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On the Use of Tryptophan to Detect Slow Orientational Motions

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For our investigations on orientational motions of membrane proteins with the method of time-resolved fluorescence anisotropy decay (TRFA), we first wanted to test the use of tryptophan fluorescence in detecting slow motions on the time scale of tens of nanoseconds and to get more insight into the possible motions of membrane proteins by investigating a simple system. We performed TRFA measurements on a short α-helical 21-amino acid peptide in different environments (Vogel, H., *et al. Proc. Natl. Acad. Sci. USA* **85,** 5067–5071, 1988). In each case, we got three relaxation time constants. The longest of these depends strongly on changes in the environment, whereas the two shorter times show only weak dependencies. So we conclude that the longest time belongs to the rotational diffusion of the entire peptide and the other to internal motions.

KEY WORDS: Tryptophan; time-resolved fluorescence anisotropy decay; slow orientational motions.

SAMPLES

Our fluorescence probe is a hydrophobic α-helical peptide, obtained from W. Beck, with the sequence H–(Ala–Aib–Ala–Aib–Ala)$_3$–Trp–(Ala–Aib–Ala–Aib–Ala)–O–Me with Aib denoting aminoisobutyric acid. We choose four environments: (i) butanol; (ii) phospholipid vesicles made of 80% POPE (1-palmitoyl-2-oleolyl-phosphatidylethanolamin) and 20% POPG (1-palmitoyl-2-oleolylphosphatidylglycerol), in buffer; (iii and iv) detergent micelles of DodOMalt (*n*-dodecylmaltosid), diluted in water (iii) and water with 0.2 *M* lactose (iv), which increases the viscosity by 20%. The latter two cases the peptide is embedded in a micelle and, therefore, has a larger effective volume. The peptide concentration was always 30 μ*M*.

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RESULTS

The method of TRFA allows measurement of the orientational motions of fluorophors. In a viscous medium, the entire system may undergo rotational diffusion with a time constant proportional to the viscosity η. The internal motions of the system should not, or only weakly, depend on η. We obtain for our above-described samples, in each case, three different correlation time constants, as shown in Table I.

Table I. Relaxation Times ϕ and Residual Anisotropy R_∞ of the Tryptophan Residue of the Peptide in Different Environments.

	Rot. time constants (ns)			
Environment	ϕ_1	ϕ_2	ϕ_3	R_∞
Butanol	0.3	1.4	3.0	0
POPE/PG	0.1	1.6	10.7	0.026
Micelles	0.6	2.1	16.5	0
Micelles + lactose	0.8	3.0	20.0	0

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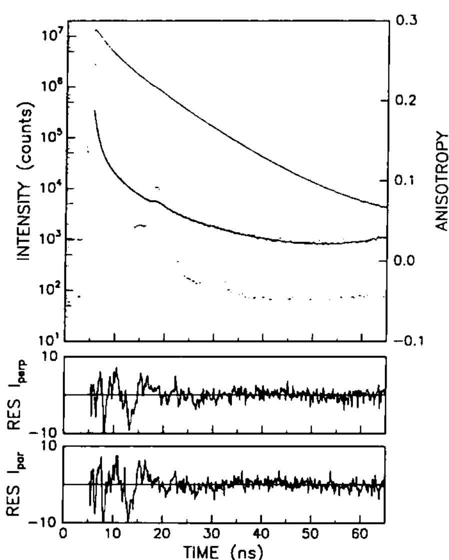

Fig. 1. Typical plot of measured data. The upper part shows the fluorescence intensity $I_{\parallel}(t)$, parallel polarized to the excitation polarization, the measured and the calculated anisotropy $R(t) = \frac{(I_{\parallel}-I_{\perp})}{(I_{\parallel}+2I_{\perp})}$, and the fluorescence standard for deconvolution. Typical fluorescence lifetimes (ns) and their relative amplitudes (in parentheses) are 1.0 (13%), 2.4 (17%), 4.8 (28%), 6.3 (42%), and 23.1 (0.3%). The lower parts show the weighted residuals of $I_{\parallel}$ and $I_{\perp}$, serving as criteria for the goodness of the calculated curves.

Additionally shown in Table I is the value of R_{∞}, the anisotropy at long times. Only for case (ii), with the peptide incorporated in the lipid membrane, does one expect a finite value of R_{∞}, due to the limited rotational motion, resolved from the incapability of the hydrophilic peptide headgroups to dip into the hydrophobic interior of the membrane.

DISCUSSION

Many difficulties of these measurements result from the short mean fluorescence lifetime of the tryptophan, approximately 3 ns. To resolve relaxational times at 20 ns, the investigated time window should be at least 60 ns. Fortunately, we found five resolvable lifetimes, four in the range up to 10 ns and one at 23 to 30 ns (depending on the environment). The latter one is present with a very low relative amplitude but serves at times greater than 50 ns for more than 90% of the detectable light. Nevertheless, to get an acceptable signal-to-noise ratio, we had measurement times around 15,000 s for each polarization direction, multiplied by a factor 2, once for the sample and another for the control sample. Due to these long measurement cycles (overall ≈24 h), the stability of the synchronously pumped cavity-dumped dye laser is a serious problem.

Our results demonstrate that there are two classes of orientational time constants. The first belongs to internal motions of the peptide, such as relative motions of the tryptophan side chain, with times in the range of a few hundred picoseconds up to 2 or 3 ns. The longer time, which depends strongly on the viscosity of the environment of the sample, should belong to the rotational diffusion of the entire peptide.

With respect to our further investigations on larger proteins in vesicle membranes, we have learned how to classify the measurable time constants. So this is another step forward in our understanding of the behavior of membrane proteins.