the direct result of the relative antiparallel orientations of the $\alpha - \beta$ bonds of the $i$th and $(i+3)$ residues as shown in Figures 23 and 24.

All of the above considerations for the interactions of the D- and L-tyrosine residues near a D-L block copolypeptide junction may be generalized to include situations in which the $A_m$ and $B_n$ blocks are composed of different polypeptides. Consider the analogous side chain interactions at a D-L junction of the $B_n A_m$ type where block $B_n$ is $(D-Tyr)_n$ and block $A_m$ is $(L-Glu-OBzl)_m$, the copolypeptide being in an $\alpha$ helical conformation of a single sense. Given a specific preferred conformation for the L-Glu-OBzl side chain and the sense of the helix, it becomes possible to examine the interactions of the side chains near the junction region. If we consider the helix to be right-handed and the side chain conformation for L-Glu-OBzl is assigned as Lra(+) (33), the interactions
will be very similar to those for the previously considered case (Table 10) where the Lra (+) side chain conformation is analogous to conformer I. If we consider that the side chain conformation of the L-Glu-OBzl is Lrt(-) (83), the interactions will be similar to those found for conformer II in Table 10. These interactions of course will not be strictly equivalent to those found for conformers I and II in Table 10 as the L-Glu-OBzl side chain is much bulkier and longer than the D- or L-Tyr side chain. This latter point is less important when we consider the $A_m B_n$ type of copolymer where the $B_n$ block is (D-Tyr)$_n$ and the $A_m$ block is (L-Glu-OBzl)$_m$, for in this case the D-Tyr and L-Glu-OBzl residues are well separated.

Side chain interactions also exist for an $A_m B_n$ or $B_n A_m$ type of junction where the configuration of the residues of the two helical blocks is the same, and a correction term, $J$, exists. However, when the configurations of the $A_m$ and $B_n$ blocks are the same, the interactions are of a more speculative nature for short side chains as such a situation is difficult to interpret sterically or otherwise. For the polypeptides with long side chains, like poly(L-Glu-OBzl), steric arguments analogous to the D-L junction case may be made with the reservation that spatial coincidences will be less severe where these occur. In addition, the interaction between the $\beta$ carbon hydrogen atoms will not occur since these are well separated at the L-L junction.

All of the various steric interactions between the side chains near the junction region contribute to $J$, but whether such interactions contribute to this free energy term in a positive or negative sense is
Table 10. Side chain interactions near the junction region (Tyr - Tyr)

<table>
<thead>
<tr>
<th>Helix Sense</th>
<th>Polarity</th>
<th>Configurations</th>
<th>Conformers</th>
<th>Interaction</th>
</tr>
</thead>
<tbody>
<tr>
<td>Right-handed</td>
<td>B_A_n_m (D-L)</td>
<td>D, L</td>
<td>1,1</td>
<td>B</td>
</tr>
<tr>
<td></td>
<td></td>
<td>D, L</td>
<td>1,11</td>
<td>B</td>
</tr>
<tr>
<td></td>
<td></td>
<td>D, L</td>
<td>11,1</td>
<td>S</td>
</tr>
<tr>
<td></td>
<td></td>
<td>D, L</td>
<td>11,11</td>
<td>S</td>
</tr>
<tr>
<td></td>
<td>A_B_m_n (L-D)</td>
<td>L, D</td>
<td>1,1</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td></td>
<td>L, D</td>
<td>1,11</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td></td>
<td>L, D</td>
<td>11,1</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td></td>
<td>L, D</td>
<td>11,11</td>
<td>0</td>
</tr>
<tr>
<td>Left-handed</td>
<td>B_A_n_m (D-L)</td>
<td>D, L</td>
<td>1,1</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td></td>
<td>D, L</td>
<td>1,11</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td></td>
<td>D, L</td>
<td>11,1</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td></td>
<td>D, L</td>
<td>11,11</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>A_B_m_n (L-D)</td>
<td>L, D</td>
<td>1,1</td>
<td>B</td>
</tr>
<tr>
<td></td>
<td></td>
<td>L, D</td>
<td>1,11</td>
<td>B</td>
</tr>
<tr>
<td></td>
<td></td>
<td>L, D</td>
<td>11,1</td>
<td>S</td>
</tr>
<tr>
<td></td>
<td></td>
<td>L, D</td>
<td>11,11</td>
<td>S</td>
</tr>
</tbody>
</table>
not certain, as steric interactions *per se* need not necessarily be unfavorable to the stability of the particular molecular conformation, and hence to conformational induction. The description of all of the nonbonded interactions upon steric principles exclusively would lead to the conclusion that spatial coincidences contribute positively to $J$, and hence disfavor conformational induction. This is a reasonable and useful manner in which to view the term $J$, since any other mechanism(s) to be invoked would very likely be far less descriptive and demonstratable. Many conformations of a polypeptide may be classed as energetically unfavorable without consideration of other kinds of interactions; however, the method breaks down to the extent that it can not discriminate between those conformations which are sterically allowed (84,85).

The difference in the free energy of formation for very long helices of the opposite helix sense, $\Delta F$, may be thought to have its origins in steric differences between these structures, but such an attempt illustrates well the real limitations of a strictly steric interpretation of interactions. The decreased stability of the left-handed $\alpha$ helix as compared with the right-handed one, has been attributed to the close contact between the nearly eclipsed $C^\beta$ and $O$ atoms of the same residue which occurs in the left-handed $\alpha$ helical conformation (13). But in the right-handed $\alpha$ helix the $C^\beta$ and the amide $H$ of the same residue are eclipsed. Therefore, as stated above, the steric interpretation can not discriminate between these conformations, as they are both allowed, and sterically equivalent. If the right-handed helix sense is the energetically preferred conformation, additional assumptions are needed to
explain this preference; the repulsive van der Waals potential is "softer" for the \( \text{CH}_2 \cdots \text{H} \) interaction than for the \( \text{CH}_2 \cdots \text{O} \) interaction, or it is easier to bend the \( \text{C}^\alpha \text{NH} \) bond angle than to bend the \( \text{C}^\alpha \text{CO} \) bond angle in order to reduce the overlap between the eclipsing groups (86). In addition, the interactions between the side chains and the helix backbone may favor a particular helix sense. Therefore, the factors which give rise to a relatively decreased stability for one sense of the helix with respect to the other remain speculative in nature. Potential energy calculations (32,33) indicate that the stabilities of the right- and left-handed helices are comparable.
CHAPTER 9. CALCULATION OF HELIX STABILITIES FROM CONFORMATIONAL INDUCTION

Whereas previously we have discussed conformational induction in a general qualitative sense only, the articulated model of the previous chapters allows the calculation of the optical rotation behavior to be expected for the occurrence of conformational induction, and by comparison with the optical behavior of the block polymers studied, affords both a reasonable test for the model and information as to the magnitudes of the induction parameters.

Since the changes in the effective residue rotations have their origins in changes in the helical dichroic absorbancies, these changes will correlate well with the changes in the effective residue rotations in magnitude and direction. Therefore, the effective residue rotation (at a single wavelength) behavior for the block polymers will reflect accurately conformational changes due to conformational induction (Chapter 5).

Consider the case of complete induction as in case (a), Figure 21. Since the differences in the free energies between the various conformational states is finite, and perhaps under certain conditions small, one would expect that state (a) would be in equilibrium with all of the other states to an extent depending upon the free energy differences of these states with respect to state (a). For the sake of simplicity, assume that state (b) is the only state which contributes significantly to such an equilibrium. The relative population of each state depends upon
the appropriate Boltzmann factor, such that,

$$\frac{[b]}{[a]} = e^{-\frac{\Delta G_{a\rightarrow b}}{RT}}$$  \hspace{1cm} (33)

where $\Delta G_{a\rightarrow b}$ is the difference in free energies with respect to the randomly coiled chain between states (a) and (b). In this instance the free energy difference is given by the following expression:

$$\Delta G_{a\rightarrow b} = (t - n)\Delta F_B + E_B^{\prime} \hspace{1cm} (34)$$

Since we require that helix reversion occurs at some critical length, $n_c$, $\Delta G_{a\rightarrow b}$ must be zero at this critical length, and therefore from Equations 21 and 34 we can derive the following relationship:

$$\Delta G_{a\rightarrow b} = \Delta F_B (n_c - n) \hspace{1cm} (35)$$

By substituting Equation 35 into Equation 33 we obtain the following Equation:

$$\frac{[b]}{[a]} = e^{-\frac{\Delta F(n_c - n)}{RT}} \hspace{1cm} (36)$$

Thus we have the relative fraction of molecules in the two states (a) and (b) as a function of the two induction parameters, $\Delta F_B$, and $n_c$.

The effective mean residue rotation observed for a block polymer will be that due to a mixture of rotational contributions from state (a) and state (b),

$$[m']_{obs} = \frac{[a]}{[a] + [b]} [m']_{(a)} + \frac{[b]}{[a] + [b]} [m']_{(b)} \hspace{1cm} (37)$$

where $[m']_{(a)}$ and $[m']_{(b)}$ are the effective mean residue rotations due
to states (a) and (b) alone respectively.

\[
[m']_A^{(a)} = \frac{m}{m+n} [m']_A^t + \frac{n}{m+n} [m']_B^t
\]  

\[
[m']_B^{(b)} = \frac{m}{m+n} [m']_A^t + \frac{t}{m+n} [m']_B^t + \frac{n-t}{m+n} [m']_B^t.
\]  

The designation of the effective mean residue rotations, \([m']_A^t\), \([m']_B^t\), and \([m']_B^t\) follows that used previously in Chapter 7 for the designation of the helix sense (' and ')), where, as an example, \([m']_B^t\) is the effective mean residue rotation of block B in the \(\alpha\) helix. These values are gotten from the direct measurements upon the long homopolymers ([m']_A^t and [m']_B^t, Table 5) and indirectly by measurements of the rotation of the short induced polymer block ([m']_A^t and [m']_B^t) and random copolymers ([m']_B^t) (Appendix A, Table 5, (18)).

By the substitution of Equation 36 into 37, we obtain

\[
[m']_{\text{obs}} = \frac{1}{1 + e^{-x}} [m']_A^{(a)} + \frac{1}{1 + e^{-x}} [m']_B^{(b)}
\]  

where we have set

\[
x = \frac{\Delta F_B (n_c - n)}{RT}.
\]  

To facilitate the comparison of the calculated and observed optical rotation behavior of the block polymers, the theoretical effective mean residue rotation curves were generated as a function of the length of the added block, \(n\), using Equation 37, allowing the range of \(t\) and \(n_c\) to vary over a specified series of values. As an additional constraint, all of the curves were required to pass through one of the experimentally determined data points. This constraint allowed the calculation of \(\Delta F_B\).
for each value of $t$ and $n_c$. By allowing the range of $t$ to include both positive and negative values, the case of state (a) in equilibrium with state (c) ($t = 0$) or state (d) ($t < 0$) was implicitly comprehended by the calculations.

The $[m']$ versus $n$ curves as calculated from the conformational induction model were compared to the experimentally obtained $[m']$ versus $n$ curves, and the induction parameters which fit the experimental curves the best were selected (Table II). The conformational induction parameters were found to be moderately sensitive to the initial constraint as cited above, and thus consecutive calculations were done changing the experimental point through which the theoretical curve was required to pass, thereby minimizing the uncertainty in the conformational induction parameters. The sensitivity of the parameters was found to be nearly inversely proportional to the relative magnitudes of the rotations of block A and block B, the errors being greatest where the magnitudes of $[m']$ for these two blocks are similar. Typical calculated conformational induction curves are shown in Figures 25-28. Appendix B contains the calculated conformational induction curves and the experimental data points for all of the block copolypeptides listed in Table II. These data are given for several values of $\Delta F_B$, $n_c$, and $t$ in order that one may estimate the uncertainty in the calculated conformational induction parameters.

Figure 26 shows the calculated curves and experimental data points for $(L\text{-Tyr})_n-(L\text{-Glu-OBzl})_{20}$ in trimethyl phosphate. The curves were calculated in a manner analogous to $(D\text{-Tyr})_n-(L\text{-Glu-OBzl})_{20}$ (Figure 25).
Table 11. Calculated conformational induction parameters

<table>
<thead>
<tr>
<th>Copolypeptide&lt;sup&gt;a&lt;/sup&gt;/Solvent</th>
<th>$n_c$</th>
<th>$t$</th>
<th>$\Delta F_B$</th>
<th>$E_B$&lt;sup&gt;b&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>(D-Glu-OBzl)&lt;sub&gt;n&lt;/sub&gt;-(L-Glu-OBzl)&lt;sub&gt;m&lt;/sub&gt;, diox.</td>
<td>11</td>
<td>0</td>
<td>0.15</td>
<td>----</td>
</tr>
<tr>
<td>(L-Asp-OBzl)&lt;sub&gt;n&lt;/sub&gt;-(L-Glu-OBzl)&lt;sub&gt;m&lt;/sub&gt;, diox.</td>
<td>20</td>
<td>3</td>
<td>0.02</td>
<td>0.34</td>
</tr>
<tr>
<td>(D-Tyr)&lt;sub&gt;n&lt;/sub&gt;-(L-Glu-OBzl)&lt;sub&gt;m&lt;/sub&gt;, TMP</td>
<td>9</td>
<td>1</td>
<td>0.18</td>
<td>1.44</td>
</tr>
<tr>
<td>(D-Tyr)&lt;sub&gt;n&lt;/sub&gt;-(L-Glu-OBzl)&lt;sub&gt;m&lt;/sub&gt;, diox.</td>
<td>13</td>
<td>1</td>
<td>0.10</td>
<td>1.20</td>
</tr>
<tr>
<td>(D-Tyr)&lt;sub&gt;n&lt;/sub&gt;-(L-Glu-OBzl)&lt;sub&gt;m&lt;/sub&gt;, quinoline</td>
<td>13</td>
<td>4</td>
<td>0.06</td>
<td>0.72</td>
</tr>
<tr>
<td>(D-Lys-Z)&lt;sub&gt;n&lt;/sub&gt;-(L-Glu-OBzl)&lt;sub&gt;m&lt;/sub&gt;, diox.</td>
<td>7</td>
<td>0</td>
<td>0.35</td>
<td>----</td>
</tr>
<tr>
<td>(D-Tyr-Z)&lt;sub&gt;n&lt;/sub&gt;-(L-Glu-OBzl)&lt;sub&gt;m&lt;/sub&gt;, diox.</td>
<td>16</td>
<td>10</td>
<td>0.32</td>
<td>1.92</td>
</tr>
<tr>
<td>(L-Tyr)&lt;sub&gt;n&lt;/sub&gt;-(L-Glu-OBzl)&lt;sub&gt;m&lt;/sub&gt;, TMP</td>
<td>10</td>
<td>1</td>
<td>0.30</td>
<td>2.70</td>
</tr>
<tr>
<td>(L-Tyr-Z)&lt;sub&gt;n&lt;/sub&gt;-(L-Glu-OBzl)&lt;sub&gt;m&lt;/sub&gt;, diox.</td>
<td>18</td>
<td>0</td>
<td>0.21</td>
<td>----</td>
</tr>
</tbody>
</table>

<sup>a</sup>Induction parameters are for the N-terminal (left) block.

<sup>b</sup>Quantities reported in kcal/residue-mole.
Figure 25. The effective molar rotation of the block copolymer (D-Tyr)$_{DP}$-(L-Glu-OBzl)$_{20}$ versus DP (degree or polymerization) of (D-Tyr) in trimethyl phosphate - experimental data (•) and curves calculated from the theory of conformational induction, where

\[ n_c = 9, \; t = 0, \; \text{and} \; \Delta F = 0.12 \; \text{kcal/mole}, \]
\[ n_c = 10, \; t = 0, \; \text{and} \; \Delta F = 0.23 \; \text{kcal/mole}, \]
\[ n_c = 9, \; t = 1, \; \text{and} \; \Delta F = 0.18 \; \text{kcal/residue-mole}. \]
Figure 26. The effective molar residue rotation of the block copolymer \((L\text{-Tyr})_{DP} - (L\text{-Glu-OBzl})_{20}\) versus DP (degree of polymerization) of \((L\text{-Tyr})\) in trimethyl phosphate - experimental data (○) and curves calculated from the theory of conformational induction, where

- \(n_c = 10, \ t = 0, \ \Delta F = 0.14 \text{ kcal/residue-mole}\)
- \(n_c = 8, \ t = 3\) or 4, and \(\Delta F = 0.37-0.42 \text{ kcal/residue-mole}\)
- \(n_c = 10, \ t = 1, \ \Delta F = 0.30 \text{ kcal/residue-mole}\)
The effective molar residue rotation of the block copolymer (D-Tyr-Z)$_{DP}$-(L-Glu-OBz1)$_{20}$ versus the DP (degree of polymerization) of (D-Tyr-Z) in dioxane - experimental data (○) and curves calculated from the theory of conformational induction, where

- $n_c = 18$, $t = 7$, $\Delta F = 0.37 \text{ kcal/residue-mole}$
- $n_c = 16$, $t = 10$, $\Delta F = 0.32 \text{ kcal/residue-mole}$
- $n_c = 18$, $t = 9$, $\Delta F = 0.34 \text{ kcal/residue mole}$
Figure 28. The effective molar residue rotation of the block copolymer $(L\text{-Tyr-Z})_{p_1}-(L\text{-Glu-OBzl})_{p_2}$ versus the DP (degree of polymerization) of $(L\text{-Tyr-Z})$ in dioxane - experimental data (○) and curves calculated from the theory of conformational induction, where

- $n = 18$, $t = 0$, $\Delta F = 0.21 \text{ kcal/mole}$,
- $n = 16$, $t = 3$, $\Delta F = 0.41 \text{ kcal/mole}$,
- $n = 16$, $t = 0$, $\Delta F = 0.60 \text{ kcal/mole}$.
Figure 29. The effective residue rotation of the L-Tyr (o) and D-Tyr (●) blocks of the copolymers \((\text{L-Tyr})_{DP}\text{-}(\text{L-Glu-OBz})_{20} \) and \((\text{D-Tyr})_{DP}\text{-}(\text{L-Glu-OBz})_{20} \) as a function of the degree of polymerization (DP) of the L-Tyr and D-Tyr blocks in trimethyl phosphate.
since the experimentally observed optical rotation behavior is not completely inconsistent with helix induction and reversion phenomenon. This was also the case for (L-Tyr-Z)_n-(L-Glu-OBzl)_20 (Figure 28).

Figure 27 shows the calculated curves and experimental data for (D-Tyr-Z)_n-(L-Glu-OBzl)_20 in dioxane. The data points are not as reliable as those obtained in the studies upon the isolated block polymers of varied compositions (Figures 25 and 26), since the rotation of the polymers was calculated from the kinetic and optical studies of the polymerization reaction (Figures 27 and 28). The conformational induction parameters of the other polymers listed in Table 11 were done in exactly the same manner as that shown in Figures 25-28, and the experimental versus calculated data for these block copolymers are given in Appendix B.

The values of the induction parameters, t, n_c, ΔF_B, and E_B' (where possible) for each polypeptide studied by this method are given in Table 11. The values of E_B' were calculated from Equation 21. Since this relationship holds only in the instance where equilibrium between states (a) and (b) are considered, E_B' may be found for only where t > 0.

If the calculated induction parameters are reasonable, they should predict the magnitudes of the helical dichroic bands of the grafted block polymers studied. The molar ellipticity, [θ], may be calculated in a manner analogous to the calculation of the effective molar residue rotation for the copolymer, [m'], (Equation 40).

\[
[θ] = \frac{1}{1 + e^{-x}} [θ]_{(a)} + \frac{1}{1 + e^{x}} [θ]_{(b)}.
\] (42)
Using the values of $\Delta F_B$, $n_c$, and $t$ from Table 11, we may compare the observed molar ellipticity to that calculated from conformational induction, with the assumption that the molar ellipticity of an $\alpha$ helix in a single sense is the same for both L and D (only as an approximation). The results of such calculations are compared with the observed molar ellipticities in Table 6 for the grafted blocks of the copolymers poly(D-Glu-OBzl)-poly(L-Glu-OBzl), poly(D-Tyr)-poly(L-Glu-OBzl), and poly(L-Tyr)-poly(L-Glu-OBzl) (Table 12).

Discussion

Previous theoretical energy calculations for isolated (single-stranded) homopolymer polyamino acids have been done by summing over the torsional, nonbonded, electrostatic, and hydrogen bonding energy terms, minimizing the energy with respect to all the backbone and side chain dihedral angles of a given residue (32,33). These investigations were confined to an examination of regular conformations near those of the right- and left-handed helices. From the relative minimum energies of the allowed conformations, the preferred conformation of several polyamino acid helices with respect to the other helical structures (and not the random coil) were assigned by Ooi, Scott, Vanderkooi, and Scheraga (32), and by Yan, Vanderkooi, and Scheraga (33). Table 13 compares the results obtained by this method with the results from the conformational induction calculations.

The values of the relative helix stabilities from potential energy calculations may be compared with those obtained from conformational
Table 12. Comparison of experimental molar ellipticities with molar ellipticities calculated from the model for conformational induction

<table>
<thead>
<tr>
<th>Block Copolymers&lt;sup&gt;a&lt;/sup&gt;</th>
<th>[θ] x 10&lt;sup&gt;-4&lt;/sup&gt; (deg cm&lt;sup&gt;2&lt;/sup&gt;/d mole)</th>
<th>209 nm (exptl.)</th>
<th>209 nm (calc.)&lt;sup&gt;b&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>(D-Glu-OBz1)&lt;sub&gt;10&lt;/sub&gt;-(L-Glu-OBz1)&lt;sub&gt;20&lt;/sub&gt;</td>
<td>-1.10</td>
<td>-0.46</td>
<td></td>
</tr>
<tr>
<td>(D-Glu-OBz1)&lt;sub&gt;20&lt;/sub&gt;-(L-Glu-OBz1)&lt;sub&gt;20&lt;/sub&gt;</td>
<td>2.40</td>
<td>2.99</td>
<td></td>
</tr>
<tr>
<td>203 nm (exptl.)</td>
<td>203 nm (calc.)&lt;sup&gt;b&lt;/sup&gt;</td>
<td></td>
<td></td>
</tr>
<tr>
<td>(D-Tyr)&lt;sub&gt;6&lt;/sub&gt;-(L-Glu-OBz1)&lt;sub&gt;20&lt;/sub&gt;</td>
<td>4.50</td>
<td>4.98</td>
<td></td>
</tr>
<tr>
<td>(D-Tyr)&lt;sub&gt;10&lt;/sub&gt;-(L-Glu-OBz1)&lt;sub&gt;20&lt;/sub&gt;</td>
<td>0.05</td>
<td>-0.36</td>
<td></td>
</tr>
<tr>
<td>(D-Tyr)&lt;sub&gt;20&lt;/sub&gt;-(L-Glu-OBz1)&lt;sub&gt;20&lt;/sub&gt;</td>
<td>-5.20</td>
<td>-7.65</td>
<td></td>
</tr>
<tr>
<td>(L-Tyr)&lt;sub&gt;10&lt;/sub&gt;-(L-Glu-OBz1)&lt;sub&gt;20&lt;/sub&gt;</td>
<td>6.00</td>
<td>-10.00</td>
<td></td>
</tr>
<tr>
<td>(L-Tyr)&lt;sub&gt;20&lt;/sub&gt;-(L-Glu-OBz1)&lt;sub&gt;20&lt;/sub&gt;</td>
<td>10.40</td>
<td>10.40</td>
<td></td>
</tr>
</tbody>
</table>

<sup>a</sup>Molar ellipticities are for the N-terminal (left) block.

<sup>b</sup>The molar ellipticities are calculated upon the assumption that [θ]<sub>D</sub> = [θ]<sub>L</sub>, and [θ]<sub>L</sub> = -[θ]<sub>D</sub>.
Table 13. Comparison of calculated energy differences between right- and left-handed α helices; potential energy versus conformational induction calculations

<table>
<thead>
<tr>
<th>Polypeptide</th>
<th>$\Delta U$ (R-L) potential energy calculations kcal/residue-mole</th>
<th>$\Delta F$ (R-L) conformational induction calculations kcal/residue-mole</th>
</tr>
</thead>
<tbody>
<tr>
<td>Poly(L-Glu-OBzl)</td>
<td>-0.31 (33)</td>
<td>-0.15</td>
</tr>
<tr>
<td>Poly(L-Asp-OBzl)</td>
<td>0.13 (33)</td>
<td>0.02</td>
</tr>
<tr>
<td>Poly(L-Tyr)</td>
<td>-0.38 (33)</td>
<td>-0.18</td>
</tr>
<tr>
<td>Poly(L-Tyr)</td>
<td>-1.84 (32)</td>
<td></td>
</tr>
</tbody>
</table>
induction, but it is of paramount importance to distinguish these energies, in that, the former energy differences are differences in the total energy, while the latter differences are differences in free energies, the distinction between these being due to the unknown conformational entropies. Therefore the total energy (essentially the enthalpy) can not predict the preferred helix sense. In fact, although energy considerations alone might favor a helix of one sense, it is yet conceivable that entropic effects could negate the energy effects to the extent of reversing the helical sense. Also, the effects of the solvent are omitted from these calculations.

In spite of the above noted distinction to be made between the methods and the rationality of assigning the helix sense from the calculated energy differences, examination of the energies reveals that the energy differences are approximately the same irrespective of the method by which they were determined. The free energy differences determined from conformational induction are smaller than those determined from potential energy calculations, and possess a greater certainty since fewer arbitrary assumptions are made. As an example, consider the helix energy differences calculated for poly-L-tyrosine. The potential energy calculations resulted in an energy difference between the right- and left-handed helix of -0.4 to -1.8 kcal/residue-mole depending upon the choice of the dielectric constant and the van der Waals radius of hydrogen (32,33). The free energy difference obtained from the conformational induction calculations was -0.06 to -0.18 kcal/residue-mole (Table 11).
The critical helix lengths obtained from the conformational induction calculations may be compared with the critical helix sizes (the minimum size or length of an isolated polypeptide molecule that will form a stable $\alpha$ helix) determined for the various polypeptides in a variety of solvents (Table 14). These helix lengths are not strictly equivalent, but afford a point of comparative reference, especially as regards the requirement that the reverted helix be stable with respect to the random coil (Chapter 7, Table 9). The comparison of the critical helix lengths, $n_c$ (Table 11), with the minimum helix sizes for the isolated helicies gives good agreement between these values, and suggests that the reverted helix is stable with respect to the random coil, since the critical length is generally equal to or greater than the critical helix size.

The calculated molar ellipticities for the various block polymers in Table 12 show fairly favorable agreement for short chain lengths, with the comparison becoming more favorable as the chain length is increased. Therefore, the initial discrepancy is most likely due to the assumption (made prior to the calculations) that the molar ellipticity of an $\alpha$ helix of a single sense is the same for both L and D residues ($[\theta]_L = [\theta]_D$).

Note that both the $(D-Tyr)_n-(L-Glu-OBz)_20$ and $(L-Tyr)_n-(L-Glu-OBz)_20$ can be fitted to the conformational induction model to give reasonable induction parameters (Figures 25, 26 and Table 11). That both curves may be fitted to the conformational induction model is not
Table 14. Critical helix sizes for several homopolypeptides

<table>
<thead>
<tr>
<th>Polypeptide</th>
<th>Solvent</th>
<th>Critical Helix size</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>poly(L-Glu-OBzl)</td>
<td>dioxane</td>
<td>8-10</td>
<td>24,25,87,88</td>
</tr>
<tr>
<td>poly(L-Glu-OBzl)</td>
<td>DMF</td>
<td>7-14</td>
<td>89</td>
</tr>
<tr>
<td>poly(L-Glu-OBzl)</td>
<td>dioxane</td>
<td>6-7</td>
<td>90</td>
</tr>
<tr>
<td>poly(L-Asp-OMe)</td>
<td>CHCl₃/DCA</td>
<td>11</td>
<td>91</td>
</tr>
<tr>
<td>poly(L-Glu-OMe)</td>
<td>DMF</td>
<td>7-9</td>
<td>92</td>
</tr>
<tr>
<td>poly(L-Glu-OEt)</td>
<td>TFE</td>
<td>7-8</td>
<td>91,93</td>
</tr>
<tr>
<td>poly(L-Glu-OEt)</td>
<td>TMP</td>
<td>6-7</td>
<td>94</td>
</tr>
<tr>
<td>poly(L-Tyr)</td>
<td>TMP</td>
<td>6-12</td>
<td>Figure 19</td>
</tr>
</tbody>
</table>
unexpected, as given the number of variables and the fitting methods, a
good fit is probable in nearly every situation. This may particularly
be seen in the case of the \( (D\text{-Tyr}-Z)_n-(L\text{-Glu-OBzl})_{20} \) where a good fit
was obtained (Figure 27) although it is known that helical induction
does not occur in this case (see Chapter 6, page 101). In Chapter 6 it
was suggested that the optical behavior of the \( (L\text{-Tyr})_n-(L\text{-Glu-OBzl})_{20} \)
is due to a chain length dependence for the L-Tyr-block. Therefore, in
light of this interpretation it is necessary to incorporate this chain
length dependence of the effective residue rotation into the conformational induction calculations for the \( (D\text{-Tyr})_n-(L\text{-Glu-OBzl})_{20} \) case. If
we consider the chain length dependence of the effective residue rotat-
on of the D-Tyr block in \( (D\text{-Tyr})_n-(L\text{-Glu-OBzl})_{20} \) (Figure 29) we see
that for long (presumably reverted) D-Tyr block lengths the absolute
magnitude of the effective residue rotation approaches that found for
the long L-Tyr block of \( (L\text{-Tyr})_n-(L\text{-Glu-OBzl})_{20} \) (Figure 29). Therefore,
a similar chain length dependence of the effective residue rotation was
assumed for both the grafted L-Tyr and the grafted reverted D-Tyr blocks
in trimethyl phosphate (see Appendix A, page 167). The conformational
induction calculations for \( (D\text{-Tyr})_n-(L\text{-Glu-OBzl})_{20} \) in dioxane and
quinoline were done assuming no chain length dependence of the effective
residue rotation since such data was not available for these solvents.
It is difficult to predict the form of the chain length dependence of
the induced helix, but since this helical segment is usually relatively
short, no drastic chain length dependence was anticipated, and there-
therefore a constant value was used for the effective residue rotation of
the induced helix. The incorporation of the chain length dependence of the effective residue rotation was found to be only a refinement upon the usual procedure for the calculation of the conformational induction parameters. Since the chain length dependence is such that for chain lengths greater than 10 residues the effective residue rotation is practically that for a long chain, and since the critical length is near 10 residues, the effective residue rotation of the inverted helix will be similar to that for long chain lengths.

The comparison of the relative helix stabilities and critical helix lengths with similar quantities obtained from other experimental and theoretical studies indicate that the calculated induction parameters are certainly credible (Tables 13 and 14). Accordingly, the conformational induction parameters, particularly the relative helix stabilities, are at least as reliable a measure of the actual quantities as those obtained from theoretical methods (32,33). The relative helix stabilities calculated by this method possess the advantage that they are based upon experimental data, rather than purely theoretical calculations.
SUMMARY

The present research was intended to study the phenomenon of conformational induction in block copolypeptides and to develop a model to describe this effect from which information about the helix stabilities and preferred helix senses of homopolypeptides might be obtained.

From the optical, infrared, and polymerization kinetic studies, and their application to the model developed for conformational induction, the following results were obtained:

A. The optical studies at a single wavelength revealed a dependence of the conformational induction phenomenon upon the nature of the solvent and the composition of the copolymer blocks. The circular dichroism studies were consistent with the phenomenon of conformational induction as described by the model.

B. The infrared spectra of block copolymers were generally consistent with the occurrence of conformation induction.

C. The infrared kinetic studies revealed that conformational induction may be in part responsible for the kinetic phenomenon observed, but conformational induction was not a necessary condition for the occurrence of the multiple propagation rates in at least one instance.

D. The difference in the free energies of formation of right- and left-handed helices (with respect to the random coil) of several polypeptides were calculated from the model for conformational induction and the optical rotation studies. The magnitudes of these parameters were comparable to those obtained by theoretical methods (32,33).
E. The determination of the helix sense for several polypeptides from the induction-reversion phenomenon gave relative helix senses in agreement with those found by other methods. The helix sense of poly-L-tyrosine could not be determined in a completely unambiguous manner, since the optical behavior of both the \((L-Tyr)_n-(L-Glu-OBzl)_20\) and \((D-Tyr)_n-(L-Glu-OBzl)_20\) could be rationalized to the presence of conformational induction. However, the greater weight of evidence favored the assignment of a right-handed helical sense for poly-L-tyrosine.
LITERATURE CITED


76. T. M. Hooker, and J. A. Schellman, Biopolymers, 9, 1319 (1970).
ACKNOWLEDGEMENTS

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Supplementary Data to the Calculation of Conformational Induction Parameters; Experimental Data

In Chapter 8 the manner of calculation of the conformational induction parameters was described (Equations 37-41). From these relationships and the values assigned to the optical rotations, $[m']_A$, $[m']_B^g$, $[m']_B^{lg}$, $[m']_n$, $m$ (the mean effective residue rotations observed for the block copolymer $B_nA_m$ for known values of $m$ and $n$) and the composition of the blocks, $n$ and $m$, data were calculated from the conformational induction model and compared to the experimental data points to obtain the conformational induction parameters (Table 11). In this section the experimental data used to obtain the parameters in Table 11 are given. The experimental mean effective residue rotations of the block copolypeptides, $[m']_n$, $m$ are given as a function of the length of the grafted $B$ block, $n$.

Copolypeptide (D-Glu-OBzl)$_n$-(L-Glu-OBzl)$_m$ in dioxane at 30°C

Initial rotation and composition values;

$[m']_A = 14.3$  \quad m = 30.8

$[m']_A^g = -62.0$  \quad n = 20.0

$[m']_B = 62.0$  \quad $[m']_n, m = 6.3$

$[m']_B^{lg} = -14.3$

Data points ($[m']_n, m$ versus $n$):

$n = 4 \quad 10 \quad 19.4$

$[m']_n, m = 19.0 \quad 18.1 \quad 6.3$
Copolypeptide \((L-\text{Asp-OBzl})_n-(L-\text{Glu-OBzl})_m\) in dioxane at 30°C

Initial rotation and composition values;

\[
\begin{align*}
[m']_A^t &= 14.3 & m &= 34.5 \\
[m']_A^u &= -62.0 & n &= 21.5 \\
[m']_B^t &= -45.0 & [m']_{n,m} &= -37.2 \\
[m']_B^u &= -205.0
\end{align*}
\]

Data points \([m']_{n,m}\) versus \(n\);

\[
\begin{array}{cccc}
n & 4 & 8 & 21.5 & 36.0 \\
[m']_{n,m} & 4.0 & -8.0 & -37.2 & -62.0
\end{array}
\]

Copolypeptide \((\text{D-Lys-Z})_n-(L-\text{Glu-OBzl})_m\) in dioxane at 30°C

Initial rotation and composition values;

\[
\begin{align*}
[m']_A^t &= 14.3 & m &= 27.8 \\
[m']_A^u &= -62.0 & n &= 11.0 \\
[m']_B^t &= 19.5 & [m']_{n,m} &= 11.9 \\
[m']_B^u &= -4.0
\end{align*}
\]

Data points \([m']_{n,m}\) versus \(n\):

\[
\begin{array}{cccccccccccc}
n & 2 & 4 & 6 & 8 & 11 & 14 & 18 & 20 \\
[m']_{n,m} & 14.6 & 14.8 & 14.4 & 13.4 & 11.9 & 11.2 & 10.5 & 10.0
\end{array}
\]
Copolypeptide (D-Tyr)$_n$-(L-Glu-OBzl)$_m$ in trimethyl phosphate at 30°C (see Figure 25)

Initial rotation and composition values;

\[
[m']_A^* = 7.3 \quad m = 22.5 \quad [m']_B^* = \frac{C_1(n)^6}{1 + B(n)^6} + A
\]

\[
[m']_A^* = -32.0 \quad n = 11.3
\]

\[
[m']_B^* = 165.0 \quad [m']_{n,m} = 0.0
\]

where, \(C_1 = -0.000172345\)

\(B = 0.000002651\)

\(A = -55.00\)

(The form of \([m']_B^*\) is meant to incorporate the chain length dependence of the reverted D-Tyr helix).

Data points \((m', n)\):

\[

<table>
<thead>
<tr>
<th>n</th>
<th>2.8</th>
<th>5.4</th>
<th>9.2</th>
<th>10.0</th>
<th>15.6</th>
<th>19.4</th>
</tr>
</thead>
<tbody>
<tr>
<td>([m']_{n,m})</td>
<td>23.5</td>
<td>27.2</td>
<td>16.0</td>
<td>9.8</td>
<td>-25.0</td>
<td>-45.0</td>
</tr>
</tbody>
</table>

Copolypeptide (L-Tyr)$_n$-(L-Glu-OBzl)$_m$ in trimethyl phosphate at 30°C (see Figure 26)

Initial rotation and composition values;

\[
[m']_A^* = 7.3 \quad m = 22.5
\]

\[
[m']_A^* = -32.0 \quad n = 9.8
\]

\[
[m']_B^* = 55.0 \quad [m']_{n,m} = 42.3
\]

\[
[m']_B^* = 220.00
\]

Data points \((m', n)\):

\[

<table>
<thead>
<tr>
<th>n</th>
<th>3.8</th>
<th>6.9</th>
<th>9.8</th>
<th>11.0</th>
<th>17.0</th>
<th>19.4</th>
</tr>
</thead>
<tbody>
<tr>
<td>([m']_{n,m})</td>
<td>13.0</td>
<td>20.5</td>
<td>42.3</td>
<td>51.0</td>
<td>85.0</td>
<td>105.0</td>
</tr>
</tbody>
</table>
Copolypeptide (D-Tyr-Z)_n-(L-Glu-OBzl)_m in dioxane at 30°C (see Figure 27)

Initial rotation and composition values;

\[
[m']_A = 14.3 \quad m = 28.8 \\
[m']_A = -62.0 \quad n = 19.0 \\
[m']_B = 240.0 \quad [m']_{n,m} = 0.0 \\
[m']_B = -350.0
\]

Data points ([m']_n,m versus n):

<table>
<thead>
<tr>
<th>n</th>
<th>2</th>
<th>4</th>
<th>6</th>
<th>8</th>
<th>10</th>
<th>14</th>
<th>16</th>
<th>20</th>
</tr>
</thead>
<tbody>
<tr>
<td>[m']_n,m</td>
<td>26.0</td>
<td>44.5</td>
<td>60.0</td>
<td>---</td>
<td>75.0</td>
<td>78.5</td>
<td>70.5</td>
<td>-13.0</td>
</tr>
</tbody>
</table>

Copolypeptide (L-Tyr-Z)_n-(L-Glu-OBz1)_m in dioxane at 30°C (see Figure 28)

Initial rotation and composition values;

\[
[m']_A = 14.3 \quad m = 30.2 \\
[m']_A = -62.0 \quad n = 15.0 \\
[m']_B = 90.0 \quad [m']_{n,m} = 67.0 \\
[m']_B = 400.0
\]

Data points ([m']_n,m versus n);

<table>
<thead>
<tr>
<th>n</th>
<th>4</th>
<th>8</th>
<th>12</th>
<th>16</th>
<th>18</th>
<th>21</th>
<th>32</th>
<th>40</th>
</tr>
</thead>
<tbody>
<tr>
<td>[m']_n,m</td>
<td>21.0</td>
<td>29.0</td>
<td>45.0</td>
<td>75.0</td>
<td>98.0</td>
<td>140.0</td>
<td>210.0</td>
<td>232.0</td>
</tr>
</tbody>
</table>
APPENDIX B

Supplementary Data to the Calculation of Conformational Induction Parameters; Comparison of Calculated and Experimental Data

In this section are presented the experimental mean effective residue rotations of the block copolypeptides of type $B_n A_m$ and mean effective residue rotations calculated from the model for conformational induction. From a comparison of the calculated and experimental mean effective residue rotations, the conformational induction parameters which gave the best fit to the experimental data were selected (Table II). The mean effective residue rotations of the block copolypeptides are given as a function of the length of the grafted B block, $n$, for fixed values of $n_c$ and for several values of $t$ and vice versa. The values of $n_c$ and $t$ presented are near those which were found to give the best fit between the experimental and calculated mean effective residue rotations, and from such a presentation of the data it is possible to estimate the relative uncertainty of the conformational induction parameters.
Copolypeptide (D-Glu-OBzl)\textsubscript{n}-(L-Glu-OBzl)\textsubscript{m} in dioxane at 30°C

Experimental Data:

\[
\begin{array}{cccc}
  n   & 4.0 & 10.0 & 19.4 \\
[m']_{n,m} & 19.0 & 18.1 & 6.3 \\
\end{array}
\]

Calculated Mean Effective Residue Rotations (\(n_c = 11\)):

\[
\begin{array}{cccccc}
  t = 1, n_c = 11, \Delta F = 0.20 \text{ kcal/residue-mole} \\
  n   & 4.0 & 8.0 & 10.0 & 14.0 & 20.0 \\
[m']_{n,m} & 19.32 & 20.54 & 19.06 & 13.06 & 5.81 \\
\end{array}
\]

\[
\begin{array}{cccccc}
  t = 0, n_c = 11, \Delta F = 0.15 \text{ kcal/residue-mole} \\
  n   & 4.0 & 8.0 & 10.0 & 14.0 & 20.0 \\
[m']_{n,m} & 18.61 & 19.22 & 17.89 & 12.97 & 5.73 \\
\end{array}
\]

\[
\begin{array}{cccccc}
  t = -1, n_c = 11, \Delta F = 0.12 \text{ kcal/residue-mole} \\
  n   & 4.0 & 8.0 & 10.0 & 14.0 & 20.0 \\
[m']_{n,m} & 17.71 & 18.02 & 16.82 & 12.60 & 5.71 \\
\end{array}
\]

Calculated Mean Effective Residue Rotations (\(t = 0\)):

\[
\begin{array}{cccccc}
  t = 0, n_c = 10, \Delta F = 0.13 \text{ kcal/residue-mole} \\
  n   & 4.0 & 8.0 & 10.0 & 14.0 & 20.0 \\
[m']_{n,m} & 18.9 & 18.10 & 16.7 & 12.23 & 5.78 \\
\end{array}
\]

\[
\begin{array}{cccccc}
  t = 0, n_c = 11, \Delta F = 0.15 \text{ kcal/residue-mole} \\
  n   & 4.0 & 8.0 & 10.0 & 14.0 & 20.0 \\
[m']_{n,m} & 18.61 & 19.22 & 17.89 & 12.97 & 5.73 \\
\end{array}
\]

\[
\begin{array}{cccccc}
  t = 0, n_c = 12, \Delta F = 0.17 \text{ kcal/residue-mole} \\
  n   & 4.0 & 8.0 & 10.0 & 14.0 & 20.0 \\
[m']_{n,m} & 19.1 & 20.47 & 19.35 & 13.97 & 5.68 \\
\end{array}
\]
Copolypeptide (L-Asp-OBzl)_n-(L-Glu-OBzl)_m in dioxane at 30°C

Experimental Data:

\[ \begin{array}{cccc}
  n &=& 4.0 & 8.0 & 21.5 & 36.0 \\
  [m']_{n,m} &=& 4.3 & -8.0 & -37.5 & -62.0 \\
\end{array} \]

Calculated Mean Effective Residue Rotations ($n_c = 20$):

\[ \begin{array}{cccc}
  t &=& 5, \\n  n_c &=& 20, \\n  \Delta F &=& 0.12 \text{ kcal/residue-mole} \\
  n &=& 4.0 & 8.0 & 20.0 & 36.0 \\
  [m']_{n,m} &=& 6.03 & 0.33 & -31.00 & -85.00 \\
\end{array} \]

\[ \begin{array}{cccc}
  t &=& 4, \\n  n_c &=& 20, \\n  \Delta i &=& 0.07 \text{ kcal/residue-mole} \\
  n &=& 4.0 & 8.0 & 20.0 & 36.0 \\
  [m']_{n,m} &=& 6.03 & -1.73 & -32.48 & -69.0 \\
\end{array} \]

\[ \begin{array}{cccc}
  t &=& 3, \\n  n_c &=& 20, \\n  \Delta F &=& 0.02 \text{ kcal/residue-mole} \\
  n &=& 4.0 & 8.0 & 20.0 & 36.0 \\
  [m']_{n,m} &=& 4.60 & -6.00 & -33.94 & -66.11 \\
\end{array} \]

Calculated Mean Effective Residue Rotations ($t = 3'$):

\[ \begin{array}{cccc}
  t &=& 3, \\n  n_c &=& 16, \\n  \Delta F &=& 0.006 \text{ kcal/residue-mole} \\
  n &=& 4.0 & 8.0 & 20.0 & 36.0 \\
  [m']_{n,m} &=& 4.10 & -7.78 & -34.48 & -58.67 \\
\end{array} \]

\[ \begin{array}{cccc}
  t &=& 3, \\n  n_c &=& 18, \\n  \Delta F &=& 0.01 \text{ kcal/residue-mole} \\
  n &=& 4.0 & 8.0 & 20.0 & 36.0 \\
  [m']_{n,m} &=& 4.21 & -7.39 & -34.47 & -60.32 \\
\end{array} \]

\[ \begin{array}{cccc}
  t &=& 3, \\n  n_c &=& 20, \\n  \Delta F &=& 0.02 \text{ kcal/residue-mole} \\
  n &=& 4.0 & 8.0 & 20.0 & 36.0 \\
  [m']_{n,m} &=& 4.60 & -6.00 & -33.94 & -66.11 \\
\end{array} \]
Copolypeptide (D-Lys-Z)\_n-(L-Glu-OBz\_1)\_m in dioxane at 30°C

**Experimental Data:**

\[
\begin{array}{cccccccc}
  n & 2.0 & 4.0 & 8.0 & 11.0 & 14.0 & 18.0 & 20.0 \\
  [m']_{n,m} & 14.6 & 14.8 & 13.4 & 11.9 & 11.2 & 10.5 & 10.0 \\
\end{array}
\]

**Calculated Mean Effective Residue Rotations \((n_c = 7):**

- **t = 1, \(n_c = 7), \Delta F = 0.54 \text{ kcal/residue-mole}**
  
  \[
  \begin{array}{cccccccc}
    n & 2.0 & 4.0 & 8.0 & 11.0 & 14.0 & 18.0 & 20.0 \\
    [m']_{n,m} & 14.70 & 14.89 & 13.32 & --- & 11.24 & 10.60 & 10.30 \\
  \end{array}
  \]

- **t = 0, \(n_c = 7), \Delta F = 0.30 \text{ kcal/residue-mole}**
  
  \[
  \begin{array}{cccccccc}
    n & 2.0 & 4.0 & 8.0 & 11.0 & 14.0 & 18.0 & 20.0 \\
    [m']_{n,m} & 14.59 & 14.62 & 13.31 & --- & 11.01 & 10.29 & 10.01 \\
  \end{array}
  \]

- **t = -1, \(n_c = 7), \Delta F = 0.20 \text{ kcal/residue-mole}**
  
  \[
  \begin{array}{cccccccc}
    n & 2.0 & 4.0 & 8.0 & 11.0 & 14.0 & 18.0 & 20.0 \\
    [m']_{n,m} & 14.39 & 14.27 & 13.84 & --- & 10.88 & 9.98 & 9.67 \\
  \end{array}
  \]

**Calculated Mean Effective Residue Rotations \((t = 0):**

- **t = 0, \(n_c = 6), \Delta F = 0.24 \text{ kcal/residue-mole}**
  
  \[
  \begin{array}{cccccccc}
    n & 2.0 & 4.0 & 8.0 & 11.0 & 14.0 & 18.0 & 20.0 \\
    [m']_{n,m} & 14.50 & 14.37 & 13.08 & --- & 11.05 & 10.31 & 10.02 \\
  \end{array}
  \]

- **t = 0, \(n_c = 7), \Delta F = 0.30 \text{ kcal/residue-mole}**
  
  \[
  \begin{array}{cccccccc}
    n & 2.0 & 4.0 & 8.0 & 11.0 & 14.0 & 18.0 & 20.0 \\
    [m']_{n,m} & 14.59 & 14.62 & 13.31 & --- & 11.01 & 10.29 & 10.01 \\
  \end{array}
  \]

- **t = 0, \(n_c = 8), \Delta F = 0.41 \text{ kcal/residue-mole}**
  
  \[
  \begin{array}{cccccccc}
    n & 2.0 & 4.0 & 8.0 & 11.0 & 14.0 & 18.0 & 20.0 \\
    [m']_{n,m} & 14.65 & 14.85 & 13.75 & --- & 10.95 & 10.27 & 20.00 \\
  \end{array}
  \]
Copolypeptide $(D$-Tyr)$_n$-(L-Glu-OBzl)$_m$ in trimethyl phosphate at 30°C (see Figure 25)

**Experimental Data:**

<table>
<thead>
<tr>
<th>n</th>
<th>2.8</th>
<th>5.4</th>
<th>9.2</th>
<th>10.0</th>
<th>15.6</th>
<th>19.4</th>
</tr>
</thead>
<tbody>
<tr>
<td>$\left[ m' \right]_{n,m}$</td>
<td>23.5</td>
<td>27.2</td>
<td>16.0</td>
<td>9.8</td>
<td>-25.0</td>
<td>-45.0</td>
</tr>
</tbody>
</table>

Calculated Mean Effective Residue Rotations ($n_C = 9$):

- $t = 1$, $n_C = 9$, $\Delta F = 0.18$ kcal/residue-mole
  - | n  | 2.8 | 5.4 | 9.2 | 10.0 | 15.6 | 19.4 |
  - | $\left[ m' \right]_{n,m}$ | 21.37 | 27.71 | 17.26 | 11.99 | -24.44 | -38.56 |

- $t = 0$, $n_C = 9$, $\Delta F = 0.13$ kcal/residue-mole
  - | n  | 2.8 | 5.4 | 9.2 | 10.0 | 15.6 | 19.4 |
  - | $\left[ m' \right]_{n,m}$ | 18.55 | 22.87 | 13.74 | --- | -21.25 | -37.55 |

- $t = -1$, $n_C = 9$, $\Delta F = 0.12$ kcal/residue-mole
  - | n  | 2.8 | 5.4 | 9.2 | 10.0 | 15.6 | 19.4 |
  - | $\left[ m' \right]_{n,m}$ | 16.58 | 20.12 | 9.89 | 5.69 | -25.36 | -41.87 |

Calculated Mean Effective Residue Rotations ($t = 1$):

- $t = 1$, $n_C = 8$, $\Delta F = 0.14$ kcal/residue-mole
  - | n  | 2.8 | 5.4 | 9.2 | 10.0 | 15.6 | 19.4 |
  - | $\left[ m' \right]_{n,m}$ | 19.74 | 23.76 | 13.86 | 9.68 | -19.90 | -34.70 |

- $t = 1$, $n_C = 9$, $\Delta F = 0.18$ kcal/residue-mole
  - | n  | 2.8 | 5.4 | 9.2 | 10.0 | 15.6 | 19.4 |
  - | $\left[ m' \right]_{n,m}$ | 21.37 | 27.71 | 17.26 | 11.99 | -24.44 | -38.56 |

- $t = 1$, $n_C = 10$, $\Delta F = 0.34$ kcal/residue-mole
  - | n  | 2.8 | 5.4 | 9.2 | 10.0 | 15.6 | 19.4 |
  - | $\left[ m' \right]_{n,m}$ | 22.70 | 32.99 | 25.47 | 18.31 | -30.45 | -41.39 |
Copolypeptide (L-Tyr)$_n$-(L-Glu-OBzl)$_m$ in trimethyl phosphate at 30°C (see Figure 26)

Experimental Data:

<table>
<thead>
<tr>
<th>$n$</th>
<th>3.8</th>
<th>6.9</th>
<th>9.8</th>
<th>11.0</th>
<th>17.0</th>
<th>19.4</th>
</tr>
</thead>
<tbody>
<tr>
<td>$[m']_{n,m}$</td>
<td>13.0</td>
<td>20.5</td>
<td>42.3</td>
<td>51.0</td>
<td>85.0</td>
<td>105.0</td>
</tr>
</tbody>
</table>

Calculated Mean Effective Residue Rotations ($n_c = 10$):

$t = 2, n_c = 10, \Delta F = 0.53 \text{ kcal/residue-mole}$

<table>
<thead>
<tr>
<th>$n$</th>
<th>4.0</th>
<th>6.0</th>
<th>10.0</th>
<th>12.0</th>
<th>16.0</th>
<th>20.0</th>
</tr>
</thead>
<tbody>
<tr>
<td>$[m']_{n,m}$</td>
<td>13.81</td>
<td>16.94</td>
<td>41.36</td>
<td>64.34</td>
<td>86.57</td>
<td>99.40</td>
</tr>
</tbody>
</table>

$t = 1, n_c = 10, \Delta F = 0.31 \text{ kcal/residue-mole}$

<table>
<thead>
<tr>
<th>$n$</th>
<th>4.0</th>
<th>6.0</th>
<th>10.0</th>
<th>12.0</th>
<th>16.0</th>
<th>20.0</th>
</tr>
</thead>
<tbody>
<tr>
<td>$[m']_{n,m}$</td>
<td>14.56</td>
<td>19.59</td>
<td>43.98</td>
<td>62.22</td>
<td>88.49</td>
<td>102.98</td>
</tr>
</tbody>
</table>

$t = 0, n_c = 10, \Delta F = 0.14 \text{ kcal/residue-mole}$

<table>
<thead>
<tr>
<th>$n$</th>
<th>4.0</th>
<th>6.0</th>
<th>10.0</th>
<th>12.0</th>
<th>16.0</th>
<th>20.0</th>
</tr>
</thead>
<tbody>
<tr>
<td>$[m']_{n,m}$</td>
<td>18.78</td>
<td>26.34</td>
<td>46.59</td>
<td>58.54</td>
<td>81.83</td>
<td>100.43</td>
</tr>
</tbody>
</table>

Calculated Mean Effective Residue Rotations ($t = 1$):

$t = 1, n_c = 8, \Delta F = 0.09 \text{ kcal/residue-mole}$

<table>
<thead>
<tr>
<th>$n$</th>
<th>4.0</th>
<th>6.0</th>
<th>10.0</th>
<th>12.0</th>
<th>16.0</th>
<th>20.0</th>
</tr>
</thead>
<tbody>
<tr>
<td>$[m']_{n,m}$</td>
<td>20.59</td>
<td>29.00</td>
<td>47.45</td>
<td>57.09</td>
<td>75.83</td>
<td>92.48</td>
</tr>
</tbody>
</table>

$t = 1, n_c = 9, \Delta F = 0.14 \text{ kcal/residue-mole}$

<table>
<thead>
<tr>
<th>$n$</th>
<th>4.0</th>
<th>6.0</th>
<th>10.0</th>
<th>12.0</th>
<th>16.0</th>
<th>20.0</th>
</tr>
</thead>
<tbody>
<tr>
<td>$[m']_{n,m}$</td>
<td>18.35</td>
<td>26.22</td>
<td>46.69</td>
<td>58.30</td>
<td>80.34</td>
<td>97.87</td>
</tr>
</tbody>
</table>

$t = 1, n_c = 10, \Delta F = 0.31 \text{ kcal/residue-mole}$

<table>
<thead>
<tr>
<th>$n$</th>
<th>4.0</th>
<th>6.0</th>
<th>10.0</th>
<th>12.0</th>
<th>16.0</th>
<th>20.0</th>
</tr>
</thead>
<tbody>
<tr>
<td>$[m']_{n,m}$</td>
<td>14.56</td>
<td>19.59</td>
<td>43.98</td>
<td>62.22</td>
<td>88.49</td>
<td>102.98</td>
</tr>
</tbody>
</table>
Copolypeptide \((L-\text{Tyr-Z})_n-(L-\text{Glu-OBzl})_m\) in dioxane at 30\(^\circ\)C

Experimental Data:

\[
\begin{array}{cccccccc}
n & 4.0 & 8.0 & 12.0 & 16.0 & 21.0 & 32.0 & 40.0 \\
[m']_{n,m} & 21.0 & 29.0 & 45.0 & 75.0 & 140.0 & 210.0 & 232.0 \\
\end{array}
\]

Calculated Mean Effective Residue Rotation \((n_c = 16)\):

\[t = 4, \ n_c = 16, \ \Delta F = 0.33 \text{ kcal/residue-mole} \]

\[
\begin{array}{cccccccc}
n & 4.0 & 8.0 & 12.0 & 16.0 & 21.0 & 32.0 & 40.0 \\
[m']_{n,m} & 23.17 & 30.56 & 41.66 & 80.79 & 146.0 & 196.0 & 221.50 \\
\end{array}
\]

\[t = 3, \ n_c = 16, \ \Delta F = 0.41 \text{ kcal/residue-mole} \]

\[
\begin{array}{cccccccc}
n & 4.0 & 8.0 & 12.0 & 16.0 & 21.0 & 32.0 & 40.0 \\
[m']_{n,m} & 23.17 & 30.34 & 30.84 & 84.14 & 153.90 & 200.58 & 225.78 \\
\end{array}
\]

\[t = 2, \ n_c = 16, \ \Delta F = 0.48 \text{ kcal/residue-mole} \]

\[
\begin{array}{cccccccc}
n & 4.0 & 8.0 & 12.0 & 16.0 & 21.0 & 32.0 & 40.0 \\
[m']_{n,m} & 23.17 & 30.25 & 38.67 & 87.50 & 143.87 & 205.87 & 230.09 \\
\end{array}
\]

Calculated Mean Effective Residue Rotation \((t = 4)\):

\[t = 4, \ n_c = 16, \ \Delta F = 0.33 \text{ kcal/residue-mole} \]

\[
\begin{array}{cccccccc}
n & 4.0 & 8.0 & 12.0 & 16.0 & 21.0 & 32.0 & 40.0 \\
[m']_{n,m} & 23.17 & 30.56 & 41.66 & 80.79 & 146.0 & 196.0 & 221.50 \\
\end{array}
\]

\[t = 4, \ n_c = 18, \ \Delta F = 0.11 \text{ kcal/residue-mole} \]

\[
\begin{array}{cccccccc}
n & 4.0 & 8.0 & 12.0 & 16.0 & 21.0 & 32.0 & 40.0 \\
[m']_{n,m} & 23.17 & 34.62 & 50.47 & 73.46 & 110.0 & --- & --- \\
\end{array}
\]

\[t = 4, \ n_c = 20, \ \Delta F = 0.07 \text{ kcal/residue-mole} \]

\[
\begin{array}{cccccccc}
n & 4.0 & 8.0 & 12.0 & 16.0 & 21.0 & 32.0 & 40.0 \\
[m']_{n,m} & 23.17 & 36.99 & 53.03 & 72.04 & 99.0 & --- & --- \\
\end{array}
\]