NON-EXPONENTIAL DECAY OF INDOLE FLUORESCENCE - THE RED-EDGE EFFECT*

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SUMMARY

The fluorescence of indole in glycerol decays exponentially at both 22° and -80°C for 280 nm excitation. Under 296 nm excitation the 22°C emission decay is exponential, but at -80°C, a second shorter lived (1.3 ns) component is also detected. The relevance of this result to the determination of protein fluorescence lifetime is described.

INTRODUCTION

Galley and Purkey have observed a red shift of the fluorescence from indole dissolved in a rigid glass when the irradiation wavelength was changed from 280 to 295 nm (1). This phenomenon, a manifestation of the "red-edge" effect (2), is related to a reduction of the energy transfer efficiency in concentrated indole solutions upon irradiation in the 300-305 nm region (3).

For 280 nm excitation the fluorescence decay of human serum albumin is non-exponential with two major components, 1.5 ns (ascribed to tyrosine) and 6.17 ns (assigned to tryptophan), and a minor tryptophan component (12.08 ns) (4). The 295 nm excited emission is also multiple, but it is unlikely that at this wavelength the fast component originates in tyrosine since the relative absorption of tyrosine is too small at 295 nm to explain the time resolved spectra presented by Wahl and Audet. Tryptophan emission in proteins can be divided roughly into three classes depending upon the environment of the chromophore: fully exposed to solvent ($\lambda_{max} \simeq 350$ nm), solvent immobilized ($\lambda_{max} \simeq 340$ nm), and hydrophobic ($\lambda_{max} \simeq 330$ nm) (5).

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Each tryptophan residue can have a different lifetime. In addition, non-exponential decay is observed from a single chromophore when the chromophore lifetime and the solvent relaxation times are of comparable magnitude (6). However, micro-environmental heterogeneity in the ground state may also be a source of multiple emissions. We now report non-exponential indole emission due to such heterogeneity.

EXPERIMENTAL

Indole (Aldrich Chemical Co., 99+%) and glycerol (Matheson, Coleman and Bell) were used without further purification. 10^{-3}-10^{-4}M solutions were degassed by three cycles of the freeze-pump-thaw technique. Lifetimes were determined with an ADP-doubled, N_2 laser pumped tunable Rhodamine 6G dye laser (7). The exciting light was passed through a 7-54 Corning glass filter and the 7-51 Corning filter was placed in front of the photomultiplier, when the fluorescence emission was monitored. The emission spectra were recorded with an instrument of conventional design using a PEK 150W Xe lamp for excitation. The spectra were corrected against a Rhodamine B quantum counter.

RESULTS AND DISCUSSION

The emission spectrum of indole in glycerol is independent of excitation wavelength (280 or 295 nm) at 22°C with λ_{max} = 332 nm. At -70°C a marked red shift is observed when the excitation is changed from 280 to 295 nm (Fig. 1). The shift in the maximum is smaller in glycerol (550 cm^{-1}) than in ethylene glycol-water solutions (~900 cm^{-1}) (1), but there is a marked broadening on the low energy side of the 295 nm indole spectrum in glycerol.

Since the fluorescence lifetimes are not long compared to the duration of the exciting light some type of convolution analysis is required. The phase plane graphical method of Demas and Adamson (8) is very useful in detecting deviations from a single exponential decay, but in order to extract multiple lifetimes from non-exponential decays, another procedure must be used. We have fitted a double exponential to the measured intensity profile by the curve simulation method (9). The phase plane plots of the -80°

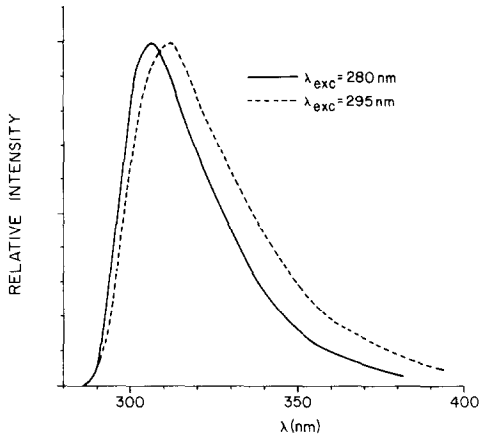

Fig. 1 Corrected emission spectra of indole in glycerol at -70°C at different excitation wavelengths.

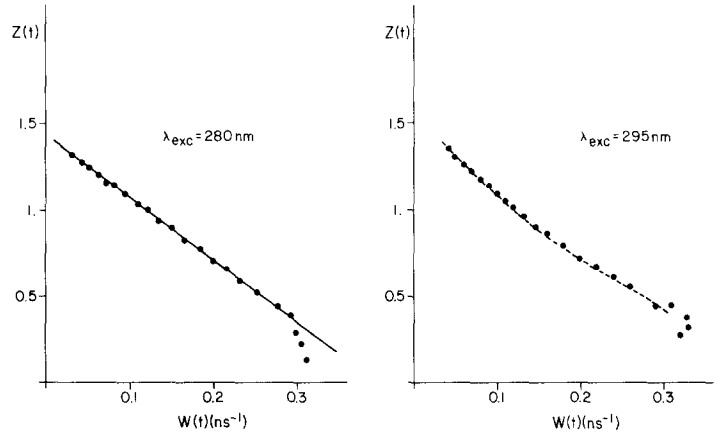

Fig. 2 Phase plane plots for emission of indole in glycerol at -80°C. A linear plot corresponds to exponential decay with the slope equal to $-\tau$. Z(t) and w(t) are defined in reference 8.

results are shown in Fig. 2. The last five points (smallest Z) can be ignored since they correspond to the shortest times after the flash initiation and the errors are large. The remaining points lie on a very good line and indicate a single exponential decay at λ_{exc} = 280 nm with τ = 4.70 ns. However with 295 nm excitation, the decay is clearly non-exponential. At 22°C the phase plane plots are linear at both 280 and 295 nm (τ = 3.6 ns). The lifetimes

Table I. Lifetimes of Indole in Glycerol

T(°C)	λ_{exc} = 280 nm τ(ns)	λ_{exc} = 295 nm
22°	3.65 ± .1	3.4 ± .1
-80°	4.7 ± 0.1	4.4 ± 0.4 1.3 ± 0.1

computed by the curve simulation convolution analysis are summarized in Table I.

The wavelength independence of the 22°C lifetime and the exponential decay indicates that the relaxation time of the glycerol solvent environment is shorter than the 3.6 ns indole lifetime. On the other hand, the -80°C results correlate with the red edge shift of the emission spectrum and can be ascribed to micro-environmental heterogeneity. In this interpretation the glycerol relaxation time is much longer than the lifetime of the indole excited state and the environment of each excited indole molecule is fixed. The lifetimes of "average" indole molecules, i.e., the overwhelming majority, are essentially the same and only a single exponential decay results at most excitation wavelengths. When the red edge of absorption is excited at 295 nm a special class of indole molecules is photoselected, viz., those with glycerol molecules oriented to maximize solute-solvent interactions. The transition energies for the red-edge species are lower than normal, hence the red shift of the emission. The red edge molecules also have a shorter lifetime, 1.3 ns. However, a substantial portion of the molecules excited at 295 nm are normal and the 4.4 ns component corresponds, within experimental error, to the normal 4.7 ns lifetime.

The results for indole in ethylene glycol-water (1:1 v/v) are analogous in all respects but one. At 20° and 281 nm excitation, τ = 4.6 ns. Under 296 nm excitation there is again a marked non-exponentiality at -75°C, but

there might also be a slight non-exponentiality even at 20°C. This may be due to the properties of the mixed solvent and we feel that homogeneous solvents are to be preferred in studies of this type.

We offer no explanation for the smaller lifetime of the red-edge species but a similar reduction in lifetime has been observed in rigid solutions of $Cr(CN)_6^{3-}$ (10). It should be kept in mind that even shorter lived species may be excited at 295 nm. The emission of such short-lived indole molecules would be "lost" in the envelope of the excitation pulse. However, in steady experiments, such short-lived emitters would contribute.

Galley and Purkey explained the failure of homotransfer in concentrated indole solutions under red-edge excitation on energetic grounds, i.e., the molecules in the red-edge do not have sufficient energy to excite the neighboring molecules (1). Although this viewpoint has merit, it should be recognized that the shorter lifetime of the red-edge moieties may also be a source of the reduced energy transfer.

The observation of multiple decays when indole in rigid environments is excited at 295 nm, suggests that caution should be exercised when interpreting emission lifetimes in proteins. In order to minimize interference from tyrosine emission, 295 nm excitation is often employed. If the emission comes from tryptophan residues well exposed to solvent, it is unlikely that the red-edge effect will be operative in fluid solutions. However, tryptophan residues in hydrophilic environments of immobilized solvent may well be subject to this effect. Perhaps the 1.5 ns component observed when human serum albumin is excited at 295 nm (4) is due to red edge tryptophan species.

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