NATURE OF MELITTIN-PHOSPHOLIPID INTERACTION

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Melittin, the major protein component of bee venom that possesses a strong hemolytic activity (1), is an amphipathic protein whose amino acid residues 1–20 are predominantly hydrophobic; residues 21–26 are hydrophilic. Determination of the molecular basis and specificity of the attractive forces involved in its interaction with membranes is essential for elucidating the mode of its biological action. In the present work we have employed the nanosecond and steady-state fluorometric properties of the single tryptophan residue of the protein (located at position 19) for probing the degree of penetration of that residue into phospholipid liposomes and the state of aggregation of the protein when bound to the liposomes.

RESULTS

Fig. 1 shows the data for fluorescence quenching by acrylamide (a nonionic, polar quencher) for melittin in tris(hydroxymethyl)aminomethane (Tris), 0.5M dibasic potassium phosphate, and when bound to egg phosphatidylcholine (EPC) bilayer liposomes. The Stern-Volmer plots exhibit upward curvature, implying that both dynamic and static quenching mechanisms operate. Thus, the relation $(F_0/F)\ \exp\ (-V[Q]) = 1 + K_{SV}\ [Q]$ is applicable (2). Here, F_0 and F are the fluorescence intensities in the absence and presence of the quencher of molar concentration $[Q]$, K_{SV} is the Stern-Volmer quenching constant, and V is the static quenching constant. By varying V, the best fit to the experimental data was obtained (linear plots in Fig. 1), yielding for Tris K_{SV} = 12.1 M^{-1}, V = 0.44 M^{-1}; for 0.5 M dibasic potassium phosphate K_{SV} = 2.9 M^{-1}, V = 0.57 M^{-1}; and for EPC K_{SV} = 2.1 M^{-1}, V = 0.46 M^{-1}. Values of 3.9×10^9, 1.4×10^9, and 1×10^9 $M^{-1}s^{-1}$ were then obtained for the second-order rate constant for dynamic quenching, k_q, from $k_q = K_{SV}/\tau_0$ (where τ_0 is the fluorescence decay time in the absence of acrylamide). The value in Tris is comparable to that for free tryptophan (6.2×10^9 $M^{-1}s^{-1}$), implying exposure of the residue to the aqueous environment. The rather high value of k_q for melittin bound to EPC liposomes implies a relatively high frequency of encounters between the hydrophilic acrylamide molecules and the residue during the lifetime of its excited state. These results suggest that the tryptophan does not penetrate deeply into the bilayer. This finding is consistent with the fact that the tryptophan is located very close to the hydrophilic part of the protein.

We investigated the state of aggregation of the protein when bound to phospholipids by comparing its optical properties with those in salt solution. Chromatographic (3) and steady-state polarization (4) studies presented evidence that the protein is tetrameric in NaCl solution, whereas it is monomeric in aqueous solution. We have employed phosphate, a component of phospholipids. Fig. 2 shows nanosecond fluorescence polarization data in 0.5 M dibasic potassium phosphate. A value of 3.5 ns was obtained for the rotational correlation time, ϕ, of the protein in that case. In contrast, a value of 1.1 ns was obtained for ϕ in Tris solution (5). Interestingly, the values

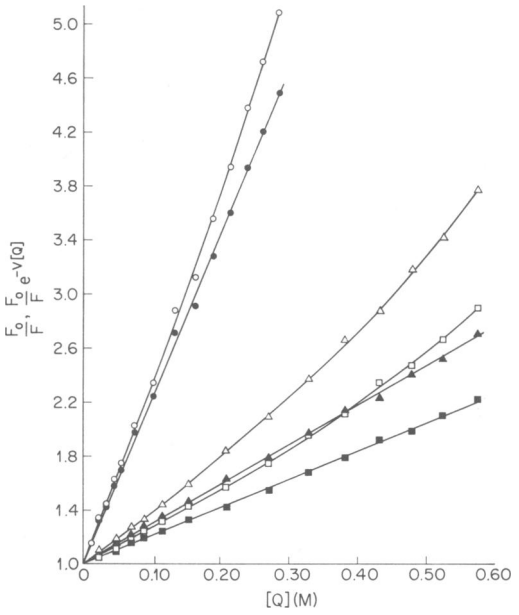

FIGURE 1 Fluorescence quenching data for the single tryptophan residue of melittin by acrylamide. F_0 and F represent the fluorescence intensities in the absence and presence of the molar concentration $[Q]$ of acrylamide, respectively, and V is the static quenching constant. Open symbols refer to Stern-Volmer plots, F_0/F vs. $[Q]$, whereas filled symbols refer to modified plots, $(F_0/F)\ \exp\ (-V\ [Q])$ vs. $[Q]$. —○—○— Tris; —●—●— modified plot for Tris; —△—△— 0.5 M dibasic potassium phosphate; —▲—▲— modified plot for dibasic potassium phosphate; —□—□— egg phosphatidylcholine (EPC) bilayer liposomes; and —■—■— modified plot for EPC. A melittin concentration of 0.17 mg/ml was employed that gave an absorbance ≈0.05 at 305 nm (the excitation wavelength, λ); acrylamide has negligible absorbance at that λ. Sonicated liposomes of 20 mg/ml in 10 mM Tris, pH 7 were prepared as previously described (12).

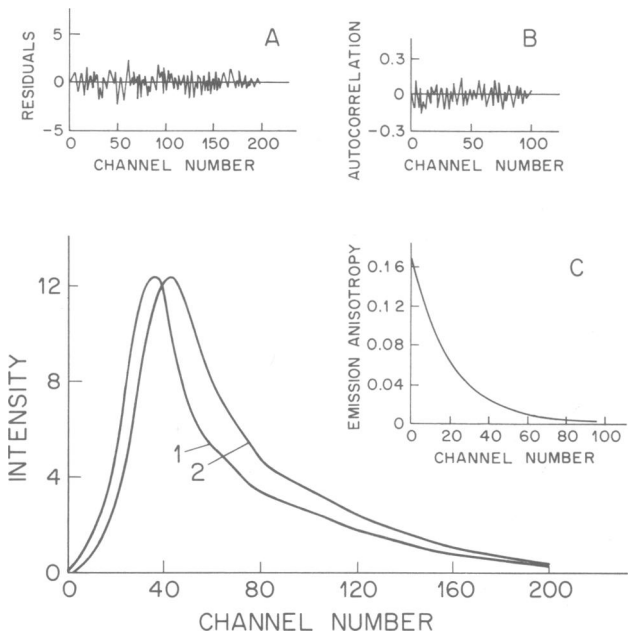

FIGURE 2 Nonlinear regression analysis of the nanosecond fluorescence polarization data for the tryptophan residue of melittin in 0.5 M dibasic potassium phosphate. Curve 1 represents the exciting light pulse profile. Curve 2 represents the plot of the experimental data for $I_V(t) - CI_H(t)$ and coincides with the convoluted curve; $I_V(t)$ and $I_H(t)$ are the vertical and horizontal fluorescence components for vertically polarized exciting light and C is a correcton factor. Inset A: Deviations between experimental and convoluted data at each channel. Inset B: Autocorrelation function of the residuals. The lack of any specific trend in A and B implies a satisfactory fit of the experimental data. Inset C: Emission anisotropy plot. Channel width = 0.168 ns. The buffer was 10 mM Tris, pH 7. The melittin concentration was 0.14 mg/ml. An interference filter of 6 nm full width at half maximum and 298 nm peak wavelength was used for excitation. The emission was viewed through a 7–37 Corning filter (Corning Glass Works, Science Products Div., Corning, N.Y.).

of ϕ for an unhydrated sphere of molecular weight 2,800 are calculated to be ~ 0.8 and 3.2 ns for the monomer and the tetramer, respectively. These results suggest that melittin aggregates, apparently as a tetramer, in the presence of phosphate. A comparison of the steady-state fluorescence spectra in phosphate solution with those of the protein bound to EPC showed a virtual coincidence (5). We have also found that to be true for the absorption spectra, which in both cases shift to the red by ~2 nm relative to the spectrum in Tris.[1] The value of $k_q = 1.4 \times 10^9\ M^{-1}s^{-1}$ in phosphate solution is comparable to that for the protein-EPC complex, $1 \times 10^9\ M^{-1}s^{-1}$. Similarities are also found in the nanosecond properties. There is a much stronger but quite similar dependence of the fluorescence spectra on time on the nanosecond scale and of the fluorescence decay times on the emission wavelength in both cases as compared to those in Tris.[1] We have also made similar observations for melittin bound to distearoyl phosphatidylcholine liposomes.[1] These observations support the notion that melittin binds to the phospholipids in an aggregated form. The magnitude of ϕ (10.1 ns in EPC liposomes [5]), however, cannot be used to estimate the number of protein molecules participating in the oligomeric aggregate, as the partial immobilization of the protein upon binding to the phospholipid gives rise to an increase in ϕ.

[1]Georghiou, S., M. Thompson, and A. K. Mukhopadhyay. Manuscript submitted for publication.

DISCUSSION

Previous workers (6–8) attributed the blue shift of the fluorescence spectrum of the tryptophan residue of melittin bound to phospholipid liposomes relative to that in aqueous solution (352–337 nm) to penetration of the residue into the hydrophobic core of the bilayer. As Eftink and Ghiron (9) demonstrated for a variety of other proteins, however, there is no linear relationship between the spectral maximum and the degree of exposure of the residue to the solvent. Interactions between the residue and neighboring polar groups of the protein would red-shift the spectrum and result in overestimation of the degree of exposure to the solvent. On the basis of the present data and recent circular dichroism data (10), we attribute the blue shift to an increase in the hydrophobicity of the environment of the residue brought about by shielding from the solvent through a combination of protein aggregation and enhancement of its α-helical content. This also explains the reduction in the k_q value relative to that in Tris. Our results support a binding mechanism involving electrostatic interactions (8, 11) between the lipid phosphate group and basic amino acid residues (lysine or argenine of the C-terminal part). The ensuing partial neutralization of the protein positive charge diminishes electrostatic repulsion, which, in turn, facilitates protein-protein hydrophobic interactions leading to aggregaton.

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REFERENCES

1. Haberman, E. 1972. Bee and wasp venoms. *Science (Wash. D.C.).* 177:314–322.
2. Weller, A. 1961. Fast reactions of excited molecules. *In* Progress in Reaction Kinetics. G. Porter, editor. Pergamon Press, Oxford. 1:187–214.
3. Talbot, J. C., J. Dufourcq, J. D. Bony, J. F. Faucon, and C. Lussan. 1979. Conformational change and self-association of monomeric melittin. *FEBS (Fed. Eur. Biochem. Soc.) Lett.* 102:191–193.
4. Faucon, J. F., J. Dufourcq, and C. Lussan. 1979. The self-association of melittin and its binding to lipids. *FEBS (Fed. Eur. Biochem. Soc.) Lett.* 102:187–190.
5. Georghiou, S., M. Thompson, and A. K. Mukhopadhyay. 1981. Melittin-phospholipid interaction: evidence for melittin aggregation. Biophys. Acta. 642:429–432.
6. Mollay, C., and G. Kreil. 1973. Fluorometric measurements on the interaction of melittin with lecithin. *Biochim. Biophys. Acta.* 316:196–203.
7. Mollay, C., G. Kreil, and H. Berger. 1976. Action of phospholipases

on the cytoplasmic membrane of *E. Coli*. Stimulation by melittin. *Biochim. Biophys. Acta*. 426:317–324.

8. Dufourcq, J., and J. F. Faucon. 1977. Intrinsic fluorescence study of lipid-protein interactions in membrane models. Binding of melittin, an amphipathic peptide, to phospholipid vesicles. *Biochim. Biophys. Acta*. 467:1–11.
9. Eftink, M. R., and C. A. Ghiron. 1976. Exposure of tryptophanyl residues in proteins. Quantitative determination by fluorescence quenching studies. *Proc. Natl. Acad. Sci. U.S.A.* 15:672–680.
10. Drake, A. F., and R. C. Hider. 1979. The structure of melittin in lipid bilayer membranes. *Biochim. Biophys. Acta*. 555:371–373.
11. Dawson, C. R., A. F. Drake, J. Helliwell, and R. C. Hider. 1978. The interaction of bee melittin with lipid bilayer membranes. *Biochim. Biophys. Acta*. 510:75–86.
12. Lianos, P., A. K. Mukhopadhyay, and S. Georghiou. 1980. Microenvironment of aromatic hydrocarbons employed as fluorescent probes of liposomes. *Photochem. Photobiol.* 32:415–419.

THE INTERACTION WITH PHOSPHOLIPIDS OF BEE VENOM MELITTIN

A Structural Study of the Peptide and Lipid Components

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The high water solubility of melittin, together with its high affinity for lipid membrane systems, makes this peptide a useful tool for the study of lipid-protein interactions.

In aqueous solution, while circular dichroism could only show the existence of either a random-coil or of a right-handed helix, high resolution ^{1}H- and ^{13}C-NMR, together with photon correlation spectroscopy, produced evidence in favor of a number of different well-defined structural

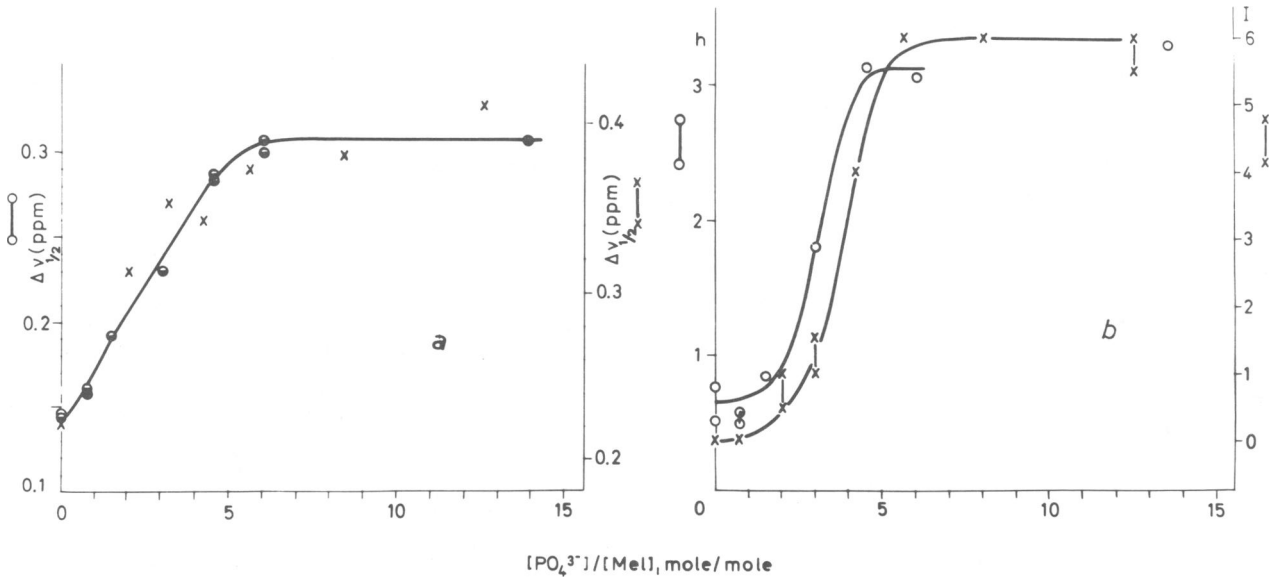

FIGURE 1 Variations of melittin methyl ^{1}H-NMR signal as a function of phosphate:melittin molar ratio. *a*, width at half-height, $\Delta\nu_{1/2}$, of the main -CH_3 band centered at ~0.9 ppm (200 MHz, 24°C: O—O; 100 MHz, 29°C: x—x); *b* relative height (at 200 MHz: O—O) and intensity (at 100 MHz: x–x) of the Ile 2 δCH_3 signal appearing at ~0.3 ppm).