Time-Resolved Spectroscopy of Tryptophan Conformers in a Supersonic Jet

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Abstract: The fluorescence decay of tryptamine and tryptophan in the gas phase was measured in a supersonic free jet. A new technique for producing CW jets of tryptophan in helium was developed in order to carry out the experiments. The fluorescence lifetimes of several conformers of tryptamine were measured. In each case the fluorescence decay was found to be exponential and the lifetimes for all conformers were identical (13.2 ± 0.3 ns). In the case of tryptophan, measurements were also made on several conformers. All decays were found to be single exponential, but in tryptophan, the fluorescence lifetime was found to vary among the conformers. Three conformers had identical lifetimes (12.9 ns), but one conformer had a lifetime of 10.4 ns. The short-lived conformer was the conformer that had previously been observed to produce an emission spectrum containing broad, red-shifted fluorescence. This red-shifted fluorescence was found to occur promptly with no measurable risetime, and the observed lifetime for this conformer did not depend on the wavelength being observed.

The amino acid tryptophan has been a frequently used probe for protein structure and dynamics.[1-3] The photophysics of tryptophan is complex and much effort has gone into attempts to provide a satisfactory description of its excited state behavior.[4-9] One perplexing aspect of tryptophan photophysics is the nonexponential fluorescence decay that is observed in aqueous solution. In the model of Fleming and co-workers,[2,8,10] it is proposed that, in solution, the tryptophan fluorescence is quenched via intramolecular electron transfer from the indole ring to acceptors such as the carbonyl group on the amino acid side chain. The quenching rate depends on the position and orientation of the indole ring with respect to the acceptors, and therefore different tyrptophan conformers exhibit different lifetimes. The form of the fluorescence decay that is observed also depends on the relative lifetimes of conformer interconversion and fluorescence. Current estimates based on molecular dynamics simulations suggest that in solution the tryptophan decay can be described as a double exponential resulting from two groups of conformers.[11] Work by Ware and co-workers, while generally supporting the conformer model, has pointed out the importance of solvent effects on the decay.[12,13] Unfortunately, direct evidence for the existence of different conformers is inaccessible in solution because of broad overlapping transitions.

The obvious approach of carrying out spectroscopic studies in the gas phase has only recently become possible.[14-17] Tryptophan has a very low vapor pressure and decomposes at temperatures well below those required for gas-phase spectroscopic studies. Rizzo et al. developed a method of vaporizing tryptophan in a supersonic pulsed jet.[15] The simplified spectra afforded by the cold jet enabled the identification of transitions from several conformers of tryptophan as well as transitions from different conformers of a number of tryptophan derivatives. The fluorescence excitation spectrum of tryptophan differed significantly from that of some simple derivatives such as tryptamine, 3-indolepropionic acid, and NATE (*N*-acetyltryptophan ethyl ester).[16-18] The dispersed emission spectrum of tryptophan revealed differences between the various conformers of tryptophan itself. In particular, one conformer (conformer A; see ref 14) gives, in addition to the usual sharp fluorescence, broad red-shifted fluorescence. Other conformers gave substantially reduced amounts of the broad red-shifted emission. Further, the yield of red-shifted fluorescence for conformer A was reduced in partially deuteriated tryptophan. This latter finding suggests that the interaction responsible for the broad fluorescence involves proton motion, and a specific model involving the neutral and zwitterionic forms of the molecule was proposed.[14]

Although the supersonic jet spectra confirm the existence of several tryptophan conformers, their differing spectra do not necessarily imply different fluorescent lifetimes for the conformers nor that individual conformers decay exponentially. The derivatives tryptamine, 3-indolepropionic acid, and NATE were also observed to exist as several conformers in the gas phase. However, although NATE, like tryptophan, has a double exponential decay in solution, tryptamine and 3-indolepropionic acid have only a single exponential decay in solution. Thus, the existence of conformers alone does not correlate to multiexponential fluorescence decays in solution. Moreover, Sipior et al.[19] have

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measured the fluorescence decay of NATE and 3-indolepropionic acid in a supersonic jet and found that in both cases different conformers had lifetimes that differed by 20–25%. Clearly, it is difficult to make connections between the time-resolved measurements in solution and the spectroscopic measurements in a supersonic jet. In order to investigate these points further, we have carried out fluorescence lifetime measurements on several conformers of tryptophan and tryptamine in a supersonic expansion. In order to carry out these measurements, a continuous supersonic expansion of tryptophan was required. The previous technique, developed for pulsed jets,[15] proved inadequate for a continuous jet, and a new technique was developed.

Experimental Section

In our previous work with tryptophan,[15] the supersonic jet source was a pulsed valve with a 1-mm orifice which discharged into a 1-mm diameter cylindrical channel, and in these experiments the duty factor was limited by the 10 Hz repetition rate pulsed lasers that were used. Because of the low duty factor, the total gas discharge through the 1-mm orifice was small enough to be handled by our pumps. In the present experiment we use a laser with much lower peak power, but the reduction in sensitivity caused by the low peak power is counterbalanced by the higher duty factor produced by the 3.8 MHz repetition rate of the laser. To take advantage of this higher repetition rate, a continuous jet was necessary, and therefore a much smaller orifice diameter was required to keep the gas discharge within the limit of the pumps. Because of the very different nozzle geometry required by the smaller orifice of the continuous jet, a new technique for volatilizing tryptophan with virtually no thermal decomposition was developed. Initially, two methods involving a continuous jet were attempted and discontinued because of their failure to produce gas-phase samples of tryptophan in adequate quantities. The first attempt involved the use of the continuous jet and a standard oven to prepare a gas-phase sample of tryptophan. Mass spectra recorded from 180 °C to 250 °C revealed only small amounts of tryptophan and large amounts of decomposition products. The next attempt extended the combined pulsed thermospray and thermal desorption techniques used in the earlier pulsed jet experiments. A continuous thermospray device was constructed consisting of a channel coaxial with the jet expansion containing tryptophan deposited on its walls. Mass spectra recorded from 150 °C to 250 °C showed only minute quantities of tryptophan. A new technique was based on the observation that an equal weight of uracil mixed with tryptophan produced the necessary quantities of gas-phase tryptophan. Specifically, 0.06 g of *d,l*-tryptophan and 0.06 g of uracil (both purchased from Sigma Chemical and used without further purification) were weighed and mixed before being loaded behind a 0.1 mm diameter nozzle.[20] The mixture was heated to 210 °C, and a continuous jet of tryptophan was produced with 3.7 atm of helium. If the temperature was controlled to ±10 °C, suitable amounts of the gas-phase sample could be maintained for up to 3 h before any decomposition products were observed. Tryptamine samples were heated in a standard oven to 140 °C.

Moderate resolution fluorescence excitation spectra were taken by using the new continuous nozzle with a pulsed laser apparatus that was previously described.[17] The apparatus for taking high resolution fluorescence excitation spectra used a CW argon ion pumped dye laser and has also been described elsewhere.[21] Fluorescence decay profiles were recorded on a subnanosecond time-correlated single-photon counting apparatus similar to one described by Chang et al.[22] The apparatus consisted of a mode-locked argon ion laser (1.3 W of 514 nm at ~76 MHz) used to synchronously pump a cavity dumped (Coherent Model 7200) dye laser. By using rhodamine 6G and dumping at 3.8 MHz, the dye laser produced at least 100 mW of tunable visible light which was focused onto a KDP doubling crystal. The UV laser light was focused into the vacuum chamber in the same manner as in the high resolution spectrometer. The fluorescence signal was imaged with an air-spaced quartz condenser lens onto a microchannel plate photomultiplier (MCP) (Hamamatsu, R2809U). The anode pulses of the MCP were amplified (Hewlett Packard 8447F), fed into a discriminator (Tennelec TC 455), and sent to a time-to-analogue converter (TAC, Ortec, 457). Different time settings on the TAC determined the time resolution. Time settings of 10.5, 57, 114, and 220 ps/channel across 512 channels of the multichannel analyzer (Tracor-Northern TN-7200) produced time windows of 4, 24, 48, and 90 ns, respectively. Instrument response functions of ~70 ps fwhm were recorded by collecting resonant scatter from argon clusters formed in an expansion of pure argon gas at a backing pressure of 85 atm through a 25 μm diameter pinhole. The fluorescence decays were fit to single-exponential functions by the method of iterative convolution.[22] The quality of fit was judged by the χ-squared criterion and by visual inspection for systematic deviations in the weighted residuals. Fluorescence decay profiles of stilbene were used as a standard. The lifetime of stilbene at 302 nm was determined to be 2.4 ± 0.1 ns in reasonable agreement with the literature values.[23]

Table I. Tryptamine Conformer Lifetimes Obtained by Time-Resolved Single-Photon Counting of the Individual Conformers as Isolated in a Supersonic Jet Expansion[a]

conformer	wavelength (nm)	lifetime (ns)
F	287.08	13.2
E	286.77	13.1
C	286.69	13.1
D	286.63	13.1
B	286.55	13.2
A	286.37	13.5

[a] All lifetimes are ±0.3 ns as determined by the maximum deviation in three temporal measurements.

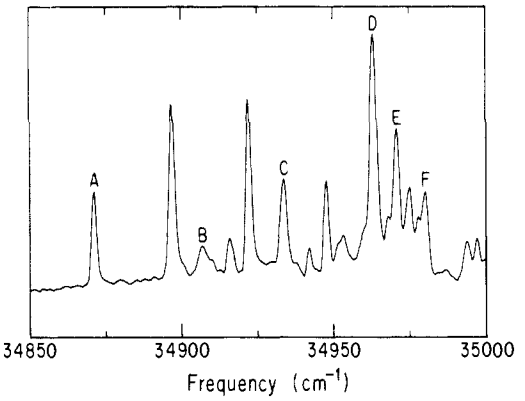

Figure 1. Gas-phase fluorescence excitation spectrum of jet-cooled tryptophan. From this conjested spectrum, six geometric conformers have been identified (A–F). The resolution of this spectrum is approximately 6 GHz.

Results

The origin region of the fluorescence excitation spectrum of tryptamine shows six bands which were assigned to different conformers by Park et al.[18] The fluorescence lifetime for each of these conformers is given in Table I. The decays all fit to single exponentials in spite of some minor contribution to the decay from the scattered excitation pulse. Excitation produces conformers at the origin of the lowest excited singlet state of each conformer, and there is only a small geometry change upon excitation.[24] Thus, the majority of the fluorescence is resonant with the excitation line, and it is impossible to eliminate the scattered excitation pulse by spectrally filtering the collected light. When data from the short-time region where the excitation pulse occurs were excluded from the fit, the calculated fit to the data was improved. No significant variation in the decay times from the average value of 13.2 ± 0.3 ns was found among the different conformers. Furthermore, the lifetimes measured at different time resolutions (from 2 to 4 decays) were identical.

The fluorescence excitation spectrum of tryptophan is shown in Figure 1, and once again the indicated conformer peaks were assigned as in previous studies.[17] In this spectrum the intensities of the different conformer peaks are somewhat different from those reported previously; however, the peak positions are identical. The differences in the intensities are probably a result of the different sample heating techniques. Mixing tryptophan with uracil in a standard oven apparently gives rise to different conformer population distributions than when using the thermospray apparatus

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Table II. Tryptophan Conformer Lifetimes Obtained by Time-Resolved Single Photon Counting of the Individual Conformers as Isolated in a Supersonic Jet Expansion

conformer	wavelength (nm)	lifetime (ns)
A	286.75	10.4
A*	≥305	10.4
B	286.46	12.9
C	286.23	13.0
D	286.02	12.8

[a] Conformer A* is the red-shifted emission from conformer A obtained by using a high-pass filter (Schott filter WG 305). All lifetimes are ±0.3 ns, except for conformer B which was ±0.5 ns, as determined by the average deviation in at least seven temporal measurements.

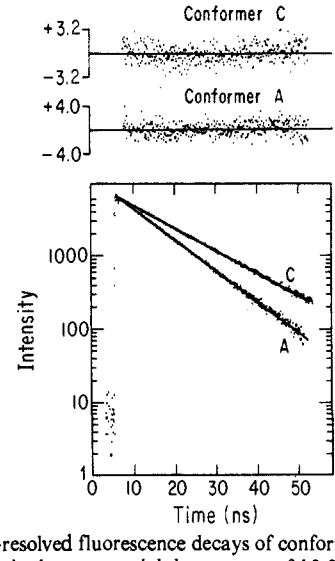

Figure 2. Time-resolved fluorescence decays of conformers C and A that have been fit to single exponential decay rates of 13.3 ± 0.4 and 10.0 ± 0.4 ns, respectively. The normalized residuals from the fits to each trace are given at the top of the figure. Each of the other conformers measured (conformers B and D) had a lifetime similar to conformer C.

employed in the pulsed jet experiment.[17] The spectrum of tryptophan is more congested with vibrational structure than that of tryptamine. This dense spectrum, coupled with the inherent spectral width of the picosecond excitation pulse, necessitated limiting the time-resolved measurements to conformers A–D.

As in tryptamine, all of the conformer decays fit well to a single exponential, but, in contrast, all of the lifetimes were not the same. The measured conformer lifetimes are presented in Table II. Inspection of Table II reveals that there is no significant difference in the lifetimes of conformers B, C, and D, these conformers having an average lifetime of 12.9 ns. Conformer A has the different lifetime of 10.4 ns. An example of fits to the data for conformer A and conformer C is shown in Figure 2. As with tryptamine, the data were taken at several different time resolutions, which covered varying time windows. At the lower time resolutions, covering time windows of 24, 48, and 90 ns, there was complete agreement for the lifetime of each conformer. With these time windows, a minimum of 1.5 lifetimes and maximum of 9 lifetimes were monitored. This time spread was ample for the obervation of any second long-time component. As seen in Figure 2, no evidence of a second long-time component was found.

In an attempt to measure any additional short-time component(s) in conformer A, several measurements were made at the highest time resolution. Some ambiguity arises in the short-time regime due to contamination of the data with scattered light from the excitation pulse. The contamination in the tryptophan spectra was much worse than in the case of tryptamine because the signal intensity from tryptophan was an order of magnitude smaller. In conformer A, however, dispersed fluorescence spectra have demonstrated that the majority of fluorescence is substantially red-shifted from the excitation pulse.[14] Thus, it was possible to preferentially monitor the red-shifted fluorescence by spectrally filtering the collected fluorescence. If the red-shifted fluorescence

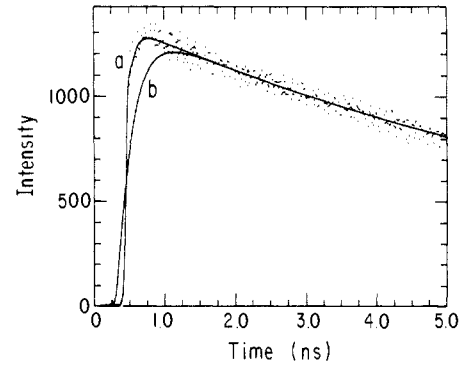

Figure 3. Time-resolved fluorescence decay of the red-shifted emission from conformer A (dots, $\lambda_{em} \geq 305$ nm, $\lambda_{ex} = 286.75$ nm). The resolution for each data point is 10.5 ps, while the overall response function of the detector is approximately 70 ps. Shown along with the data are two calculated curves that have been normalized and shifted and include the instrument response. These curves demonstrate the difference between an "instantaneous" onset of the fluorescence and a slow rise of 200 ps. Specifically, these curves have the following kinetic parameters: (a) $\tau = 8.9$ ns and (b) $A_1 = -A_2$, $\tau_1 = 200$ ps and $\tau_2 = 8.9$ ns, where $A_{1,2}$ are the preexponential amplitudes.

is due to the formation of a second state, any rise-time in the red-shifted fluorescence would then be a measure of the rate of formation of the second state. Figure 3 shows the early time behavior of the A conformer emission (≥305 nm) at high time resolution. As shown in the figure, a good fit to the data was achieved without the introduction of a rising exponential. For comparison a second calculated curve is also shown in which a 200-ps risetime has been added, and it is clear that a risetime greater than 200 ps would easily have been detected. In addition, time-resolved measurements of the red-shifted fluorescence from conformer A at low time resolution were identical with the measurements where no spectral filtering was used. Thus, both the resonant and the red-shifted fluorescence have the same single exponential decay lifetime and no measurable risetime.

Discussion

Several conclusions can be drawn from the measured lifetimes in tryptamine and tryptophan. The tryptamine measurements serve as a control experiment to help evaluate the more complicated tryptophan results. Interactions between the indole ring and the alkyl side chain in both tryptamine and tryptophan may induce quenching of the fluorescence. Further, these interactions may be geometry dependent. The different conformer peaks in tryptamine correspond to different molecular geometries,[18,24] yet no difference in fluorescence lifetime is observed among them. Thus, tryptamine is a clear example of a molecule where conformation does not affect the fluorescence decay rate of the molecule in the gas phase. These results are consistent with previous observations that tryptamine has a single exonential decay in solution.[5]

In tryptophan molecular conformation has a measurable effect on the fluorescence lifetime, the lifetime of the A conformer being substantially different from the lifetimes of conformers B–D. Further, conformers B–D have lifetimes that are similar to the tryptamine conformers. The insignificant change in the lifetime between conformers B–D and the tryptamine conformers suggests that the addition of the carboxylic acid group has little effect on the photophysics of these conformers of tryptophan. If we assume that all of the tryptophan conformers have similar radiative rates, then conformer A must have an additional nonradiative process that produces the increase in the fluorescence decay rate. This new nonradiative rate must be approximately 30% of the overall fluorescence decay rates of conformers B–D to account for this observed decrease in the fluorescence lifetime.

Connecting the isolated molecule behavior with the solution data is not straightforward. The solvent will produce both static energy shifts and dynamics. Nonetheless, there are some qualitative similarities between the solution and gas-phase behavior as well as some quantitative differences. The fluorescence decay

of tryptamine is similar in solution and in the gas phase. In both cases the decay is single exponential, and the decay lifetimes are not that different (6.14 ns in pH 7 solution[5] versus 13.2 ns in the gas phase). It should be noted that the radiative rate is proportional to the square of the index of refraction of the medium, and therefore the radiative rate is almost twice as fast in solution as it is in the gas phase. This difference in radiative rates will account for at least part of the difference between the fluorescence lifetimes in solution and in the gas phase.

The fluorescence decay of tryptophan in the gas phase shows some qualitative similarities to the decay in solution, but there are quantitative differences. The fluorescence decay from individual conformers is single exponential, and this is necessary if the conformer model of solution photophysics is correct. However, it is not sufficient proof of the validity of the conformer model. The observation of different lifetimes for different conformers is qualitatively consistent with the conformer model. However, there are quantitative differences between the solution and the gas-phase behavior. The two lifetimes observed in pH 7 solution are 3.1 and 0.5 ns,[25] while in the gas phase they are 13 and 10 ns. It has recently been proposed[11] that in room temperature solution there is a rapid averaging among the three conformers generated by rotation about the α–β bond, and that the biexponential decay is produced by the two stable conformers generated by rotation about the β–γ bond. In the low-temperature jet, it has been shown that there is no interconversion among conformers on the time scale of the fluorescence lifetime, and this difference in interconversion dynamics may account in part for the difference in solution and gas-phase lifetimes.

However, even if the different interconversion rates play some role in producing media dependent lifetimes, it seems unlikely that they are entirely responsible for these differences. Whatever the averaging dynamics, the fact remains that both solution lifetimes are substantially shorter than both gas-phase lifetimes, and this suggests that there is an additional decay mechanism operating in solution that is not operating in the gas phase. There is considerable evidence[26] that photoionization takes place in tryptophan and other substituted indoles and that ionization competes with fluorescence as a mechanism for the decay of the electronically excited state. In solution, photoionization produces a solvated electron and a solvated cation, and it seems unlikely that this process would be possible in the gas phase at the excitation energies used in our experiments.

The time-resolved measurements also provide new insights into the previously observed[14] emission spectrum of tryptophan in the gas phase. In the previous work it was observed that excitation of conformer A (now known to be the short-lived conformer) produced an emission spectrum consisting of some sharp features near the excitation frequency plus an intense broad red-shifted feature. In all of the other comformers the broad red-shifted fluorescence was very much weaker. It was proposed that the emission spectrum of conformer A was due to the existence of two electronic states: a state with geometry similar to that of the ground state (state 1) and a second state with a different geometry (state 2). Optical transitions from the ground electronic state to state 1 would be allowed by the Franck–Condon principle, while transitions to state 2 would be forbidden. The observation that the excitation spectrum and part of the emission spectrum were sharp required that these two states be separated by a barrier in the potential surface, and the observation of the broad red-shifted fluorescence required that these states be mixed.

The time dependence of the fluorescence that would be predicted by such a model would depend on the details of the coupling and on the method used to prepare the excited electronic states, and our time-resolved measurements provide some insight into these details. If the laser pulse used to prepare the excited states were short enough, the Fourier transform frequency bandwidth could span all of the mixed vibronic states, and in this case the excitation would prepare a coherent superposition of all of the mixed states. At times short compared to the dephasing time of this coherent state, the emission would be characteristic of state 1 (sharp at frequencies near the excitation frequency), while at times long compared to the dephasing time, emission from the incoherent mixed state (both sharp and broad red-shifted emission) would be observed. Under these conditions, a rise in the intensity of the broad red-shifted emission would be observed. The dephasing time depends on both the density of states and the strength of the coupling between them, neither of which can be estimated. The fact that we were unable to observe a rise in the red-shifted emission implies that either a coherent superposition was not proposed by our 20-ps laser pulse or that the dephasing time was shorter than our time resolution (around 70 ps).

In addition to the time dependent measurements we have also observed high resolution fluorescence excitation spectra of conformers A–D of tryptophan. These high resolution spectra show a rich structure consisting of a great many features each of which was of the order of 250 MHz (0.008 cm^{-1}) wide. The structure is presumably totally or partially produced by rotational structure, and the line width is largely due to Doppler broadening. While these spectra are not yet analyzed, they do shed some light on the details of state mixing. If we were creating a coherent superposition, and if the dephasing time were shorter than 70 ps, this would produce a broadening in the frequency domain of around 2 GHz. In the pulsed experiment, our frequency resolution was insufficient to observe such broadening, and therefore we cannot say if whether or not the pulsed experiment prepared a coherent state. In the CW high resolution experiment we certainly would have observed such broadening, and this implies that in the CW experiment we were not producing a coherent state. This means that tryptophan is not a statistical limit molecule[27] ($\hbar\Gamma\rho$ is not much larger than 1, where Γ is the decay rate, and ρ is the density of states).

While the existence of two coupled excited states is implied by the absorption and emission spectra of tryptophan, there is some question about the nature of these states. In our previous work we suggested that state 1, the state with geometry similar to that of the ground state, was the ^1L_b electronic state of tryptophan similar to the ^1L_b state that has been positively identified in tryptamine.[23] We proposed that state 2, the state with a geometry different from that of the ground state, was due to an interaction between the indole chromophore and a specific site on the zwitterionic amino acid side chain. Such an interaction might be similar to the intramolecular electron-transfer interaction invoked by Chang et al.[8] to explain the conformer dependent lifetimes in solution, and its strength would vary among the different conformers.

Tryptophan is known to have at least two low-lying singlet states,[7,25] a ^1L_b and a ^1L_a state, and this could be an alternative explanation of the two states required by the current results. The relative position of these two states is known to be sensitive to the solvent environment.[7,25] By analogy to solution, it is possible that molecular conformation in the isolated gas-phase molecule could influence the energy gap between the states as well, producing a mixed state in the A conformer along.

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Registry No. Tryptamine, 61-54-1; tryptophan, 73-22-3.

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