Subpicosecond Fluorescence Anisotropy Measurements of Tryptophanyl Residues in Proteins

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The fluorescence anisotropy of tryptophanyl residues in proteins is used as a probe of protein motion. We have recently shown [1] that the short time (200 fs - 10 ps) region of the fluorescence anisotropy of tryptophan in solution is dominated by an electronic relaxation process, which has the potential to obscure molecular motion. The question arises as to the relevance of this result for the behavior of tryptophanyl residues in proteins. To address this question we have examined the subpicosecond fluorescence anisotropy of tryptophanyl residues in two very different environments - exposed to the solvent in melittin, a 26 amino acid peptide, and buried in a hydrophobic region in apoazurin purified from the bacteria Pseudomonas aeruginosa. Measurements were made by ultraviolet fluorescence upconversion described elsewhere [1]. Samples were degassed and flowed in absence of air. Concentrations were adjusted so the optical density at the excitation wavelength was between 0.6 and 1.0 for a 1 mm pathlength.

Tryptophan has two low-lying singlet-states, $^1L_a$ and $^1L_b$. In melittin the tryptophanyl residue is exposed to water and $^1L_a$ is of lower energy than $^1L_b$, whereas the tryptophanyl residue in apoazurin is buried in the protein, inaccessible to water, and $^1L_b$ is lower [2, 3]. Fluorescence emitted by the tryptophanyl residue in melittin originates primarily from the $^1L_a$ state. During the first few picoseconds a fast component dominates the fluorescence and anisotropy decays as observed for tryptophan in water [1]. There are remarkably similar trends in the excitation and emission wavelength dependence for melittin and tryptophan. Figure 1 compares the parallel anisotropy components of the tryptophanyl residue in melittin to those of tryptophan in water. For tryptophan in water this fast component has been attributed to rapid level kinetics between $^1L_a$ and $^1L_b$ [1]. The characteristic shape of the parallel and
Fig. 1. Comparison of the parallel anisotropy components collected at various emission wavelengths from 300 nm excitation of the tryptophanyl residue in melittin (on the left) to those of tryptophan in water.

perpendicular (not shown) curves results from rapid decay components in both the population and anisotropy curves. We are able to simulate our results using the model described in references 1 and 4 (see figures 2 and 3) with similar parameters as those employed for the tryptophan simulations. The only parameters that are significantly altered from those for the simulation of tryptophan in water are the excitation and detection ratios, which are defined as the

Fig. 2. Comparison of the parallel and perpendicular anisotropy components collected at 330 nm from 300 nm excitation of the tryptophanyl residue in melittin (on the left) to those from a simulation of the data generated by the model discussed in the text.
fraction of absorption and emission, respectively, that is contributed by the $^1L_b$ state to the sum of contributions from both the $^1L_a$ and $^1L_b$ states. This is consistent with the differences observed between both absorption and fluorescence spectra of melittin in water and tryptophan in water. Further we report the first measurement of an initial anisotropy of 0.40 for a tryptophanyl residue in either a peptide or a protein. This result is shown in figure 3 for melittin with 300 nm excitation.

By contrast, fluorescence and anisotropy decays of apoazurin excited with 292 nm light exhibit no fast component, as observed for those of melittin, however a low initial anisotropy is observed. This is consistent with the $^1L_a$ and $^1L_b$ states being nearly degenerate (within 200 cm$^{-1}$, compared to the value of 500 cm$^{-1}$ assigned to the energy separation of states for tryptophan in water), and the interconversion being faster than our resolution.

Our results demonstrate that fluorescence anisotropy decays are convoluted with nonrotational contributions originating from a population decay from one excited singlet state to another.

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References