Eur Biophys J (1985) 13: 59–64

European Biophysics Journal

Studies of the fluorescence from tryptophan in melittin

C. D. Tran[1] and G. S. Beddard[2]*

[1] Department of Physics, Jackson State University, Jackson, MS 39217, USA
[2] Department of Chemistry, University of Manchester, Manchester M139PL, England

Accepted May 16, 1985

Abstract. The fluorescence lifetime and rotational correlation time of the tryptophan residue in melittin, as both a monomer and tetramer, have been measured between pH 6 and 11. The fluorescence decays are non-exponential and give lifetimes of 0.7 ± 0.1 ns and 3.1 ± 0.1 ns. This emission is consistent with a model in which the tryptophan residue is in slightly different environments in the protein. In a dilute solution of monomer the mean fluorescence lifetime is 2.3 ± 0.1 ns, below pH 10, but falls to 1.7 ns at higher pH. In contrast, the melittin tetramer has a mean fluorescence lifetime of only 2.2 ns at pH 6, which falls to 1.9 ns by pH 8, and falls again above pH 10 to the same value as in monomeric melittin. The behaviour between pH 6 and 8 is explained as the quenching of the Trp residue by lysine groups, which are near to the Trp in the tetramer but in the monomer, are too distant to quench. Fluorescence anisotropy decays show that the Trp residue has considerable freedom of motion and the range of "wobbling" motion is $35 \pm 10°$ in the tetramer.

Key words: Melittin, fluorescence, lifetime, anisotropy, tetramer

1. Introduction

One of the main constituents of bee venom is melittin, and the pharmacological properties of this protein are thought to arise from its ability to disrupt membrane functions by strong membrane binding. Melittin has 26 residues and contains 6 positive charges positioned at the terminal glycine, the three lysines and the two arginines, but has no negative charges. The amino-acid sequence is NH_3-Gly-Ile-Gly-Ala-Val-Leu-Lys-Val-Leu-Thr-Thr-Gly-Leu-Pro-Ala-Leu-Ile-Ser-Trp_{19}-Ile-Lys_{21}-Arg-Lys_{23}-Arg-Gln-Gln-CO-NH_2.

The solution conformation of this single tryptophan protein is of interest as it is easily influenced by its environment. At low protein concentration, Bello et al. (1982) have shown from C.D. measurements that melittin exists as a flexible, largely random extended chain with little α-helix content. Increasing the concentration of the protein or solution, ionic strength promotes the formation of water-soluble tetramers with largely α-helical conformations (Bello et al. 1982; Knoeppel et al. 1979; Faucon et al. 1979; Talbot et al. 1979). Quay and Condie (1983) have measured the equilibrium constant for the monomer-tetramer reaction and found that the decrease in free energy on tetramer formation is dominated by the increase in entropy.

The crystal structure of the tetramer has been elucidated by Terwilliger et al. (1982). Each of the melittin chains is present as a bent (at an angle of 120°) α-helix. The four proteins chains group together in the tetramer, such that the mainly hydrophobic groups, such as Trp, Leu and Ile, are on the inside. The charged residues are on the outside, next to the aqueous solvent where ion-dipole forces assist in making the tetramer soluble.

In this paper were report the determination of the fluorescence lifetimes and rotational relaxation times of monomeric and tetrameric melittin and observe transitions in fluorescence lifetimes which monitor an apparent pK of about 10.5 in dilute melittin solutions and an additional pK at 6.6 in the tetramer.

2. Experimental procedures

The melittin was purchased from Sigma Ltd. and purified by gel chromatography with Sephadex G-50, as described by King et al. (1976). The solutions of the purified, homogeneous melittin were

* To whom offprint requests should be sent

prepared in 0.02 M phosphate buffer. Melittin concentrations were determined spectrophotometrically using the extinction coefficient value of 56,900 $dm^3/M \cdot cm$ at 280 nm (Bello et al. 1982; Knoeppel et al. 1979). For each measurement fresh melittin solutions of 3.0, 8.0 or 60.0×10^{-5} M were prepared. Several pH values between 5.5 and 11 were used.

The fluorescence lifetimes and rotational correlation times were measured by the single-photon counting, delayed-coincidence method, using 300 nm excitation pulses from a frequency-doubled, picosecond, dye-laser. This technique and the data analysis methods have been described in detail elsewhere (Beddard and Tran 1985; Tran and Beddard 1982). The data analysis is briefly summarised below.

The anisotropy $r(t)$ was obtained from the observed fluorescence intensities, $I_{\parallel}(t)$ and $I_{\perp}(t)$ using the formula

$$r(t) = (I_{\parallel}(t) - I_{\perp}(t))/(I_{\parallel}(t) + 2I_{\perp}(t)). \quad (1)$$

A two-exponential function $r(t)$, Eq. (2), was used to model the experimentally observed anisotropy, $r(t)$.

$$r(t) = r_0(f \cdot \exp(-t/\varphi_a) + (1-f) \cdot \exp(-t/\varphi_b)). \quad (2)$$

The correlation time, φ_a, arises from the rotational motion of the protein and the correlation time, φ_b, from the combined motion of the Trp residue and the rotational diffusion of the whole protein. In the simplest case, the rotational correlation of the Trp residue φ_t, is related to φ_b and φ_a by;

$$1/\varphi_t = 1/\varphi_b - 1/\varphi_a. \quad (3)$$

Three sets of fluorescence anisotropy decay times were averaged and are listed in Table 2. A non-linear, least-squares method was used (Beddard et al. 1980). Analysis with convolution was not necessary for the anisotropy data since the width of the excitation pulse (250 ps) was less than the smallest lifetime measured. An example of an anisotropy decay is shown in Fig. 1.

The model of Kinosita et al. [1977] was used to analyse the restricted motion of the Trp residue within the protein. In this model the cone semi-angle (α) through which the probe molecule rotates may be calculated from the pre-exponential (f) in Eq. (2). We use α as a measure of the relative freedom of the Trp within the protein.

Fluorescence lifetime data were also analysed by using an equation of the form of Eq. (2) and the results are shown in Table 1. The lifetimes are τ_1 and τ_2 instead of φ_a and φ_b in Eq. (2). In each experiment the χ^2 parameter was significantly smaller for two exponential fitting (Eq. (2)) than for a single exponential. In Fig. 3 are shown some

Table 1. Fluorescence decay times of melittin

Concentration [M]	pH	τ_1 [ns]	f	τ_2 [ns]	τ_m [ns]
3×10^{-5}	6.2	3.10 ± 0.16	0.70	0.63 ± 0.03	2.36 ± 0.12
	6.9	3.14 ± 0.16	0.71	0.60 ± 0.03	2.40 ± 0.12
	7.9	3.20 ± 0.16	0.67	0.66 ± 0.03	2.36 ± 0.12
	8.3	3.19 ± 0.16	0.64	0.71 ± 0.04	2.30 ± 0.11
	8.9	3.15 ± 0.16	0.64	0.62 ± 0.03	2.24 ± 0.11
	9.4	3.24 ± 0.16	0.64	0.64 ± 0.03	2.30 ± 0.11
	10.3	3.15 ± 0.16	0.63	0.68 ± 0.03	2.23 ± 0.11
	10.9	2.92 ± 0.15	0.49	0.66 ± 0.03	1.77 ± 0.08
8×10^{-5}	6.3	3.17 ± 0.16	0.68	0.57 ± 0.03	2.34 ± 0.12
	7.0	3.20 ± 0.16	0.66	0.66 ± 0.03	2.35 ± 0.12
	7.5	3.11 ± 0.16	0.65	0.64 ± 0.03	2.23 ± 0.12
	7.9	3.07 ± 0.15	0.60	0.68 ± 0.03	2.15 ± 0.11
	8.6	3.04 ± 0.15	0.60	0.72 ± 0.04	2.11 ± 0.11
	8.9	3.03 ± 0.15	0.60	0.80 ± 0.04	2.14 ± 0.11
	9.3	3.05 ± 0.15	0.60	0.69 ± 0.04	2.10 ± 0.11
	9.9	3.14 ± 0.16	0.59	0.76 ± 0.04	2.15 ± 0.11
	10.4	3.04 ± 0.15	0.56	0.70 ± 0.04	2.00 ± 0.10
	11.0	2.93 ± 0.15	0.45	0.65 ± 0.03	1.67 ± 0.08
6×10^{-4}	5.5	3.05 ± 0.15	0.64	0.61 ± 0.03	2.17 ± 0.11
	6.1	3.04 ± 0.15	0.63	0.69 ± 0.03	2.17 ± 0.11
	6.8	2.92 ± 0.15	0.57	0.72 ± 0.04	1.97 ± 0.10
	7.6	2.89 ± 0.14	0.54	0.76 ± 0.04	1.91 ± 0.10
	8.0	2.85 ± 0.14	0.57	0.72 ± 0.04	1.93 ± 0.10
	8.3	2.94 ± 0.15	0.56	0.78 ± 0.04	1.97 ± 0.10
	9.8	2.92 ± 0.15	0.54	0.68 ± 0.03	1.89 ± 0.10
	11.0	2.87 ± 0.14	0.45	0.70 ± 0.04	1.67 ± 0.08

Table 2. Fluorescence anisotropy decay of melittin

[M]	pH	φ_a [ns]	f	φ_b [ns]	φ_t [ns]	α
3×10^{-5}	6.3	1.72 ± 0.09	0.36	0.52 ± 0.03	0.74	46
	7.4	1.76 ± 0.09	0.19	0.53 ± 0.03	0.76	57
	8.2	1.97 ± 0.10	0.15	0.59 ± 0.03	0.85	57
	8.6	1.80 ± 0.09	0.17	0.53 ± 0.03	0.75	54
	9.6	1.71 ± 0.09	0.49	0.51 ± 0.03	0.73	51
	10.1	1.76 ± 0.09	0.46	0.53 ± 0.03	0.75	47
	10.4	1.80 ± 0.09	0.21	0.55 ± 0.03	0.78	54
8×10^{-5}	7.0	1.87 ± 0.09	0.51	0.48 ± 0.02	0.64	38
	7.5	1.81 ± 0.09	0.57	0.52 ± 0.03	0.73	34
	7.9	2.12 ± 0.11	0.61	0.45 ± 0.02	0.57	32
	8.6	1.90 ± 0.10	0.60	0.51 ± 0.03	0.69	33
	8.9	1.95 ± 0.10	0.57	0.44 ± 0.02	0.57	34
	9.3	1.84 ± 0.09	0.53	0.61 ± 0.03	0.91	36
	9.9	2.10 ± 0.11	0.60	0.61 ± 0.03	0.86	33
	10.4	2.48 ± 0.12	0.56	0.57 ± 0.03	0.74	34
6×10^{-4}	5.5	2.30 ± 0.12	0.39	0.71 ± 0.04	1.03	44
	6.1	2.57 ± 0.12	0.58	0.76 ± 0.04	1.07	34
	6.8	2.25 ± 0.11	0.43	0.77 ± 0.04	1.16	42
	7.6	2.38 ± 0.12	0.56	0.70 ± 0.04	0.99	35
	8.0	2.45 ± 0.12	0.57	0.69 ± 0.04	0.96	35
	8.3	2.30 ± 0.12	0.57	0.70 ± 0.04	1.01	34
	9.8	2.51 ± 0.13	0.56	0.81 ± 0.04	1.20	35
	11.0	2.06 ± 0.10	0.77	0.72 ± 0.04	1.10	24

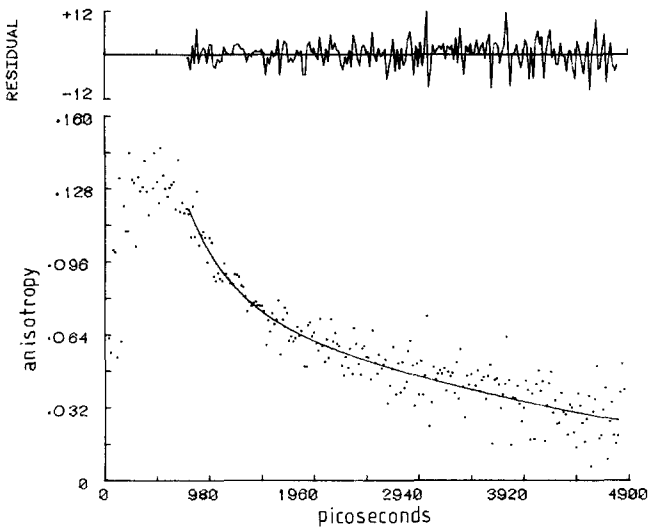

Fig. 1. Fluorescence anisotropy decay of melittin at $8 \times 10^{-5} M$ and pH 10.4. The fit (solid line) is for Eq. (2) with $\varphi_a = 2.47$ ns and $\varphi_b = 570$ ps

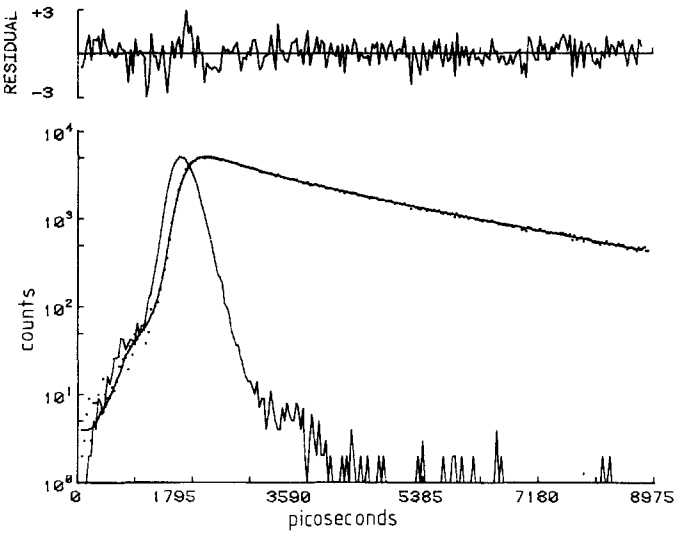

Fig. 3. Fluorescence decay of Trp_{19} residue in melittin at pH 9.9 and $8 \times 10^{-5} M$. The fit (solid line) to the data points (dots) is for a sum of two exponential functions convoluted with the instrumental response function, which is the narrow solid line. The χ^2 for these data is 1.05 with $\tau_1 = 3.16$ ns and $\tau_2 = 790$ ps

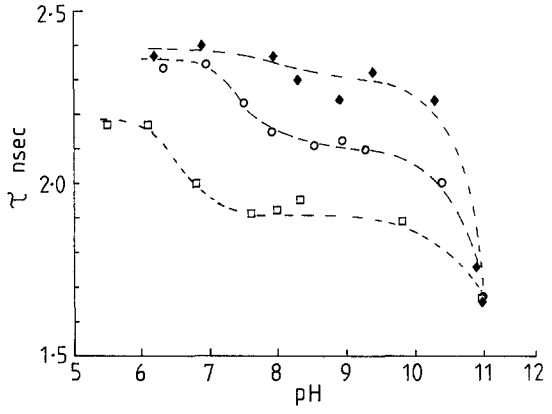

Fig. 2. Mean fluorescence decay times $\tau_m (\tau_m = f \cdot \tau_1 + (1-f)\tau_2)$ versus pH for melittin concentrations of: (top points (◆)) $3 \times 10^{-5} M$, (○) $8 \times 10^{-5} M$, and (□) $6 \times 10^{-4} M$ in 0.02 M phosphate buffer

fluorescence lifetime data taken at pH 9.9 and $8 \times 10^{-5} M$ melittin. The reduced χ^2 measured over all data points was 1.05.

3. Results and discussion

Fluorescence measurements from several small proteins, each containing a single Trp residue, have shown that two fluorescence lifetimes are needed to describe the Trp excited state behaviour (Munro et al. 1979; Beddard et al. 1980; Tran et al. 1982; Lakowicz et al. 1983; Beddard and Tran 1985). In melittin the fluorescence lifetimes, from pH 6.2 to 10.9, are 0.7 ± 0.1 ns and 3.1 ± 0.1 ns. Detailed values are given in Table 1. The lifetimes at pH 7 are similar to those previously measured by flash-lamp excitation (Georghiou et al. 1981) and by the phase method (Lakowicz et al. 1983). The mean fluorescence lifetimes (τ_m) are shown in Fig. 2 for each of the concentrations studies. The upper-most data points correspond to the most dilute solution ($3 \times 10^{-5} M$) the lower points to the most concentrated ($6 \times 10^{-4} M$).

The motion both of the probe (Trp residue), and of the whole protein is reflected in the fluorescence anisotropy decay. This was observed experimentally as well as being predicted by the theory of fluorescence depolarisation (Rigler and Ehrenberg 1973; Kinosita et al. 1977). Figure 1 shows fluorescence anisotropy data taken at pH 10.4 and $8 \times 10^{-5} M$ and analysed according to Eq. (2). The longer correlation time, φ_a, which we assign to the motion of the whole protein, and the shorter one φ_t, due to the local motion of the Trp residue, are listed in Table 2. As the melittin concentration is increased, the increases in the rotational correlation times, φ_a and φ_t, are due to aggregation changing the shape and size of the rotating protein, and also hindering the motion of the Trp residue inside the protein.

A measure of the rotational motion of the Trp residue is also given in Table 2, as the parameter α, which, according to the model of Kinosita et al. (1977), is the cone semi-angle through which the residue moves. The value of α (45–55°) is much larger than that obtained by similar measurements on cobratoxin ($\alpha = 5-10°$) (Beddard and Tran 1985), and is comparable to that of glucagon ($\alpha = 40°$)

(Tran and Beddard 1982). The value of α is almost constant over the pH range studied and, in the tetramer, has a mean value of 34°. This is to be compared with the dilute solution where $\alpha = 54°$. In the latter case the "wobbling in a cone" model is not justified as the monomeric melittin backbone is flexible and it is possible that the Trp moves together with some nearby residues since φ_t is too big for the motion of the Trp alone. The Trp molecular volume and shape would lead to a rotation time in the region of 0.17 ns which is significantly smaller than the value measured. The shorter rotational correlation time (φ_t) is also constant with pH (even though the fluorescence lifetime varies) and its mean value is 1.05 ns in the aggregated solution and 0.65 ns in the most dilute solution. These data illustrate a limited restriction on the Trp motion in the tetramer, and the closer packing of residues is also seen as a shortening of the mean fluorescence lifetime.

The limiting anisotropy (r_0), which has a value of 0.13−0.15, shows that no depolarisation occurs faster than the instrumental time resolution. The maximum r_0 value for *N*-acetyltryptophanamide in glycerol, which has a viscosity of 1750 cp at room temperature and is thus immobile on the time scale of rotational relaxation, is 0.17 at 295 nm (Lakowicz et al. 1983).

The rotational correlation times for the whole protein motion, φ_a, vary from 1.7 ns in dilute solution, to 2.3 ns in the tetramer. Some increase in φ_a is expected as the tetramer is formed, but as the rotational correlation time depends on the shape of the rotating body, this increase can be small, as changes in shape can compensate for the extra proteins in the tetramer. The calculated rotational correlation time for a sphere of 13 Å radius is 2.2 ns; similar to the lifetime measured. The monomeric melittin is unlikely to be spherical however (Bello et al. 1982; Dawson et al. 1978). The X-ray data (Terwilliger et al. 1981) shows an extended chain approximately 35 Å long and about 12 Å in diameter, which allows for some associated water molecules to move with the protein. A calculation for such a rigid rod (Barkley and Zimm 1979) gives rotational correlation times of 2.3 ns and 0.85 ns, about the long and short axes respectively. The longer time is similar to those listed under φ_a in Table 2, the shorter is about 50% larger than φ_b. The sensitivity of the calculated rotational correlation times to the assumed protein shape means that, in monomeric melittin or other flexible proteins, these type of calculations only provide a rough guide to the molecular dimensions.

The fluorescence lifetime data shows how the mean lifetime, τ_m, (Fig. 2) varies as the result of changes in the fraction (f) of Trp residues in each conformation or environment, rather than changes in the individual fluorescence lifetimes, τ_1 and/or τ_2. The τ_1 and τ_2 values are largely unaffected by pH and protein aggregation. The variation of fluorescence lifetimes with protein concentration, and the decrease in mean lifetime at about pH 7 and 10.5, may be explained using a model in which the Trp exists in different environments in the protein.

With this model we have previously argued (Beddard and Tran 1985), that the quenched fluorescence lifetimes of Trp residues in proteins, compared to the lifetime of the indole chromophore, can be explained by charge-transfer to nearby electrophilic residues. Of the groups available in a protein the amino groups are likely to be good acceptors; particularly when protonated. Amide groups and COO^- groups may also participate, but the peptide group is a poor electrophile and in the Trp residue, induces little charge-transfer from the adjacent indole chromophore. We further proposed that each fluorescence lifetime is the result of specific interactions of the Trp residue in a distinct environment in the protein (Beddard and Tran 1985). In each of these environments the Trp is not held rigidly, but undergoes limited rotational relaxation. In melittin, the correlation time for this motion varies from 0.5 ns to 0.8 ns, depending upon the protein concentration.

The decrease in mean fluorescence lifetime at pH 10.5 and in more concentrated solutions at about pH 7, is explained as charge-transfer quenching in which Trp residues are quenched by nearby electrophilic groups. This quenching could be caused by movement of the Trp residue from one existing environment to another, such as the result of pH-induced movement of the protein backbone −C−CONH− groups or a rotation of the Trp $C_\alpha - C_\beta$ bond, or could be due to additional quenchers produced by changes in pH. Quay and Tronson (1983) have recently measured the pK_a of Lys_{21} and Lys_{23} to be 6.5 and 8.6 respectively in the monomers and about 7.4 in tetramers. Bello et al. (1982) measure a pK_a of 6.8 for the terminal amino group and observe, as we do, that the apparent pK changes with protein concentration and state that this lowering of pK accompanies a structural change.

If in the tetramer, the charged amino group on the terminal glycine or on the lysine residues, is directly responsible for the fluorescence quenching, (Fig. 2) then on amine deprotonation the fluorescence lifetimes and yields should increase − as happens in tryptophan and simple derivatives (Beddard et al. 1980) − not decrease as is observed. At low pH and at the highest concentration studied, the decrease in mean fluorescence lifetime from

dilute solution (2.4 ns to 2.2 ns, Fig. 2) may reflect quenching by distant charged amino groups.

To explain our data we propose that the structural change, recognised by Bello et al. (1982) to occur at around pH 7, brings either the unprotonated terminal amino-group or the unprotonated Lys_{21} away from the protein surface and nearer to the Trp, which is in a more hydrophobic environment on the apolar side of the melittin helix. The X-ray studies (Terwilliger et al. 1981) show that the terminal $-NH_{3+}$ group in the crystal is distant from the Trp and should not contribute to the quenching. NMR studies show that the $e-NH_2$ of Lys_{23} is close to the C_6 and C_7 protons of the indole ring of the Trp_{19} residue (Brown et al. 1982). This positioning would be energetically unfavourable if the Lys were charged. In the tetramers, as both the Lys_{21} and Lys_{23} have a pK of about 7 (Quay and Tronson 1983), either could interact with the Trp. For example, the NMR data suggest that Lys_{23} is nearest to the Trp, but the pK data show that in the monomer Lys_{21} has its pK at 6.6, the same as the fluorescence quenching, whereas Lys_{23} has its pK at 8.6.

A blue-shift (to 337 nm from 353 nm) was also observed in the Trp emission maximum on forming tetramers. This has been interpreted as the Trp residue moving into an environment with a lower dielectric constant (Burstein et al. 1973) and this shift was used by Quay and Condie (1983) to obtain monomer-tetramer equilibrium constants. As was observed in human serum albumin (Munro et al. 1979) and dry keratin (Smith et al. 1980), the blue shift is accompanied by an increase in fluorescence lifetime. In melittin the data in Fig. 2 show the opposite trend and the mean lifetime is, at each pH except pH 11, lower in the aggregated protein than in the monomer. This reflects the additional specific quenching by nearby groups in the tetramer over that in the monomer and illustrates that, in a hydrophobic environment, the Trp residue need not necessarily exhibit a long fluorescence lifetime.

The quenching measured at pH 10.5 is more difficult to explain than that at lower pH as none of the groups present in the protein appear to have a pK near this pH. The indole excited, singlet state deprotonates at pH 11.5 in tryptophan and in simple peptides. $Lysine_7$ is ionised at pH 9.6 (Quay and Tronson 1983), which is too low to account directly for the quenching at pH 10.5 even if allowance is made for the fact that melittin could fold at the Leu_{13} and Pro_{14} groups (Dawson et al. 1978) and this would bring the Lys_7 near to the Trp residue. The positive charges on the protonated Arg residues at positions 22 and 24 could also influence the Trp and lower the pH for deprotonation. This can occur in the same manner as the pK for the Lys_{21} and Lys_{23} residues is lowered from the usual value of 10.53 to 6.6 and 8.6 respectively (Quay and Tronson 1983). In monomeric melittin only quenching at pH 10.5 is observed, the flexible extended chain will ensure that no pH specific quenching occurs, and the fluorescence lifetime is similar to that measured in other small proteins.

4. Summary

The fluorescence from the Trp residue in melittin decays bi-exponentially between pH 5.5 to pH 11. As melittin tetramers are formed additional quenching at pH 6.5, as well as quenching at pH 10.5, was observed. Quenching at the lower pH is attributed to charge-transfer between the Trp and nearby deprotonated lysine residues as a result of pH-induced conformational changes. The two fluorescence lifetimes are explained in terms of a conformer model in which limited rotational diffusion is allowed in each site. Fluorescence anisotropy measurements show that the Trp is very mobile on the nanosecond time scale both in the monomer and in the tretramer.

Acknowledgement. This work was supported by grants from the Science and Engineering Research Council.

References

Barkley M, Zimm B (1979) Theory of twisting and bending of chain macromolecules: Analysis of fluorescence depolarisation of DNA. J Chem Phys 70: 2991

Beddard G, Tran C (1985) Fluorescence studies of the restricted motion of tryptophan in α-Cobratoxin. Eur Biophys J 11: 243

Beddard G, Fleming G, Porter G, Robbins R (1980) Time resolved fluorescence from biological systems: Tryptophan and simple peptides. Philos Trans R Soc (London) Ser A 298: 321

Bello J, Bello H, Granados E (1982) Conformation and aggregation of Melittin: dependence of pH and concentration. Biochemistry 21: 461

Brown L, Lauterwein J, Wuthrich K (1980) High resolution proton NMR studies of self aggregation of Melittin in aqueous solution: Biochim Biophys Acta 622: 219; 622: 321

Burstein EA, Vedenkina NS, Ivkova MN (1973) Fluorescence and the location of tryptophan residues in protein molecules. Photochem Photobiol 18: 263

Dawson C, Drake A, Helliwell J, Hider R (1978) The interaction of Bee Melittin with lipid bilayer membranes. Biochim Biophys Acta 510: 75

Faucon J, Dufourcq J, Lussan L (1979) The self-association of Melittin and its binding to lipids: an intrinsic fluorescence polarisation study. FEBS Lett 102: 187

Georghiou S, Thompson M, Mukhopadhyay A (1981) Melittin phospholipid interaction, evidence for melittin aggregation. Biochim Biophys Acta 642: 429

King T, Sobotka A, Kachoumian L, Lichtenstein L (1976) Allergens of honey bee venom. Arch Biochem Biophys 172: 661

Kinosita R, Kawato S, Ikegami A (1977) A theory of fluorescence polarisation in membranes. Biophys J 20: 289

Knoeppel E, Eisenberg D, Wickner W (1979) Interactions of Melittin, a pre-protein model with detergents. Biochemistry 18: 4177

Lakowicz J, Maliwal B, Cherek H, Balter A (1983) Rotational freedom of tryptophan residues in proteins and peptides. Biochemistry 22: 1741

Munro I, Pecht I, Stryer L (1979) Subnanosecond motions of tryptophan residues in proteins: Proc Natl Acad Sci (USA) 76: 56

Quay S, Condie C (1983) Conformational studies of aqueous melittin: Thermodynamic parameters of the monomer-tetramer self-association reaction. Biochemistry 22: 695

Quay S, Tronson L (1983) Conformational studies of aqueous melittin: determination of ionisation constants of lysine-21 and lysine-23 by reactivity towards 2,4,6-trinitrobenzene-sulphonate. Biochemistry 22: 700

Rigler R, Ehrenberg M (1973) Molecular interactions and structure as analysed by fluorescence relaxation spectroscopy. Q Rev Biophys 6, 2: 139

Smith G, Thorpe M, Melhuish W, Beddard G (1980) Fluorescence from tryptophan in keratin. Photochem Photobiol 32: 715

Talbot J, Dufourcq J, DeBohy J, Faucon J, Lussan A (1979) Conformational change and self-association of melittin. FEBS Lett 102: 191

Terwilliger T, Weissman L, Eisenberg D (1982) The structure of melittin in the form 1 crystals and its implication for melittins lytic and surface activities. Biophys J 37: 353

Tran C, Beddard G (1982) Secondary structure and dynamics of glucagon in solution. Biochim Biophys Acta 709: 256