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MELITTIN-PHOSPHOLIPID INTERACTION

EVIDENCE FOR MELITTIN AGGREGATION

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Summary

The fluorescence spectra of the single tryptophan residue of melittin in 0.15 M potassium phosphate solution and when bound to egg phosphatidylcholine bilayer liposomes practically coincide and exhibit a large blue shift relative to that in aqueous solution. The rotational correlation time of the protein increases substantially in the salt solution relative to that in aqueous solution. It is inferred that the protein binds to the phospholipid in an aggregated form, most probably as a tetramer.

Melittin, the major protein component of bee venom, has a strong hemolytic activity [1]. There has been strong interest in recent years in elucidating the nature of the mode of its interaction with model membranes [2—10]. A circular dichroism (CD) study [10] reported that the α-helical content of melittin, which is very low in aqueous solution [11], increases considerably upon interaction with egg phosphatidylcholine bilayer liposomes and when in 0.15 M phosphate solution. Evidence was recently presented that melittin is monomeric at low ionic strength but associates as a tetramer at high ionic strength [12—13]. In this work we have employed the nanosecond and steady-state fluorometric properties of the single tryptophan residue of the protein for probing the state of protein aggregation when it binds to egg phosphatidylcholine bilayer liposomes.

Fig. 1 shows the fluorescence spectra of melittin in 10 mM Tris buffer, in 0.15 M dibasic potassium phosphate solution, and when bound to egg phos-

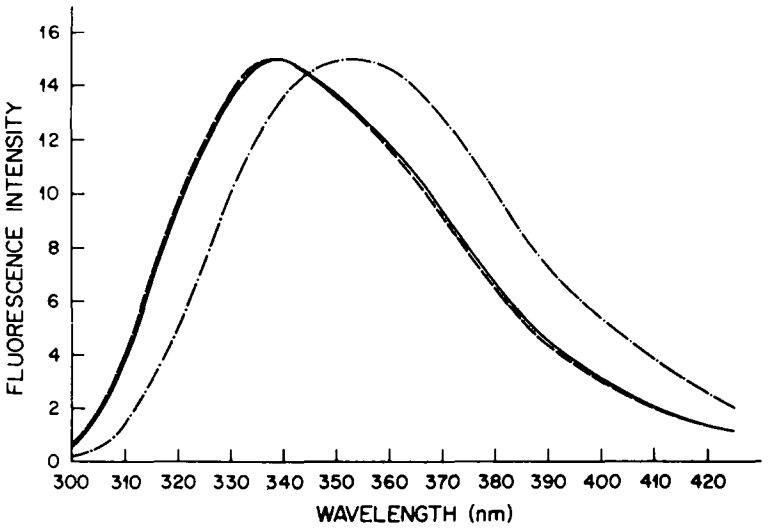

Fig. 1. Fluorescence spectra of the single tryptophan residue of melittin in (· — · — ·) 10 mM Tris, pH 7; (———) 0.15 M dibasic potassium phosphate in 10 mM Tris (pH adjusted to 7) and (- - - - -) egg phosphatidylcholine liposomes. Samples of bee venom melittin, obtained from Sigma and ICN Biochemicals, gave identical results. Chromatographically pure egg phosphatidylcholine was obtained from Sigma. Analytical reagent grade dibasic potassium phosphate and Tris were Fisher products. The preparation of the liposomes was done according to published procedures [15—17]. A 20 mg/ml lipid suspension in 10 mM Tris buffer, pH 7 (in triply distilled water) was prepared. The suspension was vigorously stirred for 20 min and then sonicated for 20 min using a Heat-Systems-Ultrasonics 20 kHz model W-375 sonifier equipped with 0.5 inch tip at 50% full power. Finally, it was centrifuged at 65 000 × *g* for 30 min. All procedures were carried out under nitrogen. Melittin was incubated for 1 h in the phospholipid solution at a concentration of 0.04 mg/ml. Steady-state and nanosecond fluorometric techniques did not detect any fluorescence from liposomes unlabelled with melittin. The fluorescence spectra were taken at room temperature with a spectrofluorometer previously described [18] and were corrected for the variation of the sensitivity of the photomultiplier-monochromator combination with emission wavelength by employing a standard lamp. The emission bandwidth was 3 nm. Excitation was at 280 nm.

phatidylcholine liposomes. It is seen that there is a large blue shift in the latter two cases and that the spectra practically coincide. This implies that the tryptophan residue reports a good degree of similarity in the modified protein conformations with the phosphate anion participating in the structural rearrangement.

Further insight has been gained by studying the nanosecond fluorescence polarization properties of the tryptophan residue. Fig. 2 shows the data for the 0.15 M phosphate solution. A value of 3.7 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. Interestingly, the rotational correlation times for an unhydrated sphere of molecular weight 2800 are calculated to be approx. 0.8 and 3.2 ns for the monomer and the tetramer, respectively. Thus, these results imply that the protein aggregates, apparently as a tetramer, in the presence of phosphate. Previous chromatographic and steady-state fluorescence polarization studies [12—13] reached a similar conclusion for melittin in NaCl solution. It is important to take also into account in this discussion salt-induced conformational changes in the protein. Melittin has been reported to have an increased α-helicity in the salt solution relative to that in aqueous solution

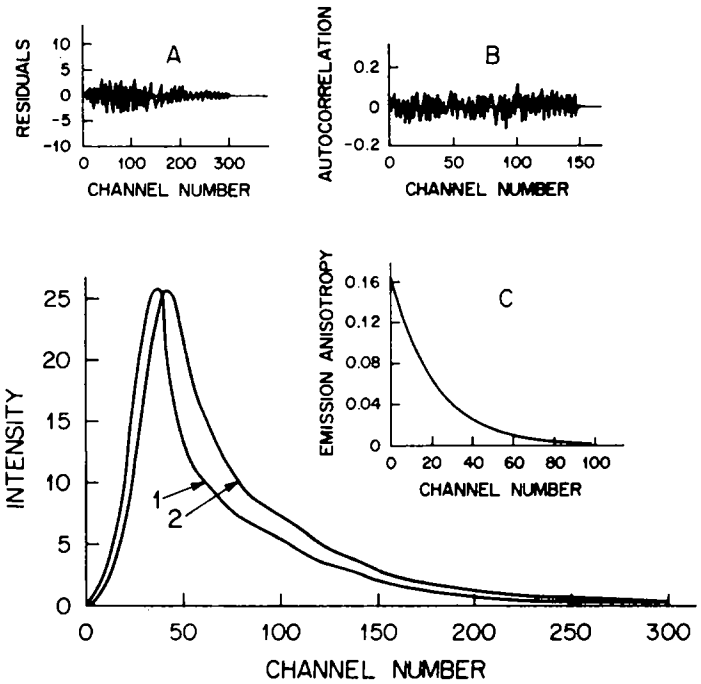

Fig. 2. Nonlinear regression analysis of the nanosecond fluorescence polarization data for the tryptophan residue of 0.14 mg/ml melittin in 0.15 M dibasic potassium phosphate, pH 7. Curve 1 represents the exciting light pulse profile. Curve 2 represents the plot of the experimental data for $I_V(t) - CI_H(t)$ and coincides with the convoluted curve; $I_V(t)$ and $I_H(t)$ are the vertical and horizontal fluorescence components, respectively, for vertically polarized exciting light and C is a correction factor close to unity calculated by combining nanosecond with steady-state emission anisotropy measurements [19]. Insert A: Deviations between experimental and convoluted data at each channel. Insert B: Autocorrelation function of the residuals. The lack of a specific trend in curves A and B shows a satisfactory fit of the experimental data. The emission anisotropy, $r(t)$, defined as $r(t) = (I_V(t) - CI_H(t))/(I_V(t) + 2CI_H(t))$, was assumed to have the form $r(t) = r_0\exp(-t/\phi)$, where ϕ is the rotational correlation time and r_0 is the zero-point emission anisotropy (at $t = 0$). The function $I_V(t) + 2CI_H(t)$, which represents the total fluorescence decay, was first analyzed and then the obtained parameters were used for the analysis of the $I_V(t) - CI_H(t)$ data. The values of 3.7 ± 0.3 ns and 0.164 ± 0.006 were obtained for ϕ and r_0, respectively. Insert C: Emission anisotropy plot. Channel width = 0.171 ns. A Pomfret interference filter of 6 nm f.w.h.m. and 298 nm peak wavelength was used for excitation, for the steady-state emission anisotropy of tryptophan is high at that wavelength [20]. The emission was viewed through a 7-37 Corning filter. Tryptophan is the only fluorophore, as melittin lacks a tyrosine residue. A 3 M 105-UV-WRMR polarizer was placed in the excitation light path and a Polaroid HNP'B polarizer in the emission path. The measurements were made in aerated solutions by employing a nanosecond fluorometer previously described [21].

[10]. As that would tend to make it more compact, one would expect a lower value of ϕ for monomeric protein in the salt solution, contrary to the present experimental observations. This argument lends further support to aggregation. The virtual coincidence between the fluorescence spectra of the protein when bound to the liposomes and when in phosphate solution (Fig. 1) implies then that the protein binds to the phospholipid in the aggregated form. The interaction could be envisioned [6,7] as involving electrostatic binding between the lipid phosphate group and basic amino acid residues (residues 21—26; the rest are predominantly hydrophobic). One melittin molecule may be interacting with a few lipid molecules, as may be gathered from the findings of a differen-

tial scanning calorimetry study [14] which estimated that one melittin molecule prevents ten phosphatidylcholine molecules from participating in the cooperative phase transition. The ensuing partial neutralization of the protein positive charge would diminish electrostatic repulsion and facilitate protein-protein interaction leading to aggregation.

The rotational correlation time for melittin interacting with egg phosphatidylcholine bilayer liposomes has been found to be 10.1 ns, consistent with molecular complex formation. The zero-point emission anisotropy values are 0.185, 0.164 and 0.185 for Tris, phosphate solution and liposomes, respectively. These are somewhat lower than the value of 0.24 which we obtained for free tryptophan in the highly viscous medium of propylene glycol at −60°C. This implies the occurrence of a relaxation process on the subnanosecond time scale.

Previous workers [2,5,6] attributed the blue shift of the fluorescence spectrum of the tryptophan residue of melittin bound to phospholipid liposomes relative to that in aqueous solution to penetration of the residue into the hydrophobic core of the bilayer. In view of the present data and the recent CD data [10], the shift can be more appropriately interpreted as arising from an increase in the hydrophobicity of the environment of the residue brought about by a combination of protein aggregation and enhancement of its α-helicity. Indeed, fluorescence quenching data from this laboratory provide support for the notion that the residue is not buried deep into the bilayer (Georghiou, S., Thompson, N. and Mukhopadhyay, A.K., unpublished results).

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