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Abstract

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Excited-State Processes in Polycyclic Quinones: The Light-Induced Antiviral Agent, Hypocrellin, and a Comparison with Hypericin

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Hypocrellin is a naturally occurring perylene quinone that possesses light-induced antiviral activity, most notably against the human immunodeficiency virus (HIV), as does the related molecule, hypericin. White-light continuum is employed to examine the excited-state processes in hypocrellin from the picosecond to the nanosecond time scales. These processes are assigned to intramolecular proton transfer, intersystem crossing, and interconversion between different conformations of hypocrellin, which is constrained to be nonplanar in its ground-state owing to its bulky side chains. The ground state of hypocrellin is suggested to be heterogeneous and to be comprised of an equilibrium between at least two tautomeric forms. The results are discussed in terms of the properties of hypericin, which bears marked similarities and differences with respect to hypocrellin, both in terms of its excited-state properties as well as its mode of induced antiviral activity.

I. Introduction

Hypocrellin and hypericin (Figure 1) are naturally occurring polycyclic quinones that have been used for centuries as folk medicines in the orient¹ and the occident,^{2,3} respectively. Hypericin is the pigment found in the common plant, St. John's wort. Hypocrellin is obtained from the mold, *Hypocrella bambusae*. Our motivation for the study of hypocrellin is that, owing to its similarities with respect to hypericin, which are at least superficial, it may provide a means of better understanding hypericin and hypericin-like molecules.

Our laboratory's interest in hypericin was stimulated by the report that it possesses antiviral activity against the human immunodeficiency virus (HIV)^{4–7} and by the discovery of Kraus and Carpenter that toxicity against the class of enveloped lentiviruses, of which HIV is a member, absolutely depends on light.^{8,9} Hypericin is in fact currently being used in clinical trials for HIV,^{10–13} and extracts from St. John's wort can be purchased in health food stores and are taken orally by individuals infected with HIV.^{14,15} Results from our group¹⁶ as well as from others¹⁷ indicate that hypocrellin also exhibits antiviral activity that absolutely depends on light. The enormous potential of such quinone species in photodynamic therapies against viruses and tumors^{18,19} prompted our investigations into the role of light in virucidal activity and into the nature of the excited-state species that are responsible for such activity.^{20–26}

The proximity of the enol and keto groups in organic molecules provides an environment that is propitious for excited-state intramolecular proton transfer or hydrogen atom transfer (in this article, we use the two terms interchangeably). Malonaldehyde,²⁷ salicylic acid,²⁸ 3-hydroxyflavone,²⁹ benzothiazole,³⁰ and tropolone³¹ are but a few of the examples of a litany of species that execute intramolecular excited-state proton transfer. The disposition and the number of enol and keto groups in hypericin are immediately suggestive of the possibility of excited-state intramolecular proton transfer, and we have interpreted our picosecond spectroscopic studies of this molecule in these terms.^{20–22} Of special relevance to the role of labile protons for light-induced antiviral activity is the observation

that hypericin retains its toxicity in the absence of oxygen and that it acidifies its surroundings upon light absorption.^{23,24} The retention of toxicity in the absence of oxygen excludes unique assignment of antiviral activity to the trivial generation of singlet oxygen—even though hypericin does generate triplets in high yield.^{38–40}

In parallel with our investigations into the origin of the light-induced antiviral activity of hypericin, we have begun a program devoted to the specific targeting and triggering of its activity by the chemiluminescent generation of light in virus-infected cells.²⁵ Preliminary results with dog cells *in vitro* indicate that chemiluminescence can be specifically induced by the presence of the infecting virus.^{25,26} We have focused our attention on the development of this type of gene therapy²⁶ because it can in principle reduce the appearance of drug-resistant mutants, which typically emerge and block the efficiency of current treatments.^{32,33}

The structural similarities of hypocrellin and hypericin would seem to suggest that hypocrellin exhibits excited-state and antiviral behavior similar to, if not identical with, that of hypericin. But this is not the case. Hypocrellin absolutely requires oxygen for antiviral activity whereas hypericin does not. Also, hypocrellin does not provide a light-induced pH drop of its surrounding, as a result of *intermolecular* proton transfer, under conditions in which hypericin does exhibit such an effect.¹⁶ Clearly, the superficial resemblance between hypocrellin and hypericin belies more profound differences that exist between them. In this article, the excited-state photophysics of hypocrellin are investigated. White-light continuum is employed to examine the excited-state processes in hypocrellin from the picosecond to the nanosecond time scales. These processes are assigned to intramolecular proton transfer, intersystem crossing, and interconversion between different conformations of hypocrellin, which is constrained to be nonplanar in its ground state owing to its bulky side chains. The ground state of hypocrellin is suggested to be heterogeneous and to be comprised of an equilibrium between at least two tautomeric forms. In the Conclusions section, these processes are summarized in a kinetic scheme and comparisons are made with hypericin.

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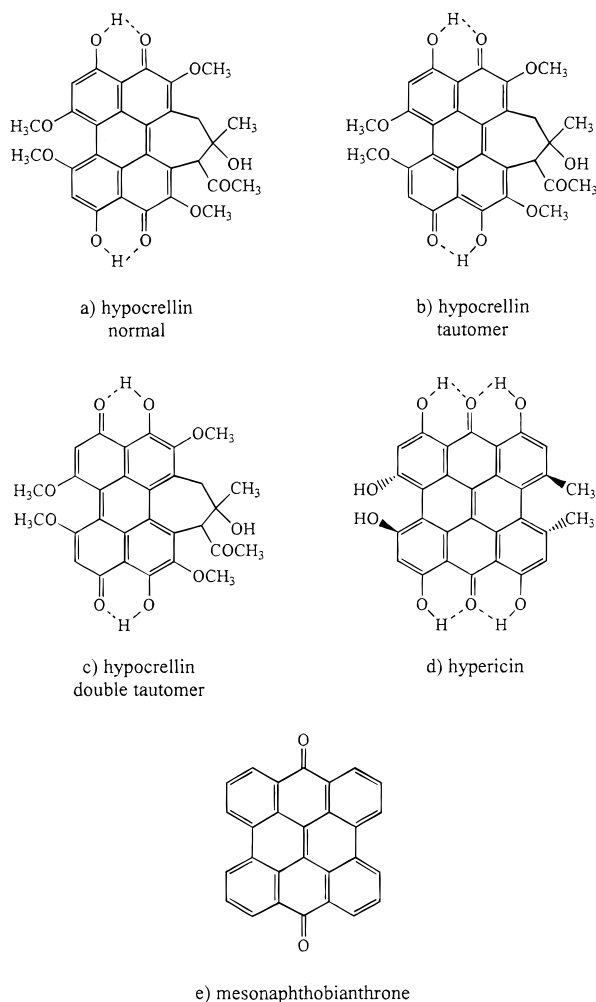


Figure 1. Two-dimensional structures of (a) the normal, that is, untautomerized, form of hypocrellin A; (b) a partially tautomerized form of hypocrellin; (c) the completely tautomerized form of hypocrellin A; (d) the normal form of hypericin; (e) the hypericin analogue, mesonaphthobianthrone. It is important to note that these structures are very misleading in that hypericin and hypocrellin are not planar molecules; interactions of the substituents on the aromatic skeleton twist these molecules out of planarity.^{36,37}

II. Materials and Methods

Hypocrellin A (to which we refer as hypocrellin) was used as received from Molecular Probes, Inc. It was >98% pure as determined by TLC and NMR (which spectra were obtained from the supplier). Given that the transients observed have amplitudes of greater than 2%, it did not appear to be cost effective to attempt to purify this very expensive compound further and lose a significant fraction of it in the process. Other indications of the purity of the sample are that its fluorescence lifetime in all solvents except H_2SO_4 is a single exponential of 1.3–1.6 ns duration and that its fluorescence excitation spectrum superimposes its absorption spectrum. Solvents were obtained from Aldrich. Methylcyclohexane and 2,2,2-trifluoroethanol, which were used in fluorescence lifetime measurements, were carefully distilled under nitrogen and over calcium sulfate. Octanol was dried by stirring overnight with magnesium sulfate. The solvents were subsequently used in transient absorption experiments after which a drop of water was added and the experiments repeated. This was done to ensure that the transients were not a result of residual water in the solvents.

All experiments were performed at ambient temperatures (22 °C). Fluorescence spectra were measured with a Spex Fluoromax with emission and excitation band-pass of 4 nm. The time-correlated single-photon-counting experiments are per-

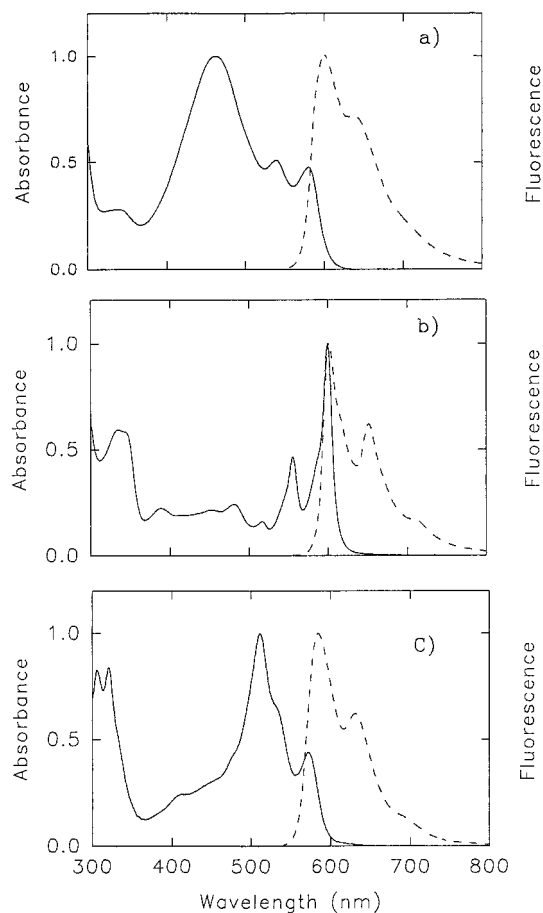


Figure 2. Steady-state absorption (solid lines) and emission (dashed lines, $\lambda_{\text{ex}} = 588 \text{ nm}$) spectra of (a) hypocrellin in ethanol, (b) hypericin in DMSO, and (c) mesonaphthobianthrone in H_2SO_4 .

formed with an apparatus described elsewhere.³⁴ The instrument response function had a full width at half maximum of 70 ps.

Pump-probe experiments with white-light continuum were performed with the apparatus described elsewhere.^{21,34} The time resolution and spectral resolution are 1–2 ps and $\pm 4 \text{ nm}$, respectively. Transient absorption spectra were obtained with a liquid-nitrogen-cooled charge-coupled device (CCD) (Princeton Instruments LN/CCD-1152UV) mounted on an HR 320 (Instruments SA, Inc.) monochromator with a grating (1200 grooves/mm) blazed at 5000 Å. The following protocol was employed. The CCD pixels were binned such as to allow simultaneous collection of both the probe and the reference beams, I and I_0 , respectively, of the transient absorption spectrometer. The signal was integrated for 5 s. Four 5 s acquisitions were averaged to give a single spectrum. Absorption spectra were constructed from $-\log(I/I_0)$. These spectra were corrected by subtraction of background spectra obtained by delaying the pump pulse to arrive after the probe and reference. The horizontal array of the CCD consists of 1152 pixels. In our optical arrangement, there were 19 pixels/nm, giving a full scale spectral range of 60 nm.

Transient spectra are presented in Figures 3 and 4. Although the two panels could, in principle, be displayed as one, covering the whole 120 nm range of our experiment, we chose not to splice the data together in this way for three reasons. First, such a presentation without the aid of colored graphics is very congested and makes it very difficult to follow a single trace through the entire region. Second, owing to slight difference in laser intensity and spatial overlap of pump and probe beams from experiment to experiment, it was not always possible to ensure an exact matchup at the blue edge of one experiment to the red edge of the other experiment that would be required in

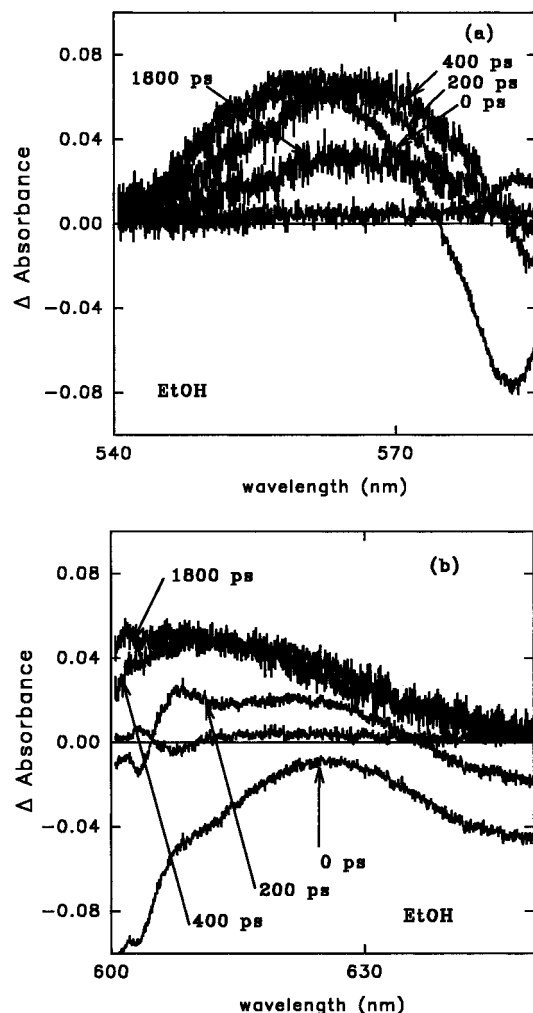


Figure 3. Time-resolved spectra of hypocrellin in ethanol. $\lambda_{\text{pump}} = 588$ nm.

order to splice the spectra together correctly. Last, the region around 588 nm is contaminated by leakage from the pump laser pulse. Distortions from the pump source are still detectable in the deviations of the flat negative time signal from zero (Figure 3).

Sample concentrations for hypocrellin were 4×10^{-5} M for steady-state and time-correlated single-photon-counting experiments and 1×10^{-3} M for transient absorption measurements. At this latter concentration, the absorption spectrum remains unchanged and thus indicates the absence of aggregation effects. Furthermore, transient absorption experiments performed at 5×10^{-4} M yield the same results.

For transient absorption measurements care was taken to prevent destruction of the sample and introduction of artifacts from high pulse powers. Typical peak pump intensities were 2.5 GW/cm^2 . At very high intensities, artifacts arise, perhaps from triplet-triplet annihilation, since hypocrellin has a substantial triplet yield.³⁵

Kinetic traces acquired at various wavelengths were fit using a global fitting procedure found in Spectra Solve. The global parameters in our case were time constants, and local parameters were amplitudes or preexponential factors. The time constants are specified and each curve is fit iteratively, varying only the local parameters, that is, the amplitudes. A local χ^2 is calculated for each local fit. After all curves are fit (typically four to eight), a global χ^2 is calculated as shown below. The global parameters are varied, and the whole process is repeated to calculate a new global χ^2 . This process is repeated until a minimum is reached

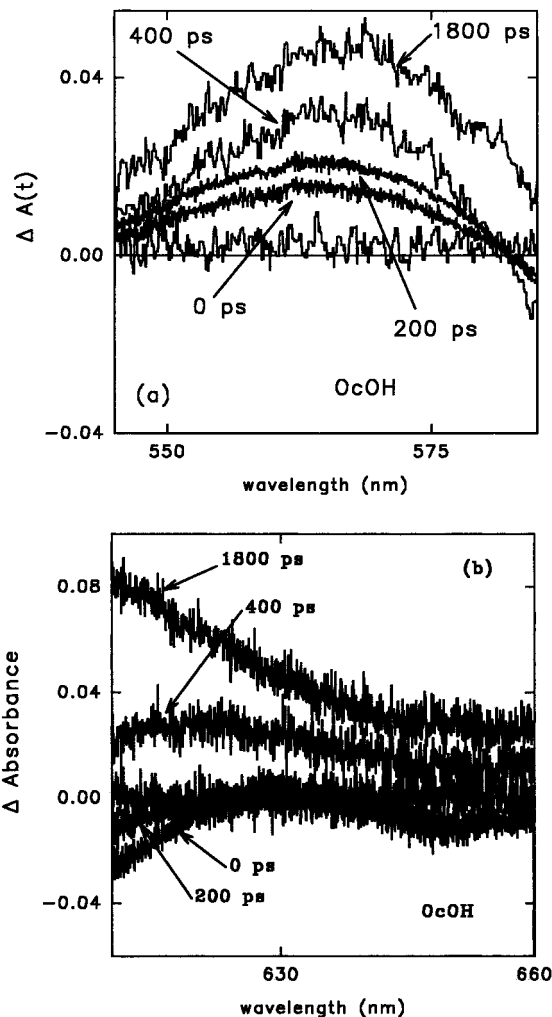


Figure 4. Time-resolved spectra of hypocrellin in octanol. $\lambda_{\text{pump}} = 588$ nm. In panel a the data obtained at negative time delay (the flat line at zero), 400 ps, and 1800 ps have been smoothed to enhance the presentation.

for χ^2 , which is defined as

$$\chi^2 = \frac{\sum_{i=1}^n \chi_{\text{local},i}^2}{n \chi_{\text{best},i}^2}$$

where $\chi_{\text{best},i}^2$ is obtained for each curve, letting all parameters local and global (that is, amplitudes and time constants) vary and n is the number of curves. The quality of the fit was determined by visual inspection of the fit and its residuals.

Four contributions are considered when fitting the pump-probe data. These are (1) a rising component in the induced absorbance, (2) an instantaneous component corresponding to appearance of signal within the pump pulse, (3) a long-lived component (assigned to a time constant of infinity for experiments on time scales up to 200 ps) corresponding to the triplet state, and (4) a decaying component in the stimulated emission. The time constants obtained in this manner are reproducible to within $\pm 10\%$.

The absorbance data are fit to the following form:

$$\Delta A(t) = a_1[1 - \exp(-t/\tau_1)] + a_2 \exp(-t/\tau_2) + a_3 \exp(-t/\infty)$$

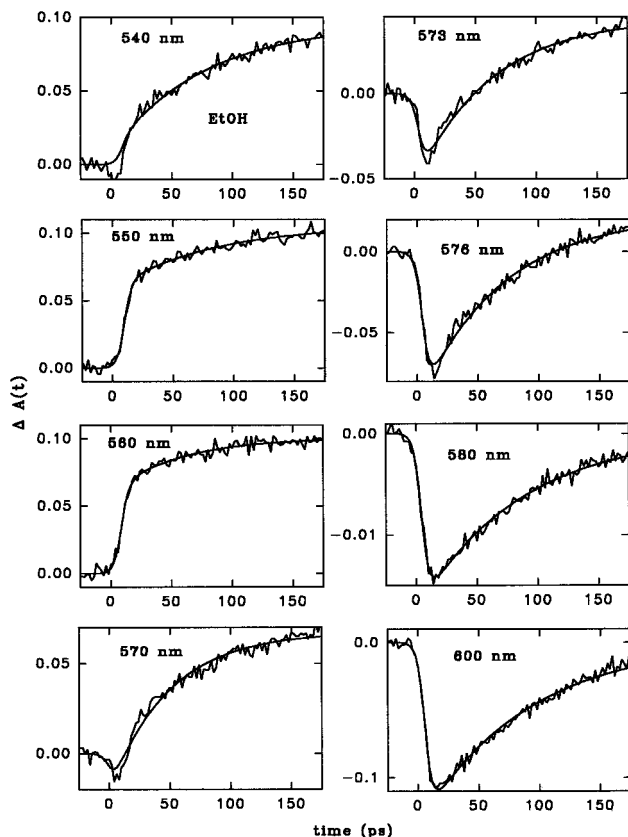


Figure 5. Kinetic traces of hypocrellin in ethanol at various probe wavelengths. $\lambda_{\text{pump}} = 588$ nm. The smooth lines passing through the data are the result of a global fit of the kinetic traces at all probe wavelengths.

The stimulated emission pump–probe data were fit to the form

$$\Delta A(t) = -a_1 \exp(-t/\tau_1) - a_2 \exp(-t/\infty)$$

Occasionally, as in Figure 5 at probe wavelengths of 573 and 576 nm, both absorption and stimulated emission need to be taken into account. It is also important to note that the interpretation of some of the kinetic traces is not unique. For example, we refer in the text to the trace in Figure 5 at a probe wavelength of 600 nm as a ~ 80 ps decay of stimulated emission. An equally acceptable interpretation is that the data reflect a fluorescent species that appears instantaneously and that does not decay on the time scale of the experiment and an absorbing species that rises in ~ 80 ps.

III. Results

Figure 2a presents the steady-state absorption and emission spectra of hypocrellin in ethanol. These spectra differ significantly from those of hypericin (Figure 2b), which exhibit a remarkable mirror image symmetry and whose emission has no Stokes shift. For comparison, we present the spectra of the analogue, mesonaphthobianthrone²¹ in H_2SO_4 (Figure 2c). In aprotic solvents this analogue has no visible absorption and no detectable emission. In strong acid, however, its spectra resemble those of hypericin and especially of hypocrellin.

Figures 3 and 4 present the time-resolved spectra of hypocrellin in ethanol and octanol, respectively. The salient features of the transient spectra are the following: (1) both stimulated emission and induced absorption are present at zero time; (2) the stimulated emission *decays* with time; (3) in addition to the component of transient absorption that is present at zero time, there is also transient absorption that *grows* in with time. Transients appearing on the picosecond and the nanosecond time

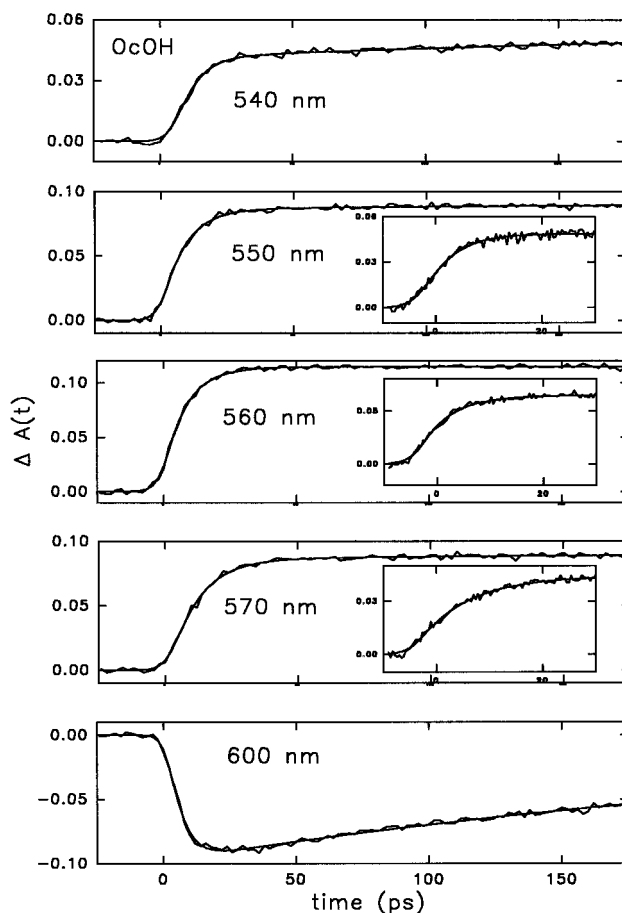


Figure 6. Kinetic traces of hypocrellin in octanol at various probe wavelengths. $\lambda_{\text{pump}} = 588$ nm. The smooth lines passing through the data are the result of a global fit of the kinetic traces at all probe wavelengths. Note that the traces presented as insets are on a 40 ps time scale.

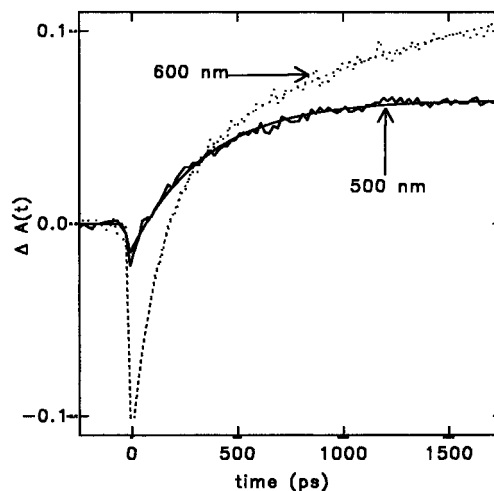


Figure 7. Kinetic traces on the nanosecond time scale of hypocrellin in ethanol at $\lambda_{\text{probe}} = 500$ nm and $\lambda_{\text{probe}} = 600$ nm; $\lambda_{\text{pump}} = 588$ nm.

scales are resolved. At some probe wavelengths, the transient absorption appears with a finite, measurable, rise time and does not decay on the time scale of the experiment (~ 2 ns).

The evolution of the above spectra and the relationship between the species giving rise to them can best be studied by monitoring the kinetics at individual probe wavelengths. These data are presented in Figures 5 and 7 for ethanol. Stimulated emission and transient absorption are observed.

Absorption transients are present at zero time and are also seen to grow in with time. The amount of transient absorbance appearing instantaneously depends on the probe wavelength.

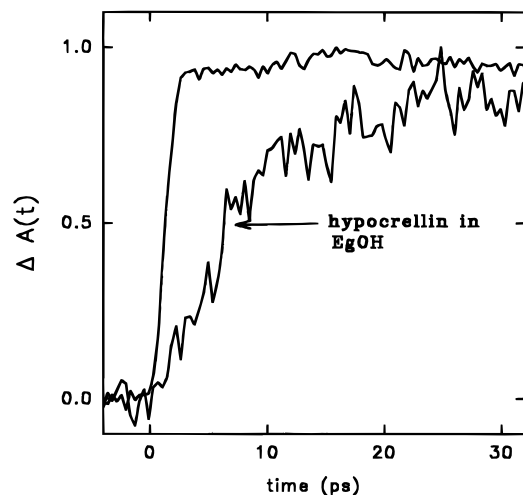


Figure 8. Transient absorption of hypocrellin in ethylene glycol. $\lambda_{\text{pump}} = 588$ nm. The ground-state bleach of the dye nile blue in ethanol is presented for comparison because it affords an instantaneous response. The rise time for the transient absorption of hypocrellin in ethylene glycol is ~ 10 ps.

Consequently, it may appear that the measured time constants depend on the probe wavelength. A global analysis of the data, however, indicates that the noninstantaneous rise time of the transient at all probe wavelengths matches, within experimental error, the decay time of the stimulated emission. This time constant is 85 ps in ethanol and 210 ps in octanol. Because of the presence of multiple components of the transient absorption and their dependence on probe wavelength, it can be misleading to use a single transient absorption trace as a measure of the intramolecular proton transfer time (see below) unless care is taken in the data analysis. The decay of stimulated emission provides a much more direct and uncomplicated measure of the proton transfer time.

In octanol, both stimulated emission and transient absorption are also observed (Figure 6). For ethanol, both an instantaneous and a slow component of transient absorption are observed. In addition to these, however, is the appearance of transient absorption that grows in on an intermediate time scale, i.e., with a time constant of ~ 10 ps in octanol. A 10 ps component is also observed for hypocrellin in the even more viscous solvent, ethylene glycol (Figure 8).

IV. Discussion

Excited-State Processes: Intramolecular Proton Transfer, Intersystem Crossing, and Conformational Relaxation. *A. Proton Transfer.* We assign the decay of stimulated emission and the concomitant rise in induced absorbance to excited-state intramolecular proton transfer. This assignment is based on the huge precedent established in other aromatic molecules,^{27–31} including hypericin.^{20–22} It is supported by the kinetic relatedness of the stimulated emission to the induced absorbance and by the observation, discussed immediately below, that the decay time of the stimulated emission is comparable to the fluorescence decay of hypocrellin in H_2SO_4 .

Hypocrellin in H_2SO_4 is weakly fluorescent. As is indicated in Figure 9, in H_2SO_4 its fluorescence decay comprises a 200 ps component that represents $\sim 60\%$ of the intensity. The fluorescence quantum yield of hypocrellin in H_2SO_4 is 0.02. The fluorescence decay of hypocrellin in all the other solvents we investigated is a single exponential and ranges from 1.3 to 1.6 ns. The fluorescence quantum yield in, for example, benzene³⁵ is 0.14.

We are unable to observe a rapid component in the fluorescence decay of hypocrellin in any nonacidic solvent. That we

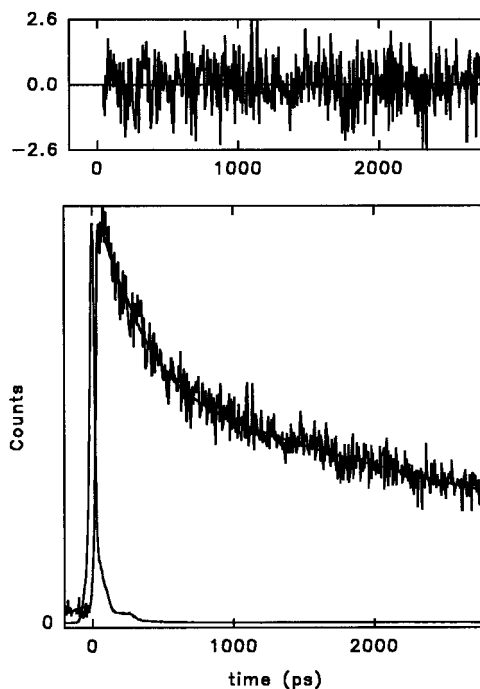


Figure 9. Fluorescence decay of hypocrellin in H_2SO_4 . A fit to a double exponential yields $F(t) = 0.54 \exp(-t/230 \text{ ps}) + 0.46 \exp(-t/4200 \text{ ps})$, $\chi^2 = 0.94$.

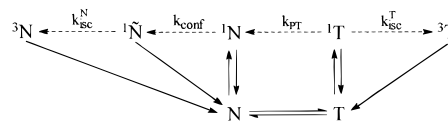


Figure 10. Kinetic scheme for the excited states of hypocrellin. k_{PT} is the back proton transfer rate; k_{conf} is the rate of conformational rearrangement⁴¹ suggested to be observed in octanol and ethylene glycol between states denoted as N and $\tilde{\text{N}}$. The intersystem crossing rates of the excited-state normal and tautomer forms are given by $k_{\text{isc}}^{\text{N}}$ and $k_{\text{isc}}^{\text{T}}$, respectively.

cannot observe, for example, an 85 ps component in the fluorescence decay of hypocrellin in ethanol (fluorescence lifetime of 1.3 ns) is surprising given the appearance of the 85 ps component in the stimulated emission (Figure 5). The point is discussed below and in the Conclusions section. We rationalize this result by postulating that hypocrellin exists in the ground state in a tautomerized form (parts b or c of Figure 1) and that this species is as weakly fluorescent as hypocrellin in H_2SO_4 . This is supported by the published X-ray structure,³⁶ which presents hypocrellin in the tautomerized form of Figure 1c.

In the excited state, this tautomeric species undergoes a “back transfer” reaction to produce the normal form (Figure 1a). This reaction occurs in 85 ps in ethanol (Figure 5). We propose that it is the normal form of hypocrellin that is strongly fluorescent and that gives rise to the ~ 1.5 ns fluorescence decay referred to above. We further propose that the fluorescence spectrum of the normal form overlaps with that of the tautomer substantially. Owing to this spectral overlap, as the normal form is produced, the rise in its emission roughly cancels out the decay of the emission of the tautomer, at least within the spectral resolution of our experiment (16 nm). See Figure 10 for more details.

B. Intersystem Crossing. Above, we have discussed the absorbance transients in terms of the appearance of a back transfer reaction occurring on a time scale of 80–200 ps to produce the normal form of the hypocrellin molecule. Examination of the transient spectra and of the kinetic traces indicates that very long-lived species are also produced. We assign these

transients to the production of triplet species. The triplet yield of hypocrellin in benzene is 0.83.³⁵

The generation of the triplet is most clearly manifested in Figures 3 and 4 and in Figure 7 where the transient absorbance appears on a nanosecond time scale at 600 nm. The time scale for these rise times is in very good agreement with the fluorescence decay time of the singlet state of hypocrellin. (Note that we have identified the long-lived fluorescent state of hypocrellin with the untautomerized or normal species, Figure 1a. See above.)

Of additional interest is the transient in ethanol in the region from 500 to 570 nm. This transient appears with both an instantaneous and a 85 ps component (Figures 5). *But examination on a 2 ns time scale (Figure 7) indicates that it does not decay.* Clearly, a contribution to the transient absorption at this wavelength must also be due to triplet–triplet absorbance. We propose that the triplet species whose formation is revealed in these kinetics arises from the decay of the hypocrellin tautomer (parts b or c of Figure 1). See Figure 10 for a detailed kinetic scheme.

We are obliged to conclude that the transient absorbance in the region of 570 nm for hypocrellin in ethanol arises from (1) singlet–singlet absorption of the normal form of hypocrellin that is produced immediately upon optical excitation, (2) the singlet–singlet absorption of the normal form of hypocrellin that is formed by excited-state back proton transfer, (3) the absorption of triplets produced from the tautomerized and the untautomerized species.

Similar assignments of the absorbing transients are made for hypocrellin in octanol.

C. Conformational Relaxation. In ethanol the subnanosecond transients at all probe wavelengths can be described by a global analysis employing instantaneous and ~ 80 ps molecular responses. The kinetics for hypocrellin in octanol are more complicated in that the absorption kinetics reveal a 10 ps component (Figure 6) in addition to the longer 210 ps component, which corresponds to the decay of stimulated emission (and consequently to the proton transfer time).

We can interpret this 10 ps component as a slow conformational change⁴¹ of the excited-state normal form (Figure 1a) of hypocrellin that is produced upon optical excitation. This component is not observed in solvents of low viscosity, but it is observed in high-viscosity solvents such as ethylene glycol (Figure 8).

The X-ray structure of hypocrellin indicates that it is not a planar molecule.³⁶ (Similar conclusions can be drawn from semiempirical calculations or, more simply, from molecular models. Hypericin is also a twisted molecule.³⁷) The large side chains protruding from the perylene quinone skeleton repel each other and prevent the molecule from acquiring a planar conformation. We propose that upon optical excitation hypocrellin converts from one twisted conformation to another. This process is too rapid to be observed with our time resolution in ethanol but is proposed to occur in 10 ps in octanol. That a time constant of ~ 10 ps is also observed in the much more viscous solvent, ethylene glycol, suggests that this process is already saturated with respect to viscosity in this solvent.

V. Conclusions

On the basis of the above results, we present the following kinetic scheme for hypocrellin (Figure 10). Both untautomerized (N) and tautomerized hypocrellin exist in the ground state. They are excited in roughly equal proportions. The 80–200 ps decays of stimulated emission correspond to an excited-state back proton transfer to form the “untautomerized” or “normal” species. Absorption transients are seen to appear on a nano-

second time scale (Figures 3, 4, and 7). They are also seen to appear in ≤ 200 ps and not to decay on a nanosecond time scale. These transients are attributed to triplet–triplet absorption. The former transients are formed from the fluorescent species of hypocrellin, the untautomerized form N, that has a ~ 1.3 ns lifetime; the latter transients are formed from the excited-state tautomer of hypocrellin, which is characterized by an 85 ps decay in ethanol and a 210 ps decay in octanol. Finally, a component of ~ 10 ps is resolved in the rise time of the transient absorbance in octanol and ethylene glycol. This transient is tentatively attributed to a conformational change between twisted forms of the aromatic ring that occurs in the excited state. These results and conclusions provide an interesting point of comparison with those for the antiviral agent, hypericin (Figure 1d).^{21,22}

As noted in the Introduction and as can be seen from Figure 2, hypericin has a mirror image symmetry between its absorption and emission spectra. Elsewhere, we rationalized this mirror image relationship in terms of the presence of ground-state tautomerized hypericin.^{21,22} Hypocrellin, on the other hand, does not possess such a mirror image relationship. The time-resolved data for hypocrellin are very suggestive of ground-state heterogeneity. For example, on a subnanosecond time scale, the transient absorbance appears with two time constants. There is a component that appears instantaneously, that is, within the excitation pulse width. And there is another component with time constants of 85 and 210 ps in ethanol and octanol, respectively.

In the context of what is known concerning hypericin and hypocrellin, the analogue mesonaphthobianthrone (Figures 1e and 2c) is of considerable interest. In aprotic solvents such as DMSO, it has no visible absorbance and no detectable fluorescence. In H_2SO_4 , however, it acquires absorption and emission spectra in the visible. In large part because of this observation, indicating that protonated carbonyl groups are responsible for the fluorescent species, *rise times* in the stimulated emission of hypericin were attributed to intramolecular proton transfer in this molecule.

Although the two-dimensional aromatic skeleton of hypericin is essentially identical with that of mesonaphthobianthrone, the absorption and emission spectra of the latter in H_2SO_4 are much more similar to those of hypocrellin than of hypericin. It is not clear how to interpret this similarity. Also, although hypericin is strongly fluorescent in H_2SO_4 ,^{21,22} hypocrellin is not (Figure 9).

Because protonated hypocrellin is weakly fluorescent, the 80–200 ps transients cited here are attributed to *back intramolecular proton transfer*, which forms the longer-lived nanosecond fluorescent species.

The stimulated emission observed in hypericin is more complicated than that in hypocrellin. This is not surprising, since there are twice as many hydroxyl hydrogens that may transfer to the carbonyl oxygen. In hypericin, the time of stimulated emission, attributed to intramolecular proton transfer, is essentially independent of solvent and occurs in 6–12 ps. There is also a 1–2-ps *decay* of stimulated emission, whose origin is less certain.^{21,22}

That the proton transfer time in hypericin is almost negligibly dependent on solvent is of considerable interest. For example, in one of the best studied excited-state proton transfer systems, 3-hydroxyflavone,²⁹ the transfer time is on the order of 100 fs in aprotic solvents. In a hydrogen-bonding solvent such as an alcohol, however, the proton transfer time is determined by hydrogen-bonding interactions between the solvent and solute. In other words, in a hydrogen-bonding solvent, the proton transfer reaction is strongly coupled to the solvent coordinate.

The independence of solvent on the proton transfer rate in hypericin suggests that the reaction is coupled to intramolecular conformational changes. The proton transfer is very likely coupled to intramolecular conformational changes in hypocrellin as well, but at this point, it is unclear why in ethanol and octanol they are almost an order of magnitude longer than those in hypericin.

In conclusion, the superficial structural similarities between hypericin and hypocrellin are belied by their different modes of antiviral activity (for example, hypericin does not require oxygen and hypocrellin does not acidify a solution in the presence of light) and by their different behaviors in the excited state. Further clarification of these excited-state photophysics will be of importance not only to the development of light-induced antiviral and antitumor therapies but also to the development of molecules that provide light-induced pH drops. Most importantly, understanding these differences will prove to be fundamental to an understanding of excited-state intra- and intermolecular proton transfer reactions.

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- (41) It is also possible that the 10 ps component represents an excited-state proton transfer from the normal species to the tautomer species, a “back-back transfer” in the context of this discussion. This argument is plausible, since 10 ps is the time scale of the proton transfer event in hypericin.^{21,22}

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