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# THE TIME RESOLVED EMISSION SPECTRA OF PEPTIDE CONFORMERS MEASURED BY PULSED LASER EXCITATION.[1]

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SUMMARY: The fluorescence decays of a number of peptides were measured using pulsed laser excitation and time correlated single photon counting techniques. In all cases double exponential kinetics were observed with the short lifetime component (0.5 - 0.85 ns) predominating over the long lifetime component (1.62 - 2.21 ns). The lifetimes varied with the peptides measured. The time resolved emission spectra of LysTrpLys shows both components have similar spectral maxima at 345 nm. It is suggested that the dual exponential kinetics originate from different conformers of the peptides.

Recently we reported that the fluorescence decay of aqueous solutions of tryptophan was described by double exponential kinetics (3.1 ns and 0.5 ns) and that the fluorescence spectrum of tryptophan could be temporally resolved into two components with $\lambda_{max}$ 350 nm and 335 nm respectively (1,2). The most consistent rationalization of these results was that the fluorescence originates from rotamers about the $C_\alpha - C_\beta$ bond of the alanyl side chain of tryptophan.

In order to assess the generality and applicability of our rationalizations for monomer systems and extend the bases for the interpretation of multi-exponential decay kinetics in proteins we have investigated the fluorescence decay of a series of short polypeptides containing a single tryptophan residue.

The low fluorescence quantum efficiencies of these peptides makes the measurement of their fluorescence decays and particularly the time resolution of their fluorescence spectra difficult using pulsed gas spark lamps. In this work we took advantage of the special features of a sync-pumped, mode-locked

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and cavity dumped dye laser system as an excitation source and achieved both the temporal and spectral resolution required for these studies.

## Materials and Methods

All peptides were purchased from Research Plus (Bayonne, N.J.) except for AlaTrpAlaGly (a generous gift from R. Williams, NRCC). Their purity was checked by two-dimensional chromatography and electrophoresis. The peptides GlyTrpGly, GluTrpGlu, LysTrpLys and AlaTrpAlaGly required purification which was achieved by preparative thin layer chromatography on cellulose. Solutions of the peptides ($5 \times 10^{-5}$ M) in 0.01 M cacodylate buffer (pH 4.9) were degassed prior to the measurement of their fluorescence decays.

The fluorescence decay measurements were performed using a frequency doubled Spectra-Physics synchronously pumped dye laser system as the excitation source ($\lambda_{ex}$ 295 nm) (3). The fluorescence decay parameters were calculated by the non-linear least squares iterative convolution technique. The adequacy of their fit and the decay model was judged by inspection of the weighted residual plots (4). The emission was detected at right angles through a monochromator (3 nm bandpass) using photon counting electronics described previously (5) but with a Tracor Northern Model 1705 multichannel analyser. The channel width was 0.0203 ns/ch.

## Results and Discussion

The laser/instrument response function (FWHM = 0.85 ns) for LeuTrpLeu, was typical of those measured for other peptides reported in this work. Figure 1a is the weighted residual plot for the "best fit" single exponential decay function for this data. The residual plot in fig. 1b resulted after deconvolution using a double exponential function. It is clear from these residual plots and the significant decrease in the root mean square residual (RMSR) from 2.32 to 1.008 that the fluorescence decay of LeuTrpLeu obeys double exponential kinetics. The lifetimes obtained at $\lambda_{em}$ 330 nm were $\tau_1$ = 2.12 $\pm$ .01 ns and $\tau_2$ = 0.70 $\pm$ .015 ns with a ratio of pre-exponentials $\alpha_1/\alpha_2$ = 0.99.

For comparison the residual plot (fig. 1c) obtained after deconvolution for a single exponential decay in the case of N-acetyltryptophanamide is nearly random with a RMSR of 1.16 and a lifetime of 2.83 $\pm$ 0.002 ns.

Double exponential fluorescence decays were observed for the other small peptides and their decay parameters are summarized in Table 1. Included in this table are results obtained with our nitrogen lamp fluorescence lifetime system. The agreement between the laser generated data and the lamp generated results is very good. This together with the N-acetyltryptophanamide results

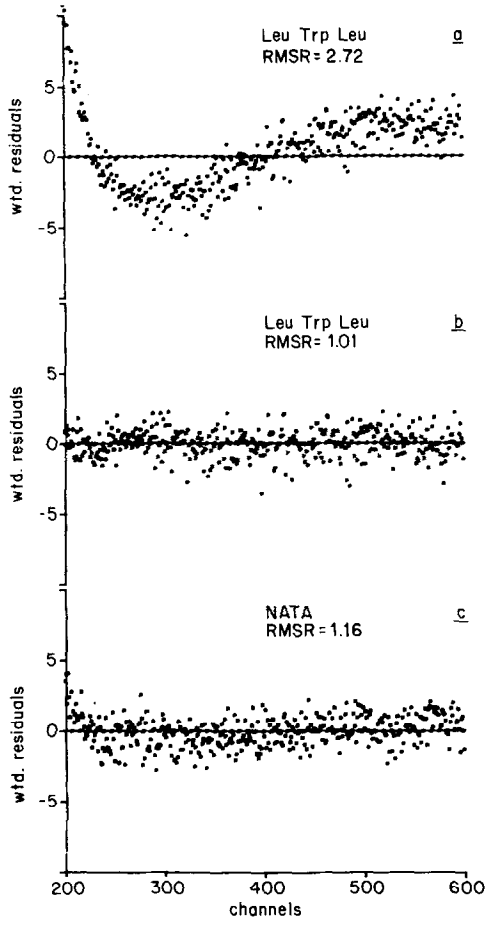


Figure 1. Weighted residual plots which are obtained from the "best fit" deconvolutions for the fluorescence decay: a) LeuTrpLeu, single exponential decay; b) LeuTrpLeu, double exponential decay; c) N-acetyltryptophanamide, single exponential decay; 20.3 ps/ch, $\lambda_{em}$, 330 nm, $\lambda_{ex}$, 295 nm.

confirms that the second exponential term in the fluorescence decay of the peptide does not originate from any instrumental artifacts.

There is very close agreement between the lifetime values at both 330 and 380 nm for all peptides except for AlaTrpAlaGly. In this latter case there is a significant difference in the value of the long lifetime component probably due to the small contribution it makes to the total fluorescence and hence higher error in its determination (4).

Recently Werner and Forster (6) reported their fluorescence decay measurements on several dipeptides, some larger peptides such as glucagon (29

Table 1. Fluorescence decay parameters[a] of peptides at 330 and 380 nm.

| Peptide | $\lambda_{em} \times nm^{-1}$ | $\tau_1 \times ns^{-1}$ | $\tau_2 \times ns^{-1}$ | R[b] |
|---|---|---|---|---|
| LysTrpLys | 330 | 2.21 | 0.85 | 0.83 |
| | 330[c] | 2.25 | 0.75 | 1.07 |
| | 380 | 2.20 | 0.89 | 0.97 |
| LeuTrpLeu | 330 | 2.12 | 0.70 | 0.99 |
| | 330[c] | 2.22 | 0.54 | 1.05 |
| | 380 | 2.14 | 0.80 | 1.06 |
| GluTrpGlu | 330 | 1.83 | 0.71 | 0.95 |
| | 380 | 1.86 | 0.77 | 1.03 |
| GlyTrpGly | 330 | 1.64 | 0.71 | 0.60 |
| | 380 | 1.71 | 0.75 | 0.57 |
| LeuTrpMet | 330 | 2.02 | 0.74 | 1.0 |
| | 380 | 1.99 | 0.71 | 1.08 |
| AlaTrpAlaGly | 330 | 2.72 | 1.02 | 0.038 |
| | 330[c] | 3.10 | 1.02 | 0.075 |
| | 380 | 3.38 | 1.06 | 0.027 |

[a] typical standard errors for $\tau_1$, were ±.01; for $\tau_2$ were 0.015; for R were ±0.05 except for AlaTrpAlaGly which was R = 0.038±.005 at 330 nm, R = 0.027±.002 at 380 nm, and R = 0.075±.02[c].

[b] ratio of pre-exponentials

[c] measured with nitrogen lamp excitation.

residues), GlyTrpGly and GlyTrpGlyGly. Their results on the latter two peptides contrast with ours (AlaTrpAlaGly being compared with GlyTrpGlyGly). We find two exponential fluorescence decaying components for these peptides while they report only a single exponential decay. Their reported lifetimes correspond with the most populous component observed in our measurements. We attribute this difference to the higher resolution of the picosecond laser/single photon counting fluorescence lifetime system and to our use of a monochromator (2 nm bandpass) rather than broad band filters for the detection of the emission.

The fluorescence spectrum of LysTrpLys was time resolved by measuring the fluorescence decay at several emission wavelengths. At all wavelenghts double exponential decay kinetics were observed with similar lifetimes at each wavelength, $\tau_1$ = 2.22 ± .07 ns, $\tau_2$ = 0.86 ± .05 ns with a small variation in the proportions of the two components. The emission spectra of both components were calculated according to expressions outlined earlier (1) and

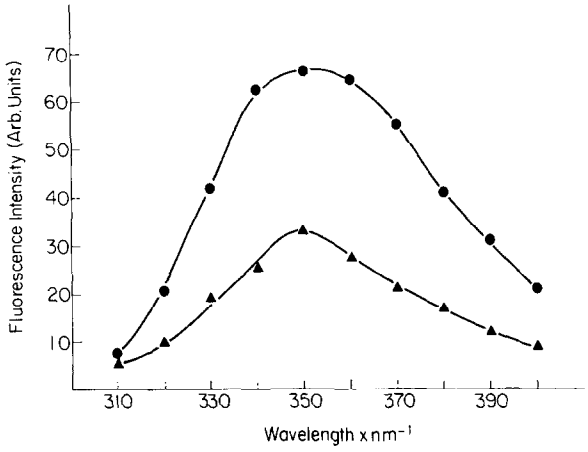


Figure 2. Time resolved fluorescence spectra of LysTrpLys at pH 4.9 26°C. Curve a, 2.2 ns component; Curve b, 0.9 ns component.

are shown in figure 2. Both have a maximum at ∿350 nm. The ratio of the concentrations of the 0.9 ns and 2.2 ns components [0.9]/[2.2] was estimated from these spectra according to the expression:

$$\frac{[0.9]}{[2.2]} = \frac{I_{0.9}}{I_{2.2}}_{max} \times \frac{2.2}{0.9}$$

where $I_{0.9}/I_{2.2}$ is the ratio of the fluorescence intensities at the spectral maximum of the 0.9 ns and 2.2 ns components. The concentration ratio was found to be 1.12.

These results contrast with those obtained for aqueous tryptophan. In the peptides the short lifetime component is present in higher concentration than the long lifetime component. Moreover in LysTrpLys both components have similar fluorescence spectra.

Following from our rationalization of the dual exponential decay observed for aqueous tryptophan fluorescence in terms of different rotamers, we suggest that the fluorescence decay behaviour in the tripeptides originates from different conformers of the indolyl ring in these molecules.

In a recent (7) nmr study of oligopeptides containing tryptophan, the fractional population of each of three rotamers about the $C_\alpha - C_\beta$ bond of the tryptophyl residue was estimated. On examination of space filling models of the most populous ground state rotamer, that with the amide carbonyl on the

α-carbon staggered between the two hydrogens on the β-carbon, the indolyl residue may readily take up a position in even closer proximity to the terminal amino group than in tryptophan. We have found (1) that the ammonium group is a very efficient quencher of indole fluorescence. Therefore we suggest the assignment of the fast decaying component as this, the most populous rotamer. This assignment is supported when we compare the fractional population of this rotamer as measured by n.m.r. (for GlyTrpGly) and of the fractional amount of the 0.9 ns component in LysTrpLys and find them to be identical (0.53).

Inspection of the ratio of pre-exponentials and lifetimes measured at 330 and 380 nm for the tripeptides given in Table 1 suggests (a) that the two components in these molecules have similar spectra and (b) that the fluorescence decay parameters are not much affected by the nature of the amino acid residues.

The results for the tetrapeptide AlaTrpAlaGly are markedly different from the tripeptides with the ratio of pre-exponentials being ∿0.03. This implies that the fractional population of the conformers in this peptide has changed significantly.

Clearly several additional experiments are suggested, particularly full spectral resolution, in order to elaborate the origin of these observations.

Our results show that N-acetyltryptophanamide is not an appropriate model for interactions of the peptide bonds with the indolyl ring in proteins. The results suggest that the dual exponential decay kinetics originate from changes in the interactions of the indole ring in different conformers of the peptide rather than for local interactions with the peptide backbone at the point where the indole ring is attached.

The results demonstrate the improved capability of a synchronously pumped, picosecond dye laser/single photon counting fluorescence decay system to resolve temporal components in peptide studies.

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