constant for internal return in 70% dioxane is estimated to be 40 times larger than k_{-d}. The fact that k_{-d} is an *apparent* dissociation rate constant for an oriented complex held together by secondary valence forces makes it impossible to accurately compare k_{-d} to the limiting values for internal return (k_{-1}). The observation of a large isotope effect of 15% does not require that k_{-d} be much less than k_{-1} since Kirby[20] has observed similar effects of 5–16% for S_N2 displacement reactions involving formaldehyde methyl phenyl acetals. The large negative ρ of −4 that is observed in 70% dioxane suggests that if the reaction *is* S_N2-like, the incoming and leaving groups are widely separated in space. This large amount of effective charge on the central carbon would be expected to have a significant effect on the zero-point energies for the two states under consideration, giving rise to the large isotope effect. If displacement reactions involving acetals commonly exhibit late, loose transition states such as this, large isotope effects such as those that are observed with glycosides would be anticipated.

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Photophysics of Aqueous Tryptophan: pH and Temperature Effects

R. J. Robbins,† G. R. Fleming,*† G. S. Beddard,† G. W. Robinson,‡ P. J. Thistlethwaite,‡ and G. J. Woolfe‡

Contribution from The Davy Faraday Laboratory of the Royal Institution, London W1X 4BS, United Kingdom, and Department of Physical Chemistry, University of Melbourne, Parkville, Victoria 3052, Australia. *Received July 26, 1979*

Abstract: The fluorescence decay kinetics of aqueous tryptophan and 3-methylindole have been determined as a function of pH and temperature by using a picosecond dye laser–single photon counting system with a time resolution of 50 ps. At pH 11, tryptophan exhibits a single exponential decay, with a lifetime of 9.1 ns at 18 °C. However, at pH 7 the decay is faster and definitely nonexponential; the values obtained from a biexponential fit to the data at pH 7 are $\tau_1 = 0.43$ ns, $\tau = 3.32$ ns, and $f = 0.19$ at 18 °C. The behavior of a 3-methylindole closely resembles that of tryptophan at pH 11. A model for the photophysics of aqueous tryptophan is presented in which the excited-state decay constant at pH 11 (where the amino acid side chain is not protonated) is given by the superposition of three independent processes: fluorescence, intersystem crossing, and photoionization; of these processes only photoionization is temperature sensitive ($E^* = 51$ kJ mol^{-1}). In the region pH 4–8, where tryptophan exists in the zwitterion form, a new nonradiative process is introduced, which involves intramolecular proton transfer from the $-NH_3^+$ group to the excited indole ring. The apparent activation energy for intramolecular quenching ($E^* = 16$ kJ mol^{-1}) suggests that it is a predominantly diffusion-controlled process. It is proposed that the nonexponential decay observed for aqueous tryptophan at pH 7 arises from transient terms in the rate constant for intramolecular quenching. Quantum yields calculated from this model compare well with experimental values.

Introduction

The fluorescence of proteins is usually dominated by that of the tryptophan residues.[1] Both the fluorescence lifetime and quantum yield of a tryptophan residue are strongly influenced by the nature of its local environment, and this sensitivity is widely exploited through the use of tryptophan as an intrinsic fluorescence probe for the structure and conformation of proteins and polypeptides in solution.[2] Interpretation of the results of such experiments requires an understanding of the excited-state decay processes in tryptophan, and their response to environmental perturbations. However, despite extensive investigations[3] two fundamental questions remain unanswered. (1) Can an isolated tryptophan residue be characterized by an exponential decay law? (2) What are the *principal* excited-state decay routes for tryptophan in aqueous solution?

Excitation of proteins that contain a few tryptophan residues per molecule yields nonexponential fluorescence decay kinetics;[4,5] the decays can be described as a superposition of a number of exponential components, each of which is assumed to correspond to a tryptophan residue (or group of residues) in a different environment. However, nonexponential decays have also been observed for a number of proteins and peptides containing a *single* tryptophan residue;[6,7] here, the nonexponentiality is attributed to the presence of multiple conformations of the molecule. Each of these explanations for nonexponentiality is based on the implicit assumption that the fluorescence decay of an isolated tryptophan residue is strictly exponential. Yet this assumption has not been verified. Recently, Rayner and Szabo[8] investigated the fluorescence decay of aqueous tryptophan at pH 7 using the conventional single photon counting technique (excitation by a weak spark lamp of 2–3 ns duration). The fluorescence emission was shown to follow a biexponential decay law with components having lifetimes of 3.14 and 0.51 ns. In a previous paper[9] we also reported the observation of a nonexponential fluorescence decay for aqueous

* Address correspondence to this author at the Department of Chemistry and James Franck Institute, The University of Chicago, Chicago, Illinois 60637.
† Davy Faraday Laboratory.
‡ University of Melbourne.

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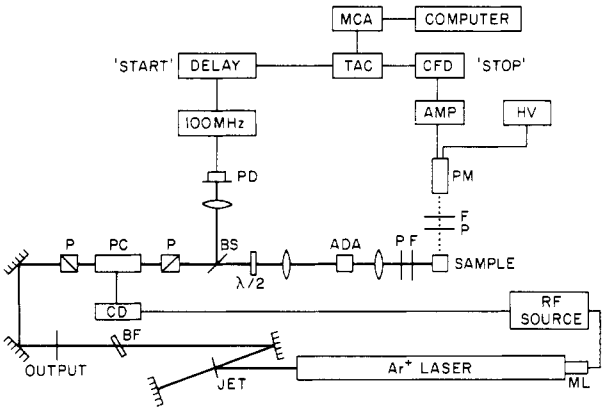

Figure 1. Block diagram of the synchronously pumped dye laser and photon counting instrument: ML, mode locker; BF, birefringent tuning element; CD, count-down logic; P, polarizers; PC, Pockels cell; BS, beam splitter; λ/2, half-wave rotator; ADA, frequency doubling crystal; F, filters; PD, photodiode; 100 MHz, leading-edge discriminator; CFD, constant fraction discriminator.

tryptophan at pH 7. Using picosecond pulses at 264 nm for excitation and an ultrafast streak camera for detection, we obtained a biexponential decay with components having lifetimes of 2.1 and 5.4 ns. These results are clearly inconsistent with those obtained by Rayner and Szabo.[8]

The use of tryptophan as an intrinsic fluorescence probe requires an understanding of the possible excited-state decay routes and their dependence on solvent, pH, temperature, and the pressure of potential quenchers. Both photophysical and photochemical processes must be considered. They include fluorescence,[10,11] intersystem crossing,[12-14] photoionization,[14-23] photodissociation (indole N–H bond fission),[24-26] and intramolecular quenching,[27-32] and, in proteins and peptides, singlet–singlet energy transfer[33,34] can be important. While the existence of these processes is widely accepted, some controversy has centered around the mechanism of the intramolecular quenching process, and whether or not monophotonic ionization is important when excitation is to the S_1 state. In a previous paper[9] we presented a model for the photophysics of aqueous tryptophan in which the excited-state decay was governed by the rates of four independent, competing processes: fluorescence, intersystem crossing, photoionization, and an intramolecular quenching process involving the amino acid side chain. However, because of experimental problems (see below), a quantitative analysis was not possible.

In view of these problems and inconsistencies, we have reexamined the fluorescence decay of aqueous tryptophan and the related compound 3-methylindole using a subnanosecond single photon counting apparatus with tunable dye-laser excitation. The results of a systematic study of the fluorescence decay of tryptophan as a function of both pH and temperature complement similar nanosecond laser flash photolysis studies by Bent and Hayon.[14] Together these results provide an insight into the origins of nonexponential decays, enable a positive identification of the intramolecular quenching mechanism, and allow a more consistent, quantitative analysis of our previously reported model for the photophysics of aqueous tryptophan.

Experimental Section

A. Chemicals. Tryptophan and 3-methylindole were obtained from Sigma and used without further purification. Samples were stored at 0 °C in a dessicator in the dark. Solutions were prepared in Analar water immediately before use, and the appropriate pH was obtained using HCl (pH 1,3), BDH buffer tablets (pH 7, 9.2), or NaOH (pH 11, 13). The purity of the samples was checked by a comparison of their absorption, emission, and excitation spectra.

B. Fluorescence Lifetime Measurements. Fluorescence lifetimes were measured with the use of the single photon counting apparatus shown in Figure 1. The laser excitation source consisted of a CW rhodamine 6G dye laser (Coherent Radiation CR590) synchronously pumped by an actively mode-locked argon ion laser (Coherent Radiation CR12). The argon ion laser produced a highly stable train of 90-ps pulses at a repetition frequency of 75.525 MHz with an average power of 800 mW while the dye laser, which had a three-plate birefringent tuning element and a 30% transmitting output mirror, produced pulses of <10 ps duration at 590 nm with an average power of 200 mW. The dye laser repetition frequency was reduced to 73.8-kHz by a low-voltage electrooptic modulator (Coherent Associates Model 28) slaved to the mode-locker rf source by home-built countdown logic. The contrast ratio between transmitted and rejected pulses was ~500:1 with an amplitude stability of ±10% for the transmitted pulses. The second harmonic (295 nm, vertically polarized) was generated with ~1% efficiency by focusing the dye-laser output into a 5-mm-long temperature tuned ADA crystal (T = 36.0 ± 0.2 °C). Residual 590-nm radiation was removed by a polarizer and a Schott UG 11 filter. Taking into account the conversion efficiency and the losses along the optical path, the energy of a single UV pulse at the sample was estimated to be ~1 pJ (~1.5×10^6 photons).

The "start" signal for the ORTEC Model 457 time-to-amplitude converter (TAC) was obtained from a portion of the laser fundamental via a Texas Instruments TIED 56 silicon avalanche photodiode and an ORTEC Model 436 100-MHz discriminator. Fluorescence emitted at right angles to excitation was detected by a Philips XP2020Q photomultiplier. In order to obtain the best time response, the standard voltage divider network was modified in a manner similar to that described by Lewis et al.[35] The single photon anode pulses (~100–400 mV into 50 Ω) were amplified in a Marconi Model TF2175 broad-band amplifier (gain 27 ± 1 dB, 2–500 MHz) and fed into an ORTEC Model 463 constant fraction discriminator, and its output used as a "stop" signal for the time-to-amplitude converter. The excitation beam was attenuated so that the ratio of "stop" to "start" pulses was always less than 2%. The TAC outputs were processed in a Tracor-Northern TN-1706 multichannel analyzer, which was interfaced to an Interdata 7/32 computer.

In the measurement of short lifetimes by the single photon counting technique, the fluorescence decay profile $f(t)$ is obtained as the convolution of the true excited state decay function $g(t)$ with the instrument response function $i(t)$:

$$f(t) = \int_0^t i(t - t')g(t')\,dt' \qquad (1)$$

The fluorescence decay profile $f(t)$ was collected by exciting the sample at 295 nm and monitoring the resultant emission using appropriate filters in front of the photomultiplier to remove scattered laser light. Polari-

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Table I. pH Dependence of the Fluorescence Lifetimes of 3-Methylindole and Tryptophan

	3-methyl-indole	tryptophan			
pH[a]	τ/ns[b]	τ_1/ns[c]	τ_2/ns[b]	f[d]	$\langle\tau\rangle$/ns[c]
1	0.77	0.29	0.80	0.27	0.66
3	8.7	0.29	3.0	0.23	2.4
7	8.9	0.43	3.3	0.19	2.8
9.2	9.3	3.0	9.0	0.56	5.6
11	8.5	*e*	9.1	*e*	9.1
13	0.84	*e*	1.2_1	*e*	1.2

[a] All measurements in aqueous solution at 18 ± 1 °C. [b] Estimated accuracy ±3%. [c] Estimated accuracy ±0.2 ns. [d] Estimated accuracy ±0.03. [e] Single exponential.

zation bias due to molecular rotation was eliminated by exciting with vertically polarized light and using an analyzer (Polaroid HNP'B) oriented at 54.7° to the vertical.[36] The instrument response function i(t) was obtained by replacing the fluorescent sample with a dilute suspension of talc, removing the filter, and collecting the scattered laser radiation. Both the scattering and fluorescent samples had an absorbance of ~0.1 at 295 nm. Under normal operating conditions the instrument response function had a full width at half-maximum of 360 ps.

The parameters describing the true excited-state decay function g(t) were extracted from the experimental data by a nonlinear least-squares curve fitting technique[37-39] using a modified version of the Marquardt algorithm.[40,41] Initially g(t) was assumed to be the form

$$g(t) = Ae^{-t/\tau} \quad (2)$$

where A is a preexponential factor and τ the fluorescence lifetime. The quality of the fit was judged from the reduced chi-squared (χ_r^2) criterion[36,41] and by visual inspection of the weighted residuals.[37-39] In those cases where a single exponential decay law was inadequate, a sum of exponentials was used

$$g(t) = A[fe^{-t/\tau_1} + (1-f)e^{-t/\tau_2}] \quad (3)$$

where τ_1 and τ_2 are the lifetimes of the two components and f is the relative weight of the shorter component ($\tau_1 < \tau_2$). No attempts were made to fit the data to more complex functions.

The response of photomultiplier tubes used in photon counting depends on the wavelength of the incident light.[35,42,43] Therefore, the instrument response function collected at 295 nm by the scattering technique will not be an exact representation of the response function i(t) required in eq 1 for deconvoluting fluorescence measurements made at longer wavelengths. Fortunately, the wavelength dependence of the XP2020 tube was very small: there was a delay of ~1 ps/nm with increasing emission wavelength, which was accompanied by small changes in the shape of the response function. The time shift of 50–60 ps was accounted for in the data analysis procedure by replacing i(t) with i(t - s) in eq 1, where s is a time-shift parameter. The change in shape of the response function was reflected by small systematic deviations on the rising edge of the decay curve (see Figure 3). Even for the shortest lifetimes reported here, this introduced negligible error.

Results

A. pH Dependence. The fluorescence decay of aqueous tryptophan was studied at room temperature (18 ± 1 °C) over the range pH 1–13 (Table I). Except at pH 11 and 13, the decays were definitely not exponential. Figure 2 shows the pH dependence of the average fluorescence lifetime $\langle\tau\rangle = f\tau_1 + (1-f)\tau_2$, which is proportional to the fluorescence quantum yield.

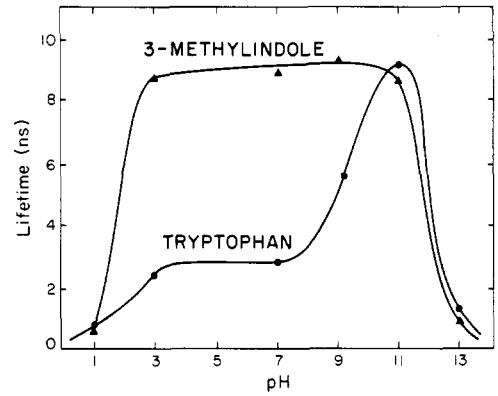

Figure 2. pH dependence of the fluorescence lifetime of 3-methylindole and the mean lifetime (where applicable) of tryptophan at 18 °C.

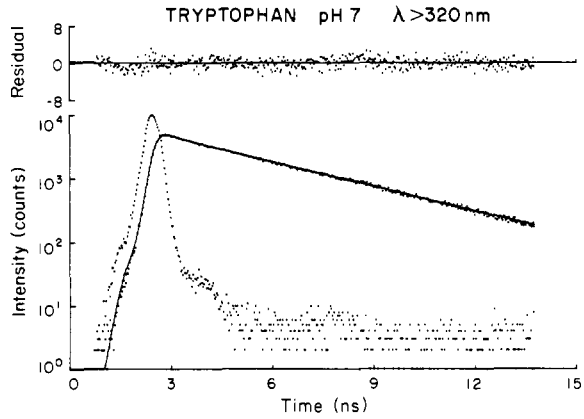

Figure 3. Fluorescence decay for aqueous tryptophan at pH 7, excited at 295 nm and monitored at λ >320 nm, plotted on a semilogarithmic scale. The solid line is the best fit of the experimental data to a biexponential decay law (eq 3). A weighted residuals plot is shown at the top of the curve. See Table I for lifetimes.

Figure 3 shows a typical fluorescence decay obtained upon excitation of an aqueous solution of tryptophan at pH 7 when the emission was monitored at λ > 320 nm (Schott WG320 filter); the sample had an absorbance of 0.1 at 297 nm and was not degassed. A biexponential fit to these data gave τ_1 = 0.43 ns, τ_2 = 3.32 ns, and f = 0.16 with χ_r^2 = 1.07 and an almost random distribution of residuals. The average values obtained from a series of ten such experiments were τ_1 = 0.43 ± 0.2 ns, τ_2 = 3.32 ± 0.12 ns, and f = 0.19 ± 0.03. Identical results were obtained for unbuffered samples and samples degassed by three freeze–pump–thaw cycles (10^{-3} torr). Trivial causes of nonexponential decays, such as scattered laser light, filter fluorescence, and aggregation, were checked and found to be absent.

The lifetimes measured for aqueous tryptophan at pH 7 (Table I) are in agreement with those obtained by Rayner and Szabo.[8] However, Rayner and Szabo also observed a dependence of the decay kinetics on emission wavelength: in the range 310–370 nm they obtained a biexponential decay with the same two lifetimes, 0.51 and 3.14 ns, at all wavelengths, but the contribution of the longer component increased with increasing wavelength until, above 380 nm, only the longer component remained.[8] We have also examined the wavelength dependence of the fluorescence decay of aqueous tryptophan at pH 7 and can qualitatively confirm these results. At λ > 400 nm (Schott GG400 filter) a single exponential decay was observed with τ = 3.30 ns, while at 340 nm (Balzer interference filter) a biexponential decay was observed with f = 0.22 and the same two lifetimes found when monitoring the total emission band.

The results obtained in this work are in contrast with our previous work:[9] a biexponential decay was also observed there, but the two components had lifetimes of 2.1 and 5.4 ns with f = 0.77. Data similar to those shown in Figure 3, but covering a much larger time span, showed no trace of a component with a lifetime

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Table II. Temperature Dependence of the Fluorescence Lifetimes of 3-Methylindole and Tryptophan

	3-methylindole	tryptophan	
T/°C[a]	τ/ns, pH 11[b]	τ/ns, pH 11[b]	τ_2/ns, pH 7[b]
9.1	10.3	10.1	4.0_2
19.8	8.0_4	8.1_8	3.1_3
29.7	5.6_7	6.3_0	2.2_9
40.4	3.7_0	4.3_0	1.8_3
49.6	2.4_9	2.9_7	1.3_5
60.6	1.4_9	1.7_8	1.0_6
74.6	0.69	0.88	0.60

[a] Estimated accuracy ±0.2 °C. [b] Estimated accuracy ±3%.

of ~5 ns. However, the fluorescence decay of a neutral aqueous solution of tryptophan recorded after irradiation for 3 h by a low-pressure mercury lamp (~10^{18} photons s^{-1} at 254 nm) contained a longer lived component. A biexponential fit to these data, which actually consisted of at least three exponential components, gave τ_1 = 2.7 ns, τ_2 = 5.3 ns, and f = 0.75 with χ_r^2 = 1.53. The similarity between these lifetimes and those obtained in our previous work[9] suggests that our original data for aqueous tryptophan at pH 7 were distorted by the presence of a fluorescent photoproduct. No attempt was made to identify the photoproduct. Rather, we were concerned with establishing conditions and experimental procedures where this problem could be avoided. This was achieved by storing the solid material at 0 °C in a desiccator in the dark, preparing samples within a few hours before use, and avoiding any unnecessary laser irradiation. After this work was completed, we received a report from Ghiggino et al.[44] that also ascribed the 5.4-ns component to a photogenerated impurity.

For purposes of comparison, we also studied the fluorescence decay of 3-methylindole, which is the most appropriate simple analogue of tryptophan (indole-3-alanine). Figure 2 shows the pH dependence of the fluorescence lifetime of aqueous 3-methylindole at room temperature (18 ± 1 °C) over the range pH 1–13. The samples were excited at 295 nm and the total emission band $\lambda > 320$ nm was monitored. At each pH the decay was well described by a single exponential; there was no evidence for a component with a lifetime of ~0.4 ns such as that observed for tryptophan at pH 7. The lifetimes are summarized in Table I.

B. Temperature Dependence. The fluorescence decays of aqueous tryptophan (pH 7, 11) and 3-methylindole (pH 11) were studied over the range ~10–75 °C (Table II). For tryptophan at pH 11, the decays were exponential at all temperatures with the fluorescence lifetime decreasing from 10.1 ns at 9.1 °C to 0.88 ns at 74.6 °C. Similar results were obtained for 3-methylindole. The fluorescence decay of tryptophan at pH 7 was definitely nonexponential, at least at room temperature, but could be described as a sum of two exponentials (see above). Although the experimental data were not accurate enough to extract a meaningful estimate of the lifetime of the shorter component at each temperature, the deviation from an exponential decay appeared to be less pronounced at the higher temperatures. The lifetime of the longer component τ_2 decreased from 4.0 ns at 9.1 °C to 0.60 ns at 74.6 °C.

Discussion

A. pH Effects: Intramolecular Quenching. The data presented in Tables I and II suggest that the photophysical behavior of tryptophan at pH 11 is very similar to that of 3-methylindole. That is, when the α-amino group is not protonated (pK_a = 9.39[45]), the side chain in tryptophan has little effect on either the radiative or the nonradiative decay rates of the indole chromophore. However, at pH 7 the fluorescence lifetime[46] (Figure 2) and quantum yield[27-30] are reduced by a factor of ~2.6 relative to their values at pH 11; the yields of triplets, radicals, and solvated electrons produced in the flash photolysis of aqueous tryptophan show the same pH dependence.[14] Therefore, the reduction in the fluorescence lifetime in the region pH 4–8 must be due to the introduction of a new nonradiative process without significantly perturbing the rates of existing processes from their values at pH 11. This process is associated with the presence of the α-NH_3^+ group and is almost certainly *intramolecular* in origin.

Table III. Deuterium Isotope Effect on Intramolecular Quenching

	ϕ[a]		k_i'[b]	
compound	H_2O	D_2O	H_2O	r[c]
3-methylindole	0.34	0.50		
tryptamine	0.30	0.46	0.4	2.3
tryptophan, pH 7	0.14	0.29	4.2	2.9
tryptophylglycine	0.11	0.17	6.2	1.5
tryptophan, pH 2.5[d]	0.08	0.12	9.6	1.5
tryptophylglycinamide	0.05	0.07	17	1.3_6
tryptophan ethyl ester	0.02_4	0.02_6	39	1.0_7
acetyltryptophan	0.23	0.30	1.4	1.0_6
Cbz-tryptophan	0.19	0.23	2.3	0.99
acetyltryptophanamide	0.15	0.18	3.7	1.0_5
indole-3-acetic acid ethyl ester	0.13	0.15	4.8	1.0_2
glycyltryptophan	0.07	0.08	11	1.0_8
indole-3-acetic acid[e]				1.0_9
indole-3-propionic acid[e]				1.1_5
indole-3-butyric acid[e]				0.99

[a] Data taken from ref 47 unless otherwise noted. [b] $k_i' = k_i/k_f$; $k_f = 5.0 \times 10^7$ s^{-1}. [c] $r = k_i(H_2O)/k_i(D_2O)$. [d] From ref 49. [e] From ref 32.

Two mechanisms for intramolecular quenching in tryptophan have been widely suggested. The first is a charge-transfer interaction, in which the excited indole ring acts as an electron donor and either a proton donated by the NH_3^+ group[30,47,48] or the carbonyl group "activated" by the presence of the α-NH_3^+ group[9,28,32] acts as an acceptor. The second mechanism involves the transfer of a proton from the NH_3^+ group to the excited indole ring.[29,31,49]

In their flash photolysis studies of aqueous tryptophan, Bent and Hayon[14] observed a very short-lived unidentified transient (τ ~ 45 ns at pH 7), which they designated T_1. The T_1 transient, tentatively assigned as a triplet state, has a maximum yield in the range pH 3–7 and decreases to zero in alkaline solutions with an apparent pK_a ~ 8.5; at pH <3 its yield also decreases.[14] The pH profile of the yield of the T_1 transient strongly suggests that it arises from the intramolecular quenching process, although its identity is uncertain. In view of the similarities between the spectra and lifetimes of the T_1 transient and the triplet state of the indole cation (formed by protonation of the excited indole ring at pH <2),[14] we suggest that the T_1 transient corresponds to the same triplet state formed by intramolecular proton transfer from the NH_3^+ group to the excited indole ring.

A large deuterium solvent isotope effect on the fluorescence quantum yield of aqueous tryptophan has been reported by a number of investigators.[31,47,49-52] However, the magnitude of the observed isotope effect reflects the average effect on the rates of all excited-state decay processes. The rate of intramolecular quenching k_i can be extracted from the quantum yield measurements, at least approximately, using the expression

$$k_i = k_f(\phi^{-1} - \phi_R^{-1}) \quad (4)$$

where ϕ is the fluorescence quantum yield in a pH range where intramolecular quenching is present and ϕ_R is the corresponding

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yield in the absence of quenching; this is taken as the quantum yield of the reference compound 3-methylindole, $\phi_R = 0.34$ in H_2O and 0.50 in D_2O at 25 °C.[47] The calculated rates for a number of indole derivatives are reported in Table III together with the ratio $r = k_i(H_2O)/k_i(D_2O)$ of the intramolecular quenching rate in H_2O to that in D_2O.

There is a large D_2O effect on the intramolecular quenching rate in neutral aqueous tryptophan ($r = 2.9$) and tryptamine ($r = 2.3$); furthermore, the T_1 transient is observed in the flash photolysis of both compounds.[14] These observations imply the existence of a common intramolecular quenching mechanism in tryptophan and tryptamine, which probably involves proton transfer from the NH_3^+ group to the excited indole ring. In the ground state, 3-methylindole undergoes protonation principally on the carbon atom at the 3 position[53] ($pK_a = -4.55$[54]). However, molecular orbital calculations[55-57] predict that the basicity of both the nitrogen atom and the carbon atom at the 3 position decreases upon excitation to either the ^1L_a or ^1L_b state, whereas the basicity of the carbon atom at the 2 position increases. Therefore, it seems most likely that the intramolecular quenching in aqueous tryptophan at pH 7 involves proton transfer from the NH_3^+ group to the 2 position of the excited indole ring.

In contrast with the results for tryptophan and tryptamine, the D_2O effect on the intramolecular quenching in indole-3-propionic acid is small ($r = 1.15$), and no T_1 transient has been detected in the flash photolysis of this compound.[14] Furthermore, the ester derivative of indole-3-acetic acid, which contains no labile protons, shows a similar intramolecular quenching efficiency to the unionized acid (Table III). Therefore, it seems probable that a charge-transfer interaction between the carbonyl group and the excited indole ring is responsible for the intramolecular quenching in these compounds. The strong correlation between the efficiency of intramolecular quenching and the electrophilicity of the carbonyl group in a series of derivatives of indole-3-acetic acid and indole-3-propionic acid[27-30,47] provides further support for this hypothesis.

Thus we suggest that two intramolecular quenching mechanisms can operate in indole derivatives under appropriate conditions. In derivatives that possess an NH_3^+ group in proximity to the indole ring, such as tryptamine and the tryptophan zwitterion, intramolecular proton transfer can occur. This process is characterized by a large D_2O effect and the presence of a short-lived T_1 transient in the laser flash photolysis experiments. In derivatives without an NH_3^+ group but with an electrophilic carbonyl group, such as indole-3-propionic acid and acetyltryptophan, intramolecular quenching can occur by a charge-transfer mechanism. This process can be identified by a negligible D_2O effect and the absence of the T_1 transient. Several of the compounds listed in Table III have an intermediate D_2O effect, the most notable examples being tryptophyglycine and tryptophan at pH 2.5 ($r = 1.5$). These molecules possess both an NH_3^+ group and an electrophilic carbonyl group (a peptide, acid, amide or ester group), and therefore both intramolecular quenching mechanisms are probably important.

B. Temperature Effects. Flash photolysis data provide evidence for two important nonradiative processes in aqueous solutions of simple indoles, such as 3-methylindole: intersystem crossing[12-14] and photoionization;[14,15,18,20,21] photodissociation appears to be unimportant in aqueous solution ($\phi \sim 0.001$).[26] The photophysical behavior of aqueous tryptophan at pH 11 is very similar to that of 3-methylindole (Tables I and II). Thus, according to our model, the fluorescence lifetime of tryptophan at pH 11 is determined by the rates of three competing processes: fluorescence (k_f), intersystem crossing (k_t), and photoionization ($k_{e_{aq}^-}$):

$$\tau^{-1} = k_f + k_t + k_{e_{aq}^-} \quad (5)$$

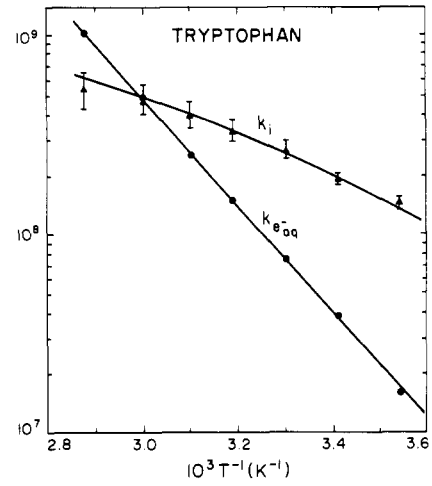

Figure 4. Temperature dependence of the rates of photoionization $k_{e_{aq}^-}$ and intramolecular quenching k_i for aqueous tryptophan, plotted in the form $\ln k$ vs. T^{-1}. The solid lines are the best fits of the data to eq 6 and 8, respectively.

The dramatic decrease in fluorescence lifetime over the temperature range 10–75 °C (Table II) implies that at least one of these processes is very temperature sensitive. It is generally accepted[30,47,58,59] that the rates of radiation and intersystem crossing are temperature independent, and therefore the observed temperature dependence must be due to variations in the photoionization rate $k_{e_{aq}^-}$. If it is assumed that $k_{e_{aq}^-}$ can be expressed by an Arrhenius equation, then the fluorescence lifetime at any temperature T is given by

$$\tau^{-1} = k^0 + A \exp[-E^*/RT] \quad (6)$$

where $k^0 = k_f + k_t$ is the sum of the rates of the temperature-independent processes, E^* is the activation energy for photoionization, and A is the frequency factor. Two separate procedures have been used to estimate these parameters.

In the first procedure, the activation energy E^* is obtained graphically from the slope of a plot of $\ln(\tau^{-1} - k^0)$ against T^{-1}. An estimate of k^0 can be obtained from the radiative rate k_f and the limiting fluorescence quantum yield at low temperatures ϕ_f^0, $k^0 = k_f/\phi_f^0$. By combining the fluorescence lifetime $\tau = 6.8$ ns for aqueous 3-methylindole at 25 °C with the corresponding fluorescence quantum yield $\phi_f = 0.34$,[30,31,47,49] we calculate $k_f = 5.0 \times 10^7\ s^{-1}$. The limiting quantum yield at low temperatures is $\phi_f^0 = 0.60 \pm 0.08$.[30,51] From these data we estimate $k^0 = 8.3 \pm 1.0 \times 10^7\ s^{-1}$ ($k_t = 3.3 \times 10^7\ s^{-1}$) for aqueous 3-methylindole. Furthermore, we assume that the rates of radiation and intersystem crossing for aqueous tryptophan are identical with those estimated for 3-methylindole. Figure 4 shows a plot of $\ln k_{e_{aq}^-} \equiv \ln(\tau^{-1} - k^0)$ against T^{-1} for aqueous tryptophan at pH 11 for this value of k^0; the plot is linear with a slope corresponding to an activation energy of 50 ± 5 kJ mol^{-1}. A similar analysis for 3-methylindole gave $E^* = 51 \pm 5$ kJ mol^{-1}.

The second procedure, which avoids the graphical manipulation, is to fit the experimental data directly to eq 6 by the method of least squares. Figure 5 shows the results obtained for aqueous tryptophan at pH 11; the calculated curve is an excellent fit to the data, with $k^0 = 8.3 \pm 0.6 \times 10^7\ s^{-1}$, $A = 5 \pm 4 \times 10^{16}\ s^{-1}$, and $E^* = 51 \pm 3$ kJ mol^{-1}. Analysis of the data for 3-methylindole gave $k^0 = 7.5 \pm 0.8 \times 10^7\ s^{-1}$, $A = 4 \pm 4 \times 10^{16}\ s^{-1}$, and $E^* = 50 \pm 3$ kJ mol^{-1}.

The results obtained by the two procedures are in excellent agreement. In particular, the agreement between the calculated temperature-independent rates ($7.5 \pm 0.8 \times 10^7\ s^{-1}$, $8.3 \pm 0.6 \times 10^7\ s^{-1}$) and the experimental estimate ($8.3 \pm 1.0 \times 10^7\ s^{-1}$) is reassuring in view of its susceptibility to systematic errors in the

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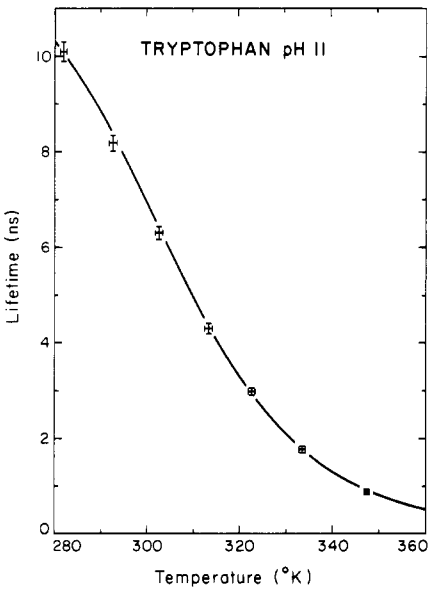


Figure 5. Temperature dependence of the fluorescence lifetime of aqueous tryptophan at pH 11; the solid line was obtained from a nonlinear least-squares fit of the data of eq 6.

data analysis,[47] and provides strong support for the hypothesis of a single temperature-dependent process. The agreement between the values of the parameters obtained for tryptophan at pH 11 and those obtained for 3-methylindole confirms the similarity of these two systems, although the photoionization rate $A \cdot \exp[-E^*/RT]$ is consistently a little higher for 3-methylindole over the entire temperature range.

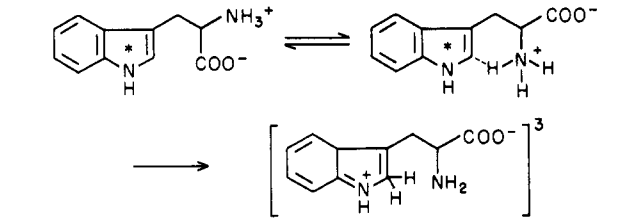
The activation energy for the photoionization of aqueous 3-methylindole obtained in this work, $E^* = 50 \pm 3$ kJ mol^{-1}, agrees well with the value of 54 ± 6 kJ mol^{-1} reported by Kirby and Steiner.[47] Similar activation energies have been reported for other indole derivatives in aqueous solution, including indole (45–52 kJ mol^{-1}),[30,47,58-60] 1-methylindole (48–55 kJ mol^{-1}),[58,59] 5-methoxyindole (44 kJ mol^{-1}),[59] and indole-3-acetate (53 kJ mol^{-1}).[47] These results suggest that the temperature-dependent process, which we identify with photoionization, has an activation energy close to 50 kJ mol^{-1} for all simple indole derivatives in aqueous solution.

According to our model, the only change in the region pH 4–8 is the introduction of an intramolecular quenching process without perturbing the rates of the other processes from their values at pH 11. Thus, for aqueous tryptophan at pH 7, the rate of the intramolecular proton transfer process is given by

$$k_i = \tau^{-1}(\text{pH } 7) - \tau^{-1}(\text{pH } 11) \quad (7)$$

The calculated intramolecular quenching rate is plotted in Figure 4 in the form ln k_i vs. T^{-1}. A straight-line fit to these data leads to an activation energy of 16 kJ mol^{-1} and preexponential factor $A = 1.5 \times 10^{11}$ s^{-1}. However, a careful inspection of the data reveals a curvature in the plot of ln k_i vs. T^{-1} in excess of that expected from the magnitude of the experimental errors. This raises the question of the dynamics of the intramolecular process and, in particular, the validity of expressing k_i in the form $A \cdot \exp[-E^*/RT]$. The apparent activation energy (16 kJ mol^{-1}) corresponds closely to that expected for diffusion in water. Thus we envisage the intramolecular quenching process as a diffusion-controlled reaction; in the ground state there is a nearly random distribution of conformations of the amino acid side chain, so that intramolecular quenching can occur only subsequent to diffusion of the side chain into the correct conformation for the proton-transfer step. A possible mechanism is shown in Scheme I where the product species is identified with the T_1 transient of Bent and Hayon[14] as discussed previously.

Scheme I



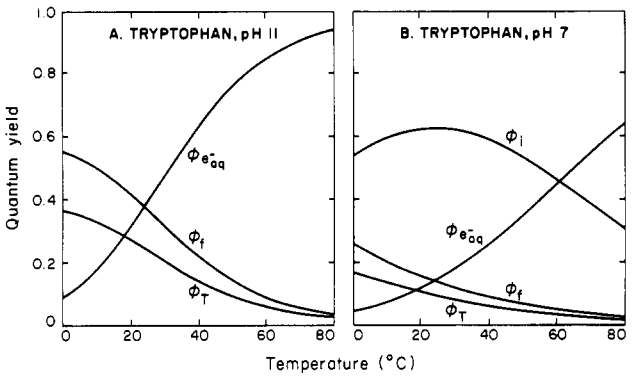


Figure 6. Calculated quantum yields of fluorescence (ϕ_f), intersystem crossing (ϕ_t), photoionization ($\phi_{e_{aq}^-}$), and intramolecular quenching (ϕ_i) for aqueous tryptophan at pH 7 and 11, as a function of temperature.

In the steady state, the rate of diffusion-controlled intramolecular quenching is expected to be of the form

$$k_i = BT/\eta \quad (8)$$

where η is the solvent viscosity and B is a proportionality constant. The best fit of the experimental data to this expression gives $B = 6.8 \times 10^5$ cP K^{-1} s^{-1}, with no systematic deviations between the experimental and calculated points (Figure 4).

Our results for aqueous tryptophan at pH 7 definitely show the existence of two temperature-dependent nonradiative processes: photoionization with an activation energy of ~50 kJ mol^{-1} and diffusion-controlled intramolecular quenching with an apparent activation energy of ~16 kJ mol^{-1}. The activation energies of 25–35 kJ mol^{-1} reported by other investigators[47,51,61] result from the superposition of these two processes, which cannot normally be separated because of experimental uncertainties and the limited temperature range available to aqueous solutions. In fact, a plot of ln $(\tau^{-1} - k^0)$ against T^{-1} for our data for tryptophan at pH 7 gives a straight line within experimental error with a slope corresponding to an activation energy of 30 kJ mol^{-1}. The same explanation would hold for tryptamine and the other indole derivatives studied by Kirby and Steiner[47] where activation energies considerably less than 50 kJ mol^{-1} were also obtained.

C. A Self-Consistent Model of Tryptophan Photophysics. The discussion in section A provides a qualitative picture of the photophysics of aqueous tryptophan. According to our model, the behavior of tryptophan at pH 11 closely resembles that of 3-methylindole, where the principal nonradiative decay routes are intersystem crossing and photoionization. For tryptophan in the region pH 4–8 a new nonradiative process is introduced, which involves intramolecular proton transfer from the NH_3^+ group of the amino acid side chain to the excited indole ring; here the function of the carboxyl group is probably to enhance the acidity of the α-NH_3^+ group ($pK_a = 9.39$;[45] cf. 10.2 for tryptamine[62]). Then at pH <3 the protonated carboxyl group ($pK_a = 2.38$[45]) becomes the center for another nonradiative process, which involves an intramolecular charge-transfer interaction between the excited indole ring and the electrophilic carbonyl group.

The analysis of the temperature effects (section B) enables a quantitative calculation of the quantum yields of all competing

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excited-state decay processes in aqueous tryptophan. Figure 6A shows a plot of the quantum yields of fluorescence, intersystem crossing, and photoionization for tryptophan at pH 11, calculated from the rate constants estimated above; i.e., $k_f = 5.0 \times 10^7\ s^{-1}$, $k_t = 3.3 \times 10^7\ s^{-1}$, and $k_{e_{aq}^-} = A \exp[-E^*/RT]$ with $A = 5 \times 10^{16}\ s^{-1}$ and $E^* = 51\ kJ\ mol^{-1}$. A similar calculation, but including the intramolecular quenching process ($k_i = BT/\eta$ with $B = 6.8 \times 10^5$ cP $K^{-1}\ s^{-1}$), gave the corresponding quantum yields for aqueous tryptophan at pH 7 shown in Figure 6B. This calculation takes into account only the longer component of the nonexponential decay observed at pH 7. However, the error introduced by neglecting the shorter component is less than 20%, and therefore these results are still of value for comparison with experimental quantum yields, which often have uncertainties of this magnitude.

Fluorescence. The fluorescence quantum yield of aqueous tryptophan at pH 7 calculated in the above manner decreases monotonically from 0.201 at 10 °C to 0.024 at 80 °C with a value of 0.137 at 25 °C. There have been many determinations of the fluorescence quantum yield of tryptophan in neutral solution,[10,11,30,62-66] and it now seems certain that the absolute yield at 25 °C lies in the range 0.13–0.14. The calculated quantum yield $\phi_f = 0.137$ at 25 °C is in excellent agreement with these results. The temperature dependence of the fluorescence quantum yield of tryptophan in neutral solution has also been studied.[47,51,62] Eisinger and Navon[51] obtained yields of 0.204 at 10 °C and 0.023 at 80 °C, in agreement with the calculated values of 0.201 and 0.024, respectively.

The calculated fluorescence quantum yield of aqueous tryptophan at pH 11 is 0.366 at 25 °C. The reported quantum yields[27,30,31,49,62,67] are widely scattered in the range 0.3–0.6 owing, in part, to the different quantum yields assumed for aqueous tryptophan at pH 7 as a fluorescence standard. After adjusting the experimental data relative to $\phi_f = 0.13_5$ for tryptophan at pH 7, the quantum yields lie in the range 0.26–0.41 at ~25 °C with an average value of 0.36. The residual scatter in the results probably arises from differences in both pH and temperature in the measurements. Nevertheless, the average value $\phi_f = 0.36$ at 25 °C agrees well with the calculated quantum yield of 0.366.

Intersystem Crossing. For aqueous tryptophan at pH 7, the calculated triplet yield ϕ_t decreases from 0.133 at 10 °C to 0.016 at 80 °C with a value of 0.090 at 25 °C; the corresponding yields at pH 11 are 0.327 (10 °C), 0.242 (25 °C), and 0.023 (80 °C). Although triplets have been identified as transient species in the flash photolysis of tryptophan,[12-14] absolute triplet yields have not been reported. However, the absolute yields can be estimated from the data of Bent and Hayon[14] using a comparison technique,

$$\phi_t = \left(OD^0_t \epsilon_{e_{aq}^-} / OD^0_{e_{aq}^-} \epsilon_t\right) \phi_{e_{aq}^-} \qquad (9)$$

where OD_t^0 and $OD^0_{e_{aq}^-}$ are the optical densities at time zero for the triplet and solvated electron absorptions, ϵ_t and $\epsilon_{e_{aq}^-}$ are the extinction coefficients of these species at the monitoring wavelength, and $\phi_{e_{aq}^-}$ is the yield of solvated electrons. Taking ϵ_t(450 nm) $= 8 \times 10^3$ L mol^{-1} cm^{-1},[68] $\epsilon_{e_{aq}^-}$ (650 nm) $= 15.4 \times 10^3$ L mol^{-1} cm^{-1},[18] and $\phi_{e_{aq}^-} = 0.08$[14] at 25 °C, we calculate $\phi_t = 0.10$ for aqueous tryptophan at pH 7 and 0.21 at pH 11. These results are in reasonable agreement with the calculated yields of 0.090 and 0.242, respectively. Bent and Hayon[14] also studied the temperature dependence of the relative triplet yield for aqueous tryptophan at pH 7. Assuming that ϵ_t is temperature independent, a similar calculation gives $\phi_t = 0.15$ at 10 °C and 0.019 at 80 °C, compared with the values of 0.133 (10 °C) and 0.016 (80 °C) calculated from our model for the photophysics of aqueous tryptophan.

Photoionization. The initial products in the photoionization of aqueous tryptophan are the solvated electron e_{aq}^- and the radical cation Trp^+, which may subsequently deprotonate to yield the neutral radical.[18] Figure 6 shows the temperature dependence of the calculated photoionization yield, $\phi_{e_{aq}^-}$ or ϕ_{Trp^+}, for aqueous tryptophan at pH 7 and 11. At 25 °C, the calculated yields are 0.146 (pH 7) and 0.392 (pH 11). Since the initial work of Grossweiner and Joschek,[15] there has been considerable controversy over the absolute photoionization yield.

Using the conventional microsecond flash photolysis technique with broad-band excitation ($\lambda > 210$ nm), Grossweiner and Usui[69] obtained $\phi_{e_{aq}^-} = 0.06$ for aqueous tryptophan at pH 7, and 0.09 at pH 11.8. Pailthorpe et al.[20] used the same technique but confined the excitation flash to $\lambda > 270$ nm and obtained $\phi_{e_{aq}^-} = 0.008$ at pH 10–12. Using a steady-state photolysis technique based on the oxidation of Fe^{2+}, Steen[19] found that the photoionization yield of aqueous tryptophan at pH 1–3 was constant for excitation within the first absorption band 260–290 nm ($\phi_{e_{aq}^-} = 0.015$), but increased dramatically for excitation below 240 nm. The fluorescence quantum yield shows a similar decrease for excitation below 240 nm,[19,65] whereas the fluorescence lifetime is independent of excitation wavelength in the range 220–320 nm.[70] On the other hand, Amouyal et al.[23] oserved a threshold in the excitation wavelength dependence of the photoionization yield of neutral aqueous tryptophan. They used steady-state photolysis, in the presence of N_2O, to scavenge the hydrated electron, and found that ionization was absent for excitation at $\lambda > 275$ nm, but increased rapidly with decreasing excitation wavelength.[23]

Recent laser flash photolysis studies with nanosecond time resolution have produced much higher electron yields. Bent and Hayon[14] obtained electron yields of 0.08 (pH 7) and 0.21 (pH 11) at 25 °C; log–log plots of electron yield against flash intensity gave slopes of 1.2–1.5, which were interpreted as indicating a predominantly monophotonic process. Bryant et al.[13] made the important observation that the electron and radical coproduct decay by a rapid back-reaction, leading to residual yields after several microseconds that are significantly smaller than the initial photoionization yield. This could explain the low yields deduced from steady-state[16,17,19,23] and conventional flash photolysis experiments.[20] Unfortunately, the high intensities used in the laser flash photolysis work introduces the possibility of a consecutive biphotonic electron ejection mechanism,[21,71] in which the excited singlet state absorbs the second photon. High intensities can also lead to "saturation" and, in the case of a biphotonic process, the yield-intensity dependence is complex; the dependence is quadratic at low intensity, linear at intermediate intensities, and saturates at higher intensities.[21] In the flash photolysis of aqueous tryptophan, Lachish et al.[21] obtained a slope of 1.4 from a log–log plot of electron yield against excitation intensity, which they considered to indicate a predominantly biphotonic ionization mechanism. Recently, Baugher and Grossweiner[18] obtained a strictly linear yield–intensity relationship for aqueous tryptophan at pH 7 with an electron yield $\phi_{e_{aq}^-} = 0.10 \pm 0.01$ and a slightly higher radical yield $\phi_{Trp^+} = 0.12 \pm 0.01$. However, at pH 10.9 the e_{aq}^- yield had a nonlinear intensity dependence, indicating a significant biphotonic contribution.[18] In any case, the electron and radical yields should be identical. Yet Baugher and Grossweiner[18] found that ϕ_{Trp^+} was slightly greater than $\phi_{e_{aq}^-}$ over the entire pH range. The Trp^+ yield can also be estimated from the data of Bent and Hayon:[14] using eq 9 with ϵ_{Trp^+} (580 nm) $= 2.9 \times 10^3$ L mol^{-1} cm^{-1} [13] gives $\phi_{Trp^+} = 0.12$ at pH 7 and 0.32 at pH 10–12, in agreement with the results of Bauger and Grossweiner.[18] The difference between $\phi_{e_{aq}^-}$ and ϕ_{Trp^+} may indicate another

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(70) Alpert, B.; Jameson, D. M.; Lopez-Delgado, R.; Schooley, R. *Photochem. Photobiol.* **1979**, *30*, 479–481. These authors find single exponential decay at all excitation wavelengths, confirming the absence of any 5.4 ns component. However, they fit only the portion of the fluorescence after the excitation pulse has completely decayed (about 4 ns after the maximum of the fluorescence of their Figure 1). This procedure would not reveal the presence of the short component (0.43 ns) in the tryptophan fluorescence decay obtained in this work and that of Rayner and Szabo.[8]
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Table IV. Calculated Quantum Yields at 25 °C

	3-methyl-indole	tryptophan[a]	
		pH 11	pH 7
ϕ_f	0.340	0.366 (0.36)	0.137 (0.13_s)
ϕ_T	0.171	0.242 (0.21)	0.090 (0.10)
$\phi_{e_{aq}^-}$	0.489	0.392 (0.32)	0.146 (0.12)
ϕ_i	-	-	0.627 (0.65)

[a] Figures in parentheses are the "best" experimental estimates; see text for details.

pathway for the formation of Trp^+ radicals, or it may indicate a rapid decay route for the hydrated electron that is complete within the duration of the laser pulse (~10 ns).

Despite the current uncertainty about the absolute photoionization yield, there is reasonable agreement between the Trp^+ yields of 0.12 (pH 7) and 0.32 (pH 11) obtained from the laser flash photolysis experiments[14,18] and the calculated yields of 0.146 and 0.392, respectively.

Intramolecular Quenching. Figure 6B shows the calculated quantum yield ϕ_i for the intramolecular quenching of aqueous tryptophan at pH 7. In the temperature range 10–35 °C ϕ_i is almost constant with a maximum yield of 0.627 at 25 °C but decreases more rapidly above 35 °C owing to competition between intramolecular quenching and photoionization. There have been no direct experimental determinations of the intramolecular quenching yield. However, from the pH dependence of the T_1 transient observed by Bent and Hayon,[14] we suggested that this species results from the intramolecular quenching process. This assertion is confirmed by the temperature dependence of the T_1 transient (see Figure 7 in ref 14); the T_1 yield is constant in the range 10–35 °C, but drops rapidly at higher temperatures. It was suggested above that the T_1 transient corresponds to a triplet state of the indole cation, similar to (if not identical with) that observed in very acidic media[14] (1–3 M H_2SO_4). If we assume that this triplet is generated with unit efficiency at pH <0 where its yield is constant,[14] then the quantum yield for intramolecular quenching at pH 7 is estimated to be 0.65 at 25 °C. This estimate is in good agreement with the calculated yield $\phi_i = 0.627$ at 25 °C.

Summary of Yields. The experimental estimates of the quantum yields for fluorescence, intersystem crossing, photoionization, and intramolecular quenching for aqueous tryptophan at 25 °C are summarized in Table IV along with yields calculated from our model. In general, the agreement between the experimental and calculated yields is good, considering the difficulty of obtaining reliable flash photolysis data on a nanosecond time scale and the approximations involved in extracting absolute yields from these data.

An important feature of our model is the assumption that the introduction of the intramolecular quenching process at pH 7 does not alter the rates of radiation, intersystem crossing, and photoionization from their values at pH 11. A quantity that we can use to test this assumption, and which obviates the need for absolute quantum yields, is the ratio of the quantum yield for each process at pH 11 to the corresponding yield at pH 7. This ratio should be constant at a given temperature. The model predicts a ratio of 2.7 at 25 °C, compared with the experimental ratios of 2.7, 2.0, 2.6, and 2.6 for the yields of fluorescence, triplets, solvated electrons, and Trp^+ radicals, respectively. The agreement between the calculated and experimental ratios provides further support for our model.

D. Nonexponential Decays. For 3-methylindole, the fluorescence decays were exponential under all of the conditions studied. This implies that the nonexponential decay observed for neutral aqueous tryptophan is not an intrinsic property of the indole chromophore, but is associated with the presence of the amino acid side chain. On the other hand, Rayner and Szabo[8] attributed the nonexponential fluorescence decay of tryptophan to dual emission from the ^1L_a and ^1L_b states. However, the absence of any variation of the decay kinetics (or emission spectrum) of aqueous tryptophan with excitation wavelength[8] indicates that the ^1L_a and ^1L_b states are in thermal equilibrium. An alternative mechanism leading to the nonexponential decay for tryptophan is discussed below.

Nonexponential fluorescence decays can arise in two distinct ways. The nonexponentiality can be due to heterogeneity in the molecular population, with each subset of molecules having a characteristic exponential decay. A trivial example of this type of behavior would be the simultaneous excitation and independent decay of a mixture of two molecules in solution. A second cause of nonexponentiality is an intrinsic time dependence in one or more of the nonradiative decay rates. For example, in intermolecular energy migration by the Forster mechanism, the initial energy transfer rate has a time dependence of the form $k(t) \propto t^{-1/2}$.

In a previous paper[9] we also reported a nonexponential fluorescence decay for tryptophan in neutral aqueous solution, which was interpreted in terms of two solvent-trapped conformers. It was proposed that in one conformation the carboxyl group was in close contact with the indole ring and intramolecular quenching was efficient, while in the other, the carboxyl group was more remote and quenching less efficient.[9] The heterogeneity associated with multiple molecular conformations has also been proposed to explain the nonexponential decays observed for a number of proteins and polypeptides.[6,7] However, for a small molecule like tryptophan it is difficult to see how these two stable conformations would come about.

An alternative, albeit related, way of interpreting these nonexponential decays is suggested by the results of the present work. It was shown that the apparent activation energy for intramolecular quenching of tryptophan fluorescence is characteristic of a diffusion-controlled reaction. Therefore, it was suggested that intramolecular quenching can occur only subsequent to diffusion of the amino acid side chain into the correct conformation for the proton-transfer step. The kinetics of translational diffusion-controlled (intermolecular) reactions have been extensively studied, both theoretically and experimentally. A solution of Fick's second law for this system, including the transient terms, gives a time-dependent quenching rate,[72]

$$k(t) = 4\pi R' D[Q](1 + R'/\sqrt{\pi D t}) \qquad (10)$$

Here, D is the mutual diffusion coefficient for the reactive particles, R' is related to the encounter distance, and [Q] is the concentration of quencher. Taking $D \sim 5 \times 10^{-5}$ cm^2 s^{-1} and $R' \sim 3$ Å as reasonable *guesses* for quenching of tryptophan by the attached NH_3^+ group, a calculation based on eq 10 predicts that the quenching rate will fall to within 10% of the steady-state value in ~600 ps. Although this equation is not strictly applicable to intramolecular reactions, it does suggest that diffusion-controlled intramolecular quenching may have an intrinsic time dependence of the correct order of magnitude to explain the nonexponential decays observed experimentally.

The fluorescence decay profile represents the sum of all the competing decay processes; however, at pH 7 this decay is dominated by the intramolecular quenching process, and it is the transient behavior of this process, we suggest, that determines the form of the fluorescence decay curve and produces the observed nonexponentiality. A suggested mechanism for intramolecular quenching by proton transfer is given in Scheme I. The nonexponential decay thus reflects the dynamic nature of the system. The nonexponential decays observed for proteins containing a single tryptophan may therefore contain information about the fluctuations in protein structure which are important, for example, for an understanding of enzyme function.[73]

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Chemical Properties of the Fulvene Radical Cation: A Cycloaddition with 1,3-Butadiene

David H. Russell and M. L. Gross*

Contribution from the Department of Chemistry, University of Nebraska, Lincoln, Nebraska 68588

Abstract: The chemical reactivity of the fulvene radical cation has been measured and compared with those of the benzene and various acyclic isomeric radical cations. This was accomplished by studying their reactivities in ion–molecule reactions observed using ion cyclotron resonance spectrometry (ICR). The fulvene radical cation exhibits considerably different properties from the other $[C_6H_6]^{+\cdot}$ isomers, and this is interpreted in terms of a unique structure for ionized fulvene which is not subject to ring opening or isomerization to benzene. One of the reactions of the fulvene cation, that with 1,3-butadiene, was examined in detail using dueterium and C-13 labeling. The intermediate complex has been assigned as a cycloadduct formed by an ionic analogue of a [6 + 4] cycloaddition.

In this paper, we report a study of the chemistry of ionized fulvene in order to compare its chemical properties with other $[C_6H_6]^{+\cdot}$ isomers. The structures of stable and decomposing C_6H_6 radical cations have been the subject of numerous experimental and theoretical studies.[1,2] Even with this large volume of experimental data, a clear and consistent understanding of the structure(s) and chemical properties of various C_6H_6 ions is only now beginning to emerge. Our present understanding of this important gas-phase ion has evolved because of the utilization of a large number of experimental methods including high-pressure mass spectrometry,[3] ion cyclotron resonance (ICR),[2a,4] defocused metastable methods,[5] collision-induced dissociation (CID),[6] isotopic labeling[7] (both ^2H and ^{13}C), photoion–photoelectron coincidence spectroscopy (PIPECO),[8] emission spectroscopy,[9] and ionization/appearance energy measurements.[10]

Our work is directed at the question of whether structure is preserved upon ionization of organic molecules, and we have chosen C_6H_6 isomers as one model system for investigating this point. The answer to this question depends on the barrier heights for isomerization on various potential energy surfaces. Our experimental approach is to investigate the chemical properties of $[C_6H_6]^{+\cdot}$ as a function of neutral precursor structure. Of the various techniques mentioned above, high-pressure mass spectrometry, CID, and ICR are appropriate for the required studies of stable or nondissociating ions. We have chosen to study chemical reactivity in near-thermal ion–molecule reactions using ICR.

For highly activated dissociating ions, the possibility exists for isomerization and/or rearrangement reactions because activation energies for these processes can be lower than for fragmentation channels. Competitive or dominant isomerization is particularly important for systems which have high activation energies for dissociation. The molecular ion of benzene is a good example because the lowest energy fragmentation barrier is nearly 5 eV. Moreover, the ground-state heats of formation for the acyclic and cyclic C_6H_6 cation isomers are below the threshold for benzene decomposition. Thus, it is possible for a variety of C_6H_6 radical cations to interconvert at energies below the dissociation threshold.

However, we have found that the ion–molecule reaction chemistry of C_6H_6 radical cations which have insufficient energy to dissociate is consistent with unique structures for ionized benzene and acyclic isomers.[2a] Specifically, C_6H_6 ions from benzene react with 2-propyl iodide to form $C_9H_{13}^+$, whereas acyclic

(1) "Stable" ions refer to those of insufficient energy to decompose within a few milliseconds; "decomposing" ions have been activated to decompose at times less than a few milliseconds.

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