Monophotonic Ionization of 7-Azaindole, Indole, and Their Derivatives and the Role of Overlapping Excited States

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Abstract
7-Azaindole undergoes monophotonic ionization just as its counterpart, indole. This result suggests that 7-azaindole is qualitatively more similar to indole than has previously been recognized. The appearance of the solvated electron for zwitterionic and anionic 7-azatryptophan and for 7-azaindole in water and methanol is complete within 1 ps, which indicates that the fluorescent state whose lifetime is >100 ps cannot be the source of the electron. The origin of the electron is related to the presence of closely spaced or overlapping excited states in 7-azaindole, which is another similarity that this chromophore bears with respect to indole. The fluorescence quantum yield of 7-azaindole is shown to be excitation wavelength dependent. The excitation-wavelength dependence and the temperature dependence of the fluorescence quantum yield of 7-azaindole are explored and related to the production of the solvated electron. The implications of these observations for the use of 7-azatryptophan as an alternative to tryptophan as a probe of protein structure and dynamics are discussed.

Keywords
derivatives, electronic properties, fluorescence, ionization, molecular structure, proteins, azaindoles, indole, monophotonic ionization, overlapping exited states, organic compounds

Disciplines
Chemistry

Comments
Monophotonic Ionization of 7-Azaindole, Indole, and Their Derivatives and the Role of Overlapping Excited States

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Abstract: 7-Azaindole undergoes monophotonic ionization just as its counterpart, indole. This result suggests that 7-azaindole is qualitatively more similar to indole than has previously been recognized. The appearance of the solvated electron for zwitterionic and anionic 7-azatryptophan and for 7-azaindole in water and methanol is complete within 1 ps, which indicates that the fluorescent state whose lifetime is >100 ps cannot be the source of the electron. The origin of the electron is related to the presence of closely spaced or overlapping excited states in 7-azaindole, which is another similarity that this chromophore bears with respect to indole. The fluorescence quantum yield of 7-azaindole is shown to be excitation wavelength dependent. The excitation-wavelength dependence and the temperature dependence of the fluorescence quantum yield of 7-azaindole are explored and related to the production of the solvated electron. The implications of these observations for the use of 7-azatryptophan as an alternative to tryptophan as a probe of protein structure and dynamics are discussed.

Introduction

7-Azaindole is the chromophoric moiety of the nonnatural amino acid, 7-azatryptophan, which we have proposed as an alternative to tryptophan as an optical probe of protein structure and dynamics1-4 (Figure 1). One observation that renders 7-azatryptophan preferable to tryptophan as an optical probe is that its fluorescence lifetime over most of the pH range is single exponential when emission is collected over the entire band.2,5 On the other hand, the fluorescence lifetime of tryptophan at neutral pH is nonexponential.6 Characterization of the 7-azaindole chromophore as an optical probe requires an understanding of its nonradiative pathways of deactivation.

We have reported the monophotonic production of solvated electrons in 7-azaindole.5 This observation is particularly intriguing because it indicates that its photophysics is more similar to those of indole, which also undergoes monophotonic ionization,7 than had previously been recognized. In fact, it is the ability of the indole moiety of tryptophan to undergo excited-state charge transfer to side-chain acceptors at various separation distances that has been suggested as the explanation for the nonexponential fluorescence decay in tryptophan.6 A fundamental question, then, is if photoionization is also a significant nonradiative process in 7-azatryptophan, why is its fluorescence decay a single exponential of 780 ps?1,2 In order to begin to address this question, in this article we discuss the monophotonic ionization of the 7-azaindole chromophore in detail, and we relate this ionization to the excited-state manifold of 7-azaindole.

The arguments and conclusions that will be presented here depend on several diverse observations of the photophysics of indole and 7-azaindole. We summarize them here.

1. Indole in water and methanol, 7-azaindole in water and methanol, 5-methoxynindole in water (methanol was not used as a solvent), and their analogs (investigated mostly in water) photionize monophotonically and instantaneously to produce the solvated electron.5,7,8 Unless otherwise indicated, the results reported here are obtained using water as the solvent. Because the fluorescent state of these analogs is always characterized by an average lifetime of at least hundreds of picoseconds, the solvated electron cannot originate from the lowest excited singlet.

2. Fluorescence-excitation anisotropy spectra of 7-azaindole in propylene glycol glasses indicate, as for indole,1,9 the presence of closely spaced 1La and 1Lb electronic states in 7-azaindole whose transition dipole moments are at large angles to each other.3

3. We demonstrate here that the fluorescence quantum yields, φF, of indole, 7-azaindole, and 5-methoxynindole, are all strongly dependent on excitation wavelength.

Figure 1. Structures of indole, 7-azaindole, zwitterionic tryptophan, zwitterionic 7-azatryptophan, 5-methoxynindole.
4. The excitation-wavelength dependence of $\Phi_F$ is used to assign the $1L_s$ state to the photoionizable channel in these compounds.

5. In zwitterionic tryptophan, the presence of the $-\text{NH}_3^+$ and $-\text{CO}_2^-$ side-chain groups brings considerable complexity to the indolyl photophysics. In particular, nonexponential fluorescence decay is observed. These side chains are not, however, a source of nonexponential fluorescence decay in zwitterionic 7-azatryptophan.\(^2\)

We argue that these data can be synthesized and comprehended by the following working hypotheses.

1. The $1L_s$ state is photoionizable, and the $1L_s$ state is not.
2. The $1L_s$ state is coupled to charge-transfer states, which are the source of the nonexponential fluorescence decay in tryptophyl derivatives.
3. Wave packet evolution away from the initial Franck-Condon region provides a means of rationalizing these data.

With this summary in mind, we shall now present and discuss our results in more detail.

### Experimental Section

7-Azaindole (7AI) (Sigma) was purified by flash chromatography using ethyl acetate as a solvent. Indole, tryptophan (Trp), 7,8-dihydroxytryptophan (Sigma), 5-methoxyindole (5MeOOak (Aldrich), stilbene (Sigma), rhodamine B (Eastman Kodak Co.), $p$-terphenyl (Sigma), and quinine sulfate monohydrate (Aldrich) were used without further purification. N-(methyl-7-azaindole)(1M7AI) and 7-methyl-5H-pyrido[2,3-$d$]pyridine (1M7AI) were prepared as described elsewhere.\(^3\)

For steady-state anisotropy determinations, all compounds were dissolved in propylene glycol, and experiments were performed as discussed elsewhere.\(^1\)

#### Table 1. Fluorescence Quantum Yields

<table>
<thead>
<tr>
<th>Compound</th>
<th>$\lambda_{\text{exc}}$ (nm)</th>
<th>$\Phi_F$ (pm)</th>
<th>$\Phi_F$ (literature)</th>
</tr>
</thead>
<tbody>
<tr>
<td>indole</td>
<td>270</td>
<td>0.45 ± 0.05</td>
<td>0.45 [13,20, 0.27 [21], 0.23 [18]</td>
</tr>
<tr>
<td>tryptophan</td>
<td>278</td>
<td>0.18 ± 0.01</td>
<td>0.13 [14], 0.12 [15], 0.2 [16], 0.14 [17,18], 0.06 [19]</td>
</tr>
<tr>
<td>7-azaindole</td>
<td>289</td>
<td>0.056 ± 0.007</td>
<td>0.03 [20]</td>
</tr>
<tr>
<td>5-methoxyindole</td>
<td>270</td>
<td>0.43 ± 0.02</td>
<td>0.22 [21]</td>
</tr>
</tbody>
</table>

* Data reported from our laboratory were obtained at 24.5 ± 0.5 °C. \(^b\) The fluorescence quantum yield measurements were performed by varying the excitation wavelength in increments of 5 nm. The excitation wavelength that yields the maximum fluorescence quantum yield is therefore only approximate. \(^c\) These quantum yield values were obtained over a range of 23–27 °C or simply at “room temperature.” We assume that most of these measurements were made at the absorbance maxima since excitation wavelengths were not given in the references. For purposes of comparison, we note that at the absorbance maxima of indole (270 nm) and of 7-azaindole (290 nm), we obtain fluorescence quantum yields of 0.45 ± 0.05 and 0.039 ± 0.002, respectively. \(^d\) The wavelength dependence of the fluorescence quantum yield of zwitterionic tryptophan was not investigated for this work. The value we report here was determined at 280 nm.

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and fit very well to a straight line. We obtain the solvated electron at 650 nm in units of New York, 1970. Data from this text for the extinction coefficient of the Thistelthwaite, P. J.; Woolfe, G. J. J. Spectroscopy be even smaller. The differences are small but reproducible. It is important to recognize that the magnitude of the difference between a fluorescence excitation 5-methoxyindole in water all differ from their respective absorption spectra. Significant. Similarly, the contribution due to absorption by triplets should higher energies. If we take the quantum yield of photoionization as 0.2, then the maximum one 2.5. The amplified laser pulse width is deconvoluted from the molecular response and is best described by a double-sided exponential of 0.8 ps full width at half-maximum.

(1.538–1.329), and since the emission spectra of the indoles do not change with excitation wavelength, no correction for the index of refraction was required in comparing the fluorescence quantum yield at one excitation wavelength with respect to another. We investigated the possibility that the variations in the plots of vs resulted from absorption by excited-state transients such as solvated electrons or triplets. For example, the extinction coefficient for a solvated electron is about 1000 M⁻¹ cm⁻¹ at 400 nm (and decreases toward higher energies). If we take the extreme case and assume that all of the ground-state population is projected into the excited state and if we take the quantum yield of photoionization as 0.2, then the maximum correction for absorption by electrons is less than 1%. Under normal conditions of excitation, the absorption by solvated electrons is insignificant. Similarly, the contribution due to absorption by triplets should be even smaller.

The fluorescence excitation spectra of indole, 7-azaindole, and 5-methoxyindole in water all differ from their respective absorption spectra. The differences are largest in 5-methoxyindole. In indole and 7-azaindole, the differences are small but reproducible. It is important to recognize that the magnitude of the difference between a fluorescence excitation spectrum and an absorption spectrum can be greatly decreased because in order to compare the two they must be normalized. (A change between the two spectra will also be underestimated—or annulled—by normalization if it occurs in a region where the spectra change rapidly with wavelength, as occurs on the red edges of the indole spectra.) This normalization usually takes the form of setting the value of the optical density and the fluorescence intensity to be arbitrarily equal at a given wavelength.

As a final indication of self-consistency, we note that curves similar to the ones displayed in Figure 5a–c can be constructed directly from the excitation spectra. If the emission spectral shape does not change with respect to excitation wavelength (we do not observe such a change), then the integrated area of the emission (the fluorescence quantum yield) is proportional to the emission intensity. When the fluorescence intensity at a given emission wavelength is monitored in order to obtain an excitation spectrum, the quantum yield over the range of excitation wavelengths is essentially what is observed if for each fluorescence intensity measured an accurate correction is made for the sample absorbance. Such a correction is similar to that used to obtain standard quantum yields, except that no factor to account for the fluorimeter lamp intensity is included since this has already been accounted for when obtaining the excitation spectrum itself. Of course, if the absorption and excitation spectra are exactly superimposable, this procedure yields a plot of the quantum yield that is independent of the excitation wavelength.

Fluorescence lifetime measurements were performed with the time-correlated single-photon counting apparatus described elsewhere. In the course of this article, we make a distinction between anisotropies obtained from two types of experiments: is the anisotropy obtained from steady-state, low-temperature measurements; is the limiting anisotropy obtained from time-dependent measurements in the liquid phase.

Pump–probe transient absorption measurements were performed with a system based on an Antares 76s cw–modelocked Nd:YAG synchronously pumping a Coherent 701-2 dye laser with 1 W of 532-nm radiation (Figure 2). The output of the dye laser was amplified to 1–2 mJ at 30 Hz with a Continuum regenerative amplifier that is seeded with residual 1064-nm radiation from the Antares. Half of the amplified dye-laser pulse train is focused into a cell containing water to form a white light continuum that is used as a probe beam. The remainder of the pulse train is focused into a crystal of KDP to obtain the appropriate ultraviolet wavelength for excitation.

The pulsewidth of the amplified pulse train is determined by measuring the apparent rise time of the induced transmission or absorption of a standard (Figure 2). In the pump-probe experiments discussed here, the typical full scale is 800 ps. It is thus crucial that the translation stage (optical delay line) is adequately aligned so as to avoid drift in the overlap of the pump and probe beams during the course of the experiment.
...where does not occur or 7-azatryptophan (Figure 3). Geminate recombination of the electron
tryptophan at pH 0.25 M absorbance of the solvated electron produced from zwitterionic tryptophan
pH 12.3; using an excitation wavelength of 304 nm. Identical results are obtained with
identical to the value of the single-exponential decay time we obtain for
absorption change in the absence of scavenger is 0.16; in the presence of
(c) Zwitterionic 7-azatryptophan, pH 6.8, and anionic 7-azatryptophan,
Figure 3. Transient absorption arising from the solvated electron at 23
° C. (a) Zwitterionic tryptophan, pH 6.8; λ_{ex} = 304 nm, λ_{probe} = 650 nm. The
decaying signal is obtained in the presence of 0.25 M KNO3. The upper trace is flat on the time scale of the measurement. The maximum absorption change in the absence of scavenger is 0.16; in the presence of 0.25 M KNO3, it is 0.05. The two traces are normalized to have the same maximum absorption change. Similar behavior is observed for 7A1, 1M7A1, and 7M7A1. (b) Tryptophan, pH 12.3; λ_{ex} = 294 nm, λ_{probe} = 580 nm. The data are fit to 10% of a rising component of duration 3.2 ns. Identical results are obtained with λ_{ex} = 304 nm. This rise time is identical to the value of the single-exponential decay time we obtain for tryptophan at pH 12.3 by means of time-correlated single-photon counting. (c) Zwitterionic 7-azaindole, pH 6.8, and anionic 7-azatryptophan, pH 12.3; λ_{ex} = 294 nm, λ_{probe} = 580 nm. Identical results are obtained using an excitation wavelength of 304 nm.

The quantum yield of photoionization was determined at various excitation wavelengths using the relation

$$\Delta A_e = \epsilon_e I \phi_e \tau_{photo} = \rho \epsilon_e \phi_e I_{pdf} (1 - 10^{-\phi_e})$$

where \( \epsilon_e \) is the extinction coefficient of the solvated electron at the probe

Table 2. Solvated Electron Yield (\( \phi_e \)) vs Excitation Wavelength (\( \lambda_{exc} \))

<table>
<thead>
<tr>
<th>compound</th>
<th>excitation wavelength (nm)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>294</td>
</tr>
<tr>
<td>Trp (pH 6.5)</td>
<td>0.14 ± 0.05</td>
</tr>
<tr>
<td>7A1 (pH 6.9)</td>
<td>0.08 ± 0.03</td>
</tr>
<tr>
<td>5MeOTrp (pH 7.3)</td>
<td>0.23 ± 0.04</td>
</tr>
</tbody>
</table>

* The probe wavelength for all measurements is 665 nm. At this wavelength the extinction coefficient for the reference compound, nile blue in ethanol, is approximately equal to that of the solvated electron: \( 1.8 \times 10^6 \text{ M}^{-1} \text{ cm}^{-1} \). All measurements were performed at 23 °C. The absence of a value indicates that the measurement was not performed.

The quantum yield of the solvated electron is demonstrated by the transient quenching in the presence of 0.25 M KNO3.

The slope is 1.2 ± 0.3 indicating a monophotonic ionization.

Table 2 indicates that the quantum yield of the solvated electron is excitation-wavelength dependent for tryptophan. For 7-azaindole and 5-methoxyindole, because the electron yield is less than that in tryptophan and because of experimental error, the excitation-wavelength dependence is obscured. The data for tryptophan indicate that the electron yield is higher toward the red edge of the absorption spectrum and are thus consistent with the diminished fluorescence quantum yield at those wavelengths (Figure 5). The yields for the solvated electron production for a variety of compounds, relative to that for tryptophan, are listed in Table 3.
Ionization of 7-Azaindole, Indole, and Their Derivatives

Table 3. Quantum Yield of Solvated Electron*  

<table>
<thead>
<tr>
<th>compound</th>
<th>$\phi_e$</th>
<th>compound</th>
<th>$\phi_e$</th>
</tr>
</thead>
<tbody>
<tr>
<td>tryptophan, pH 6.8</td>
<td>1.00</td>
<td>7MAI (N$_2^+$), pH 12.3</td>
<td>0.30</td>
</tr>
<tr>
<td>indole, pH 6.4</td>
<td>0.86</td>
<td>7MAI (N$_2^+$), pH 8.8</td>
<td>0.16</td>
</tr>
<tr>
<td>7AI (N$_2^+$, N$_2^+$), pH 6.3</td>
<td>0.35</td>
<td>7MAI (NH$_2^+$), pH 3.3</td>
<td>0.14</td>
</tr>
<tr>
<td>7AT (N$_2^+$, N$_2^+$), pH 6.7</td>
<td>0.28</td>
<td>7A (N$_2^+$)</td>
<td>0.68</td>
</tr>
<tr>
<td>7AT (N$_2^+$, N$_2^+$), pH 2.1</td>
<td>0.12</td>
<td>7A (N$_2^+$)</td>
<td>0.17</td>
</tr>
</tbody>
</table>

* All yields are reported relative to that of zwitterionic tryptophan at 23°C. $\phi_{el}$ = 0.04, $\Delta_{el}$ = 0.36, 0.45.  

The motivation for measuring ionization yields for 7-azaindole in methanol was to determine whether the solvated electron originates from the normal or the tautomer form of 7-azaindole. If it originates from the tautomer form, the rise time for the solvated electron would be expected to be equal to the formation time for the tautomer, 140 ps. Such a rise time for the solvated electron is not observed.

B. Excited States. Low-temperature, steady-state fluorescence excitation anisotropy studies of 7-azaindole in propylene glycol indicate that, like indole, its absorption spectrum is composed of two overlapping electronic transitions whose transition dipole moments are at large angles to each other. The fluorescence excitation anisotropy spectrum and the decomposed fluorescence excitation spectra of 7-azaindole are displayed in Figure 6. The measurements of Bulska et al. suggested the presence of $L_1$ and $L_2$ bands in 7-azaindole.

Valeur and Weber resolved the fluorescence excitation spectrum of indole into overlapping $L_1$ and $L_2$ bands at $\pm 58^\circ \text{C}$ in propylene glycol and reported the $L_1$ transition to be quite structured with maxima at 282.5 and 289.5 nm. The $L_2$ transition is broader and absorbs farther to the red than does the $L_1$ transition. At wavelengths longer than 295 nm, only absorption resulting from the $L_2$ transition is observed. The excitation anisotropy of 7-azaindole is qualitatively similar to that of indole, but it possesses less pronounced structure. Anisotropy minima at 293.5 and 300.5 nm and a relative area at 297.0 nm give rise to the structure in the resolved excitation spectra.

Neither 7-azaindole, nor indole, nor 5-methoxyindole yield a limiting steady-state anisotropy, $\phi_e$, of 0.4 at any excitation wavelength. The control experiment, stilbene, produced $\phi_e = 0.4$ at $\Delta_{el} = 336$ nm. Our results for stilbene are qualitatively similar to those of Bobrovich et al., who measured the excitation polarization spectrum of stilbene in butanol at 77 K.

The excitation-wavelength dependence of the photoionization process indicates that conventional methods of decomposing fluorescence excitation spectra of indole-like molecules into $L_1$ and $L_2$ absorption spectra based on measurements of polarized emission are inappropriate. A fluorescence excitation spectrum can only be considered to represent the absorption spectrum if $\phi_e$ is independent of the excitation wavelength.

C. Excitation Wavelength Dependence of the Fluorescence Quantum Yield. If the instantaneous appearance of the electron has its origin in the upper of the two electronic states illustrated in Figure 6b, then the fluorescence quantum yield of 7-azaindole is expected to be excitation-wavelength dependent. The data presented in Figure 5 indicate that this is the case for 7-azaindole, indole, and 5-methoxyindole. The control experiment was performed for rhodamine B in ethylene glycol, for which no excitation-wavelength dependence on the fluorescence quantum yield was observed. The observation of an excitation-wavelength dependent fluorescence quantum yield is of significance because it is consistent with the excitation-wavelength dependence of the electron yield. These two observations argue cogently, along with the log–log plot (Figure 4), for the monophotonic ionization of 7-azaindole, indole, and 5-methoxyindole, and their derivatives. The photoionization of indoles has been studied extensively. The measurements of Steen and Pigault suggest an excitation-wavelength dependence of the electron yield, but their data are not precise enough to demonstrate that this dependence exists at wavelengths greater than 250 nm.

![Figure 4. Plot of the logarithm of $\Delta_{el}$ against the logarithm of the pump intensity for 7-azaindole. The plot yields a straight line with a slope of 1.2 ± 0.3, indicating a monophotonic ionization.](image)

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Discussion

A. Temperature and Excitation-Wavelength Dependence of the Fluorescence Quantum Yield. The low-temperature fluorescence anisotropy data indicate the presence of closely spaced excited states in indole, 7-azaindole, and their derivatives. The instantaneous appearance of the solvated electron indicates that at least one of the excited states is dissociative. The fluorescence quantum yields of the compounds investigated here are very low if at least not at—the absorption maximum, and subsequently decrease toward the blue edge of the absorption spectrum. This is in contrast to the fluorescence lifetime, which we observe to be constant over the range of wavelengths accessible to our laser system (285–310 nm). For example, our results for 7-azaindole indicate that the electron is generated monophotonically and instantaneously. Time scale of ≤130 fs is required for the electron to become “solvated”. The excitation-wavelength dependent fluorescence quantum yield, $\phi_F(\lambda)$, is correspondingly diminished by the instantaneous production of electrons. This is especially clear in the case of indole and tryptophan (Figure 5 and Table 2).

The wavelength dependence of $\phi_F$ and $\phi_e$ led us to investigate the role of both temperature and excitation wavelength on $\phi_F$ and $\phi_e$. These measurements were performed to address the following questions. Assuming that the variation in $\phi_F$ arises only from changes in the yield of solvated electrons, by performing measurements of the fluorescence quantum yield as a function of both excitation wavelength and of temperature, can we obtain any information concerning the barrier—if there is one—to photoionization? Alternatively, can we obtain any information concerning the interaction of bound and dissociative excited-state surfaces?

Our experiences were based on the suggestions in the literature that photoionization is a thermally activated process. It is possible (as opposed to the case illustrated in Figure 1a) that one dissociative surface intersects the bound state near its minimum (no barrier); and the other, at a higher energy (large barrier). Alternatively, because the Franck–Condon factors for predissociation are favorably only for the vibrational levels of the bound state that coincide with the crossing of the dissociative state, the rate of photoionization increases at both low and high excitation energies (Figure 1a). The former scheme predicts that excitation to vibrational levels lying between the two crossing points will yield solvated electrons with different activation energies depending on the energy separation between the initially excited vibrational level and the upper crossing point.

**The data compiled in Table 4 indicate, however, that the Arrhenius parameters are essentially independent of excitation wavelength**—although some fluctuations are observed for indole. The treatment of these temperature and excitation-wavelength dependent data is as follows. Because the solvated electron is produced on a time scale that is instantaneously compared to steady-state or conventional time-correlated single-photon counting experiments, the apparent fluorescence quantum yield obtained in either of these two latter experiments must be corrected by the electron yield at “zero time”; thus,
Arrhenius prefactors greater than $10^{13}$ are consistent with experiments where the temperature dependence of the quantum yield is determined only by the long-lived component, and $\phi_p(\lambda)$ = $[1.0 - \phi_r(\lambda)]k_R$ $\tau_2$, where $k_R$ is the radiative rate of the fluorescent species. Our measurements in water indicate that the radiative rate, $k_R$, is $1.05 \times 10^8$ s$^{-1}$ for indole; $3.3 \times 10^7$ s$^{-1}$, for 7-azaindole; and $1.07 \times 10^8$ s$^{-1}$, for 5-methoxyindole. We define $\phi_r(\lambda)$ = $1.0 - \phi_p(\lambda)$ and $\tau_2 = (k_R + k_0 + k(T))^{-1}$, where for simplicity we have assumed that there is only one temperature-dependent nonradiative process, $k(T)$, and only one temperature-independent nonradiative process, $k_R$, such as intersystem crossing. We have set $k_0$ equal to the rate for intersystem crossing, $3.3 \times 10^7$ s$^{-1}$. We find that $k_R = 1.2 \times 10^{13}$ s$^{-1}$, 7-azaindole ($\lambda_{ex}$ = 288 nm), $E_R = 9.3$ kcal/mol, $A = 1.2 \times 10^{13}$ s$^{-1}$; 5-methoxyindole ($\lambda_{ex}$ = 285 nm), $E_R = 2.4$ kcal/mol, $A = 7.3 \times 10^{10}$ s$^{-1}$; 5-methoxyindole ($\lambda_{ex}$ = 288 nm), $E_R = 7.8$ kcal/mol, $A = 6.1 \times 10^{10}$ s$^{-1}$.

Because the fluorescence detected in our experiments is collected after the photoionization event, and because the steady-state and the time-resolved measurements yield identical activation energies, we conclude that the majority of photoelectrons are produced by means of a temperature-independent pathway and that the observed activation energies reveal the presence of another nonradiative process that is thermally activated. The photoelectrons are most likely produced by a temperature independent pathway, such as tunnelling. It is possible, however, that thermal activation of the "fluorescent" state produces solvated electrons with the reported activation parameters. The yield of solvated electrons from the "fluorescent" state is not expected to be more than 10% of that produced instantaneously from the Franck-Condon region (Figure 3b).
Lee and Robinson have performed an interesting, detailed, and original analysis of the nonradiative processes of indole in water/methanol mixtures using fluorescence lifetime and fluorescence quantum yield measurements. It is useful to compare and contrast our results and conclusions with theirs. Because of the agreement between lifetime measurements obtained with a neodymium/phosphate-glass laser and quantum yield measurements, they conclude that the nonradiative processes they observe result from monophotonic events. By analogy with previous work on photoionizing compounds such as anilino/naphthalene sulfonic acid (ANS) and from evidence in the literature indicating that indole produces photoelectrons, Lee and Robinson assume that the nonradiative process measured in their experiments is photoionization. Since they do not directly monitor absorption from the solvated electron, their assignment of the nonradiative process is tentative. As we note in the previous paragraph and elsewhere in this article, we can never observe more than 10% of a contribution of solvated electron whose rise time coincides with the decay time of the fluorescent state. Below we suggest the importance of abstraction of the N$_1$ proton in deactivating the fluorescent state. Regardless of the identity of the nonradiative process for indole observed in the experiments of Lee and Robinson, it is clear that it is very sensitive to solvent. For example, from the temperature dependence of the nonradiative rate they obtain activation energies of 10.4 and ~0.0 kcal/mol in water and methanol, respectively. Assuming that the nonradiative process is photoionization, Lee and Robinson suggest that in methanol trapping of the photoelectron by a solvent cage is unfavorable and that a rapid recombination occurs that reforms ground-state indole. The optimum cage is proposed to be formed from about four water molecules. With regard to our photoionization measurements of 7-azaindole, the electron yield in water is greater by about a factor of 2 than in methanol. If this reduction in yield results from recombination with the parent cation, the back reaction must occur in <1 ps (beyond our time resolution). We observe no geminate recombination 150 ps subsequent to photoionization. Similarly, for indole in butanol, no recombination is observed on the 30-ps time scale investigated subsequent to photoionization.

Our conclusions are in contrast to those of Feitelson and Bent and Hayon, which have significantly influenced the interpretation of indole photophysics over the past 20 years. Feitelson proposed that the production of photoelectrons from indole was temperature dependent based on indirect measurements of hydrogen evolution. Bent and Hayon performed direct flash photolysis measurements of the solvated electron. They observed that the optical density change, $\Delta A$, at 650 nm due to the solvated electron produced from indole at pH 7.5 is temperature dependent.

Figure 4 of their article presents these raw data. A plot of $\Delta A$ vs $1/T$ yields an apparent activation energy of 0.37 kcal/mol. A plot of $\ln(\Delta A)$ vs $1/T$ yields an apparent activation energy of 4.9 kcal/mol, when the absorbance data have been corrected for the shift of the spectrum of the solvated electron with temperature. The interpretation of these results is not straightforward. The quantum yield of photoelectrons is, by definition

$$\phi_e = \frac{k_e}{k_e + \sum_i k_i}$$

where the $k_i$ represent all the other processes, radiative and nonradiative, that deactivate the excited state. $\phi_e$ is proportional to $k_e$ only if $k_e < \sum_i k_i$; this is unlikely since in 7-azaindole the solvated electron appears in $\leq 130$ fs and, in every other example studied here, within a 1-ps pulse width. It is therefore inappropriate to attach any physical significance to the activation energies obtained from the temperature dependence of the absorbance change resulting from the solvated electron.

The temperature dependence observed by Bent and Hayon for the electron yield may be attributed to changes in the ground-state absorption spectrum with temperature. As can be seen for indole in Figure 8, raising the temperature decreases the shoulder at 290 nm and increases the absorbance in the short-wavelength region of the spectrum. Such a temperature-dependent absorption increase occurs at 265 nm, the excitation wavelength used by Bent and Hayon. The increase in electron yield that they report may thus be attributed to shifts in the spectra resulting from the $1^A \rightarrow 1^L_a$ and $1^A \rightarrow 1^L_b$ transitions.

If the observed activation energy in indole derivatives does not reflect thermally activated photoionization, what nonradiative process is involved? Glasser and Lami have proposed that in the vapor-phase dissociation of the N-H bond is an important nonradiative process. Detailed information on the $1^L_a$ and $1^L_b$ states in jet-cooled indoles has become accessible. Wallace and co-workers undertook an investigation of 2,3-dimethylindole, which is known to have a low-lying $1^L_b$ state in all media. Their work yielded evidence that the $1^L_b$ state was strongly coupled to the $1^L_a$ state, from which dissociation of the N-H bond could occur. These results deal with site-specific hydrogen-bonding interactions in the gas phase. We have performed proton inventory experiments of 7-azaindole in water and have suggested that abstraction of the N$_1$ hydrogen may be an important.
Ionization of 7-Azaindole, Indole, and Their Derivatives


temperature-dependent, nonradiative process in indoles, especially 7-azaindole. Barkley and co-workers have recently discussed hydrogen abstraction as a possible nonradiative pathway in indoles.

We suggest that the excitation-wavelength dependence of the fluorescence quantum yield reflects the coupling (most likely vibronic, see below) of the zero order \( \mathbf{L}_{a} \) and \( \mathbf{L}_{b} \) states. These results argue against the utility of using zero-order pictures of \( \mathbf{L}_{a} \) and \( \mathbf{L}_{b} \) states to describe the photophysics of indole and its derivatives. Similar conclusions have been obtained by Wallace and co-workers and Fleming and co-workers.

B. The Effect of Closely-Spaced Excited States. First Cross et al. and then Szabo demonstrated how the presence of two excited electronic states whose energy gap is close to \( kT \) can influence the short time anisotropy decay and hence give rise to apparently anomalously low \( r(0) \) values if the anisotropy measurement is not performed with sufficient time resolution. Subsequently, Fleming and co-workers experimentally observed these effects. Subpicosecond resolution reveals \( r(0) \approx 0.4 \) and rapid components of the anisotropy decay in the range of 1–4 ps.

In the specific case where there are two closely-spaced excited states, \( \mathbf{L}_{a} \) and \( \mathbf{L}_{b} \), the measured anisotropy decay function is a function of both wavelength and time:

\[
\frac{r(\lambda, t)}{r(\lambda, 0)} = \frac{k_{a}g_{a}(\lambda)K_{a}^{(2)}(t)}{k_{b}g_{b}(\lambda)K_{b}^{(2)}(t)} \]

where \( k_{a,b} \) are the radiative rate constants, \( K_{a,b}^{(2)}(t) \) are the population (fluorescence) decay laws, and \( g_{a,b}(\lambda) \) are the emission line shapes, whose integrals are normalized to unity, of \( \mathbf{L}_{a} \) and \( \mathbf{L}_{b} \) states. Thus, when two closely-spaced states can be reached from the ground state in an optical transition (or if the upper state can be thermally populated), the observed anisotropy decay is a superposition of the individual anisotropy decays. The form of the observed anisotropy decay will depend strongly on the extinction coefficient connecting the ground electronic state to \( \mathbf{L}_{a} \) or \( \mathbf{L}_{b} \), which will determine \( k_{a,b}^{(2)}(t) \). The decay will also depend on the relative contribution of emission from the two states that is detected at a given wavelength.

Using the level scheme depicted in Figure 9, the following rate constants are defined:

\[
k_{a} = k_{b} = k_{R}^{(1)} + k_{R}^{(2)}
\]

Here we assume that \( k_{R}^{(1)} = k_{R}^{(2)} \). We also assume that the sum of the rate constants of the nonradiative processes depleting \( \mathbf{L}_{a} \) and \( \mathbf{L}_{b} \), neglecting internal conversion is the same for both levels:

\[
k_{a}^{NR} + k_{b}^{NR} = k_{a}^{NR} = k_{b}^{NR}
\]

\( k_{a}^{NR} \) is the rate of \( \mathbf{L}_{a} \) to \( \mathbf{L}_{b} \) internal conversion. \( k_{b}^{NR} \) is the rate at which \( \mathbf{L}_{b} \) is thermally populated by \( \mathbf{L}_{a} \) and is determined using the appropriate \( \mathbf{L}_{a} \) energy gap and an estimated value of \( k_{ab} \).

In order to evaluate the limiting anisotropies obtained from steady-state and time-domain measurements, we employ the following relationships:

\[
k_{a}(t) = \left( \frac{1}{2}Q \right)\left[ K_{a}^{(0)}(Q - \delta) + 2k_{ab}K_{b}^{(0)} \right] \exp(\delta t) + \left( \frac{1}{2}Q \right)\left[ K_{a}^{(0)}(Q + \delta) - 2k_{ba}K_{b}^{(0)} \right] \exp(-\delta t)
\]

\[
k_{b}(t) = \left( \frac{1}{2}Q \right)\left[ K_{b}^{(0)}(Q + \delta) + 2k_{ab}K_{a}^{(0)} \right] \exp(\delta t) + \left( \frac{1}{2}Q \right)\left[ K_{b}^{(0)}(Q - \delta) - 2k_{ba}K_{a}^{(0)} \right] \exp(-\delta t)
\]

\[
\delta = k_{a} + k_{ab} - k_{b} - k_{ba}
\]

\[
Q = \delta^{2} + 4k_{ab}k_{ba}^{1/2}
\]

The initial populations obtained from subpicosecond anisotropy and fluorescence decay measurements are summarized in Table 5. In every case, the result of the lifetime measurement indicates a much larger \( \mathbf{L}_{a} \) population than would have been obtained from a consideration of only the anisotropy measurement. It is this discrepancy that we address in the next section.

C. Modeling Anisotropy Data (Inclusion of Photoionization at "Zero Time"). We have collected a large body of data on the monophotonic, instantaneous ionization of 7-azaindole, indole, and their derivatives and demonstrated that this ionization is excitation-wavelength dependent. We have also observed that the \( \mathbf{L}_{a} \) state is preferentially and dynamically solvated with respect to higher excited states in 7-azaindole.

<table>
<thead>
<tr>
<th>( \lambda_{ex} ) (nm)</th>
<th>( \alpha_{1} )</th>
<th>( \alpha_{2} )</th>
<th>( \alpha_{1}/\alpha_{2} )</th>
<th>( K_{a}^{(0)} )</th>
<th>( K_{b}^{(0)} )</th>
</tr>
</thead>
<tbody>
<tr>
<td>305</td>
<td>0.77</td>
<td>0.23</td>
<td>3.35</td>
<td>0.43</td>
<td>0.01</td>
</tr>
<tr>
<td>303</td>
<td>0.66</td>
<td>0.34</td>
<td>1.94</td>
<td>0.70</td>
<td>0.03</td>
</tr>
<tr>
<td>300</td>
<td>0.69</td>
<td>0.31</td>
<td>2.23</td>
<td>0.62</td>
<td>0.05</td>
</tr>
<tr>
<td>297</td>
<td>0.65</td>
<td>0.35</td>
<td>1.86</td>
<td>0.72</td>
<td>0.11</td>
</tr>
<tr>
<td>294</td>
<td>0.59</td>
<td>0.41</td>
<td>1.44</td>
<td>0.92</td>
<td>0.16</td>
</tr>
<tr>
<td>292</td>
<td>0.59</td>
<td>0.41</td>
<td>1.44</td>
<td>0.92</td>
<td>0.18</td>
</tr>
<tr>
<td>290</td>
<td>0.77</td>
<td>0.23</td>
<td>3.35</td>
<td>0.43</td>
<td>0.40</td>
</tr>
</tbody>
</table>

\( \alpha_{1} \) and \( \alpha_{2} \) are the experimentally determined preexponential factors for the excited-state population (fluorescence) decay obtained by Fleming and co-workers with subpicosecond resolution. \( K_{a}^{(0)} \) is the initial relative population of the \( \mathbf{L}_{a} \) state predicted from their reported values of \( \alpha_{1} \) and \( \alpha_{2} \) by eqs 18 and 19. The last column labeled \( K_{b}^{(0)}(0) \) is the initial relative population of the \( \mathbf{L}_{a} \) state required by Fleming and co-workers to fit their experimental anisotropy decay. In each case \( \lambda_{ex} = 335 \) nm.
gaps among the indoles. Because the solvated electron appears within 1 ps for excitation wavelengths as red at 305 nm for moment of excitation, that is, immediately upon excitation, or at 5-methoxyindole, it is clear that are excitation-wavelength dependent (Figure 9).

In 5-methoxyindole, one of the largest absorption spectra with temperature is suggested to explain the temperature dependence of the electron yield observed by Bent and Hayon. We propose that most of the photoionization occurs only at the ground-state of indole derivatives: (a) indole, (b) 7-azaindole, (c) 4). 5-Methoxyindole also possesses one of the largest factors into the description of the anisotropy decay of indole and 5-methoxyindole, 10 is based on the observation of preferential solvation of the temperature dependence of the electron yield observed by Bent and Hayon. We tentatively attribute the photoionization to 1. In 5-methoxyindole, Lb is the lowest excited singlet. 1° is photoionizable. 79 is the lowest excited singlet. 1° for the following reasons.

1. The quantum yields for fluorescence and photoionization are excitation-wavelength dependent (Figure 6 and Tables 2 and 4). If both states were photoionizable, the excitation-wavelength dependence would be expected to be much weaker or absent.

2. In 5-methoxyindole, Lb is the lowest excited singlet, 10 5-Methoxyindole also possesses one of the largest Lb-La energy gaps among the indoles. Because the solvated electron appears within 1 ps for excitation wavelengths as red at 305 nm for 5-methoxyindole, it is clear that Lb is photoionizable.

We propose that most of the photoionization occurs only at the moment of excitation, that is, immediately upon excitation, or at time, the initially prepared wavepacket will be displaced, and photoionization will no longer be possible. Furthermore, at subsequent times, for indole, 7-azaindole, and their derivatives, the Lb state will be stabilized or solvated with respect to the Lb state. This preferential solvation event occurs in ~300 fs for 7-azaindole in methanol.8 If for λex = 305 nm at t = 0 the initial populations in the Lb and La levels are Kb(0) = K(0) = 0.5 and λex = 0.3 (Table 5), the populations at a later time, t ~ 0, must be correspondingly diminished by the depletion produced by photoionization. Thus at a later time, the normalized populations are K(t ~ 0) = 0.2/0.7 = 0.28 and K(t ~ 0) = 0.5/0.7 = 0.72. At λex = 300 nm where φe = 0.4, the corresponding populations are K(t ~ 0) = 0.17 and K(t ~ 0) = 0.83. Given the resolution of any currently realizable time-resolved experiment, these residual populations not depleted by photoionization are in fact taken as the zero time populations measured in steady-state experiments or by time-correlated single-photon counting.

Figure 9 presents the anisotropy decay data of Fleming and co-workers for tryptophan at λex = 305 and 300 nm. Superimposed on their results are our simulations using the time zero populations of the Lb and La states corrected for photoionization. Our simulation is identical with the global fit to the experimental data when noise is taken into account.

The important conclusion to be drawn from this exercise is that the amount of the Lb excited-state population in indole, 7-azaindole, and their derivatives that possess small Lb-La energy gaps is more than that estimated from subpicosecond anisotropy decay measurements or from steady-state anisotropy measurements. We have discussed this point elsewhere.3 The agreement of the zero time populations of our simulation with those obtained from the subpicosecond lifetime measurements12 (Table 5) illustrates this point.

Crucial to our analysis are the observations that the solvated electron is produced instantaneously (<1 ps) and that the fluorescence quantum yield is also excitation-wavelength dependent for molecules whose lowest lying excited state is believed to be Lb and in which there is a relatively large Lb-La energy gap: 5-methoxy- and 5-hydroxyindole.
different stable conformations between the indole ring and the peptides bearing amino acid, tryptophan. Essentially all tryptophan derivatives counting measurements, will be 3.8 ns.

data (Table V). Obtained from the global analysis of the experimental population decay experimental anisotropy decay data and are closer to the populations we suggest that 'La is capable of photoionization as long as it is at excitation wavelengths of 305 and 300 nm, respectively. At 300 nm, coupling will decrease and this state, giving rise to fluorescence decay;50 we have measured its lifetime at 20 ps.

The description of the excited states of indole, 7-azaindole, and their derivatives proposed above is useful insofar as it can be used to explain or at least to rationalize the photophysics of more complex molecules—among which is the naturally occurring amino acid, tryptophan. Essentially all tryptophan derivatives and peptides bearing nonrigid side chains display nonexponential fluorescence decay.6,48 A notable exception is N-acetyltrypophanamide (NATA), which affords a single exponential fluorescence decay of about 3 ns at 20 °C.6,48,52 5-Hydroxytryptophan has also been observed to have a single exponential fluorescence decay.6,48,52 we have measured its lifetime at 20 °C and neutral pH to be 3.8 ns.

We have suggested that this nonexponential fluorescence decay arises from two (or more) side-chain orientations with respect to the indole moiety. Each orientation is characterized by a different rate of charge transfer from the indole donor to the side-chain acceptor.6 The existence of stable conformational isomers (conformers) of tryptophan is supported by NMR data.6,48,52 Molecular dynamics simulations of Engh et al.52 have suggested different stable conformations between the indole ring and the side chain in tryptophan. Levy and co-workers6,48,52 have observed different conformers of tryptophan and tryptophan-containing compounds in supersonic jets.

The fluorescence decay of zwitterionic tryptophan is fit well to two exponentially decaying components, namely to the function

\[ K(t) = 0.22 \exp(-t/620 \text{ ps}) + 0.78 \exp(-t/3200 \text{ ps}) \]

Measurement of the lifetime as a function of emission wavelength reveals that the short-lived component is blue-shifted with respect to the

**Figure 10.** Comparison of the global fit (---) of the subpicosecond fluorescence anisotropy data for tryptophan of Fleming and co-workers12 with a simulation (---) taking into account "zero time populations" of the $1^{1}$La and $1^{1}$Lb states that are corrected for photoionization. As we discuss in the text, "zero time" refers to the population of the excited states that remain after the instantaneous photoionization event. The figure indicates that we are able to simulate very well the results of Fleming and co-workers by using $1^{1}$Lb populations at "zero time" of 0.28 and 0.20 at excitation wavelengths of 305 and 300 nm, respectively. At 300 nm, we use $K(0) = 0.20$ instead of 0.17 because it gives better agreement with the experimental data and because 0.20 is within the bounds of experimental error in our electron yield measurements (Table II). The $1^{1}$Lb populations that we estimate from our electron yield measurements are significantly larger than the populations required to fit globally the experimental anisotropy decay data and are closer to the populations obtained from the global analysis of the experimental population decay data (Table V).

**Conclusion**

The existence of stable conformational isomers (conformers) of tryptophan is supported by NMR data.6,48,52 Molecular dynamics simulations of Engh et al.52 have suggested different stable conformations between the indole ring and the side chain in tryptophan. Levy and co-workers6,48,52 have observed different conformers of tryptophan and tryptophan-containing compounds in supersonic jets.

The fluorescence decay of zwitterionic tryptophan is fit well to two exponentially decaying components, namely to the function

\[ K(t) = 0.22 \exp(-t/620 \text{ ps}) + 0.78 \exp(-t/3200 \text{ ps}) \]

Measure-
The two components obtained in the fluorescence decay of zwitterionic tryptophan and essentially all tryptophan analogs and tryptophan-containing peptides have temperature dependencies that yield identical Arrhenius activation energies, $E_a$, but different Arrhenius prefactors.6

Figure 11 provides a means of summarizing the data presented in this article on the photoionization of the excited states of indoles and also provides a qualitative explanation of the nonexponential fluorescence decay kinetics of zwitterionic tryptophan. Figure 11 considers the indolyl photophysics in the context of a three-dimensional energy surface. The energy is a function of an ionization coordinate determined by an R*$+e^-$ distance and a charge-transfer-to-side-chain coordinate. The body of data on indole and its derivatives can be rationalized by taking the $1\text{L}\alpha$ surface to be highly asymmetric in two coordinates and by using propagating wavepackets54 to describe excited-state dynamics. Comparison of Figure 11a,b shows the $1\text{L}\beta$ surface to be dissociative in the ionization coordinate and bound in the side-chain or charge-transfer coordinate.

In indole and 7-azaindole derivatives, because $1\text{L}\alpha$ and $1\text{L}\beta$ lie so close to one another, upon light absorption significant populations of each are excited (Table 5, Figures 9 and 11). The wavepacket projected onto the $1\text{L}\alpha$ surface can either slide down the dissociative chute along the ionization coordinate (Figure 11a) or spill over into the $1\text{L}\alpha$ surface if the $1\text{L}\alpha$ surface crosses the $1\text{L}\beta$ surface near its minimum (Figure 11b). Thus, about a femtosecond after excitation, the initially prepared population is either photoionized or localized in bound states. This time corresponds to the inverse bandwidth54 of the indole absorption spectrum.

Fluorescence measurements performed with steady-state or time-correlated, single-photon counting apparatus will only probe areas of the potential energy surfaces illustrated in Figure 11b,c owing to their limited time resolution. The regions of the potential surface displayed in Figure 11b,c are no longer susceptible to instantaneous ionization. It is known, however, that the presence of electrophilic side-chain groups in tryptophan induces nonexponential fluorescence decay from, presumably, the $1\text{L}\alpha$ state. The activation barrier for this process is determined by the intersection of the surface for the charge-separated state with that of the $1\text{L}\alpha$ state. If there is a distribution of $1\text{L}\alpha$ states with different frequencies for the side chain mode, it is possible to obtain crossings between the $1\text{L}\alpha$ and the charge-separated states that all possess the same activation barrier (Figures 11b,c). The rate of passage from $1\text{L}\alpha$ to the charge-separated state will now be determined by the curvature55 of the $1\text{L}\alpha$ surface. The larger the curvature or the higher the frequency of the side-chain mode, the larger the rate for the charge-transfer process.

The absence of nonexponential fluorescence decay in NATA may now be explained if all its conformers have similar side-chain frequencies and intersect the charge-separated surface at the same position. The absence of nonexponential fluorescence decay in 5-hydroxytryptophan, where $1\text{L}\beta$ is the fluorescent state, can be attributed to a much higher barrier crossing between the $1\text{L}\alpha$ and the charge-separated states.

For tryptophan at high pH, the carboxylic acid is deprotonated, and the amino group is uncharged; hence, neither group is a good charge acceptor,5 the fluorescence lifetime becomes insensitive to the side chain distribution, and single exponential fluorescence decay arises.6 We observe a finite rise time of $\sim$3 ns for the appearance of the 10% of the solvated electron produced at pH 12.3 in tryptophan (Figure 4b). This rise time corresponds to the 3.2-ns fluorescence decay at pH 12.3. Similarly, Bent and Hayon5 observe about 10% of the solvated electron produced at pH 10.3 in tryptophan to appear with an $\sim$8-ns time constant. Again, this rise time agrees with the fluorescence decay time of the anionic tryptophan. An explanation for this behavior is that when the charge-transfer states are no longer accessible at high pH, thermal activation can transfer population from the $1\text{L}\beta$ state back to the dissociative $1\text{L}\alpha$ surface. Instantaneous rise times for the solvated electron are observed at high pH for 5-hydroxytryptophan and all other compounds investigated here. In 5-methoxy- and 5-hydroxyindole and 5-hydroxytryptophan, the $1\text{L}\alpha$ state is the lowest and hence is the fluorescent state.

Zwitterionic 7-azatryptophan is expected to have the same distribution of conformers in aqueous solution as zwitterionic tryptophan; and although zwitterionic 7-azatryptophan has its $1\text{L}\beta$ state lying slightly below its $1\text{L}\alpha$ state,3 it is characterized by a single exponential fluorescence decay over most of the pH range when emission is collected over most of its band.1,2,5 This distinguishing characteristic of 7-azatryptophan, which contributes greatly to its amenability as a fluorescent probe,1,4 shall be discussed elsewhere.

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