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THE STRUCTURE OF MELITTIN IN LIPID BILAYER MEMBRANES

A.F. DRAKE[a] and R.C. HIDER[b]

[a]*Department of Chemistry, King's College, Strand, London, W.C.2 and* [b]*Department of Chemistry, Essex University, Colchester, Essex (U.K.)*

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Summary

0.15 M inorganic phosphate dramatically increased the α-helix content of melittin in aqueous solution.

When melittin interacted with egg yolk phosphatidylcholine liposomes in the absence of inorganic phosphate, it was converted to an α-helix rich form, as postulated by Dawson et al. (Dawson, C.R., Drake, A.F. Helliwell, J. and Hider, R.C. (1978) Biochim. Biophys. Acta 510, 75—86).

Dawson et al. [1] have demonstrated that melittin fails to form an extensive α-helical structure in both aqueous NaCl (0.15 M) and ethanol. In contrast, melittin in SDS micelles exists in a predominantly α-helical form [1]. Although SDS is usually considered to be a powerful protein denaturant, it has been shown that erythrocyte membrane glycoproteins are not denatured by SDS when incubated below 30°C. Instead, the detergent binds to the existing protein conformation [2]. Indeed SDS micelles have been used as a model for biological membranes [3]. Consequently, it was argued by Dawson et al. [1] that melittin, by analogy with its properties in SDS, could adopt an α-helical configuration in biological membranes.

Recently Strom et al. [4] have produced conflicting evidence for this concept using phospholipid vesicles (egg phosphatidylcholine/dicetylphosphate, 85:15, by weight) prepared in phosphate buffer (0.15 M, pH 7.4). They report that the absolute ellipicity of melittin over the 200—240 nm range decreases in magnitude when introduced into the lipid structure. In fact, the CD for melittin in aqueous solution as reported by Strom et al. [4] can be interpreted as being relatively rich in α-helix. This result is quite different to that reported by Dawson et al. [1] and apparently contradicts the conclusion of Jentsch [5], that in aqueous solution there is little or no

α-helix in melittin. Consequently, it was decided to measure the CD spectrum of melittin in the presence and absence of 0.15 M sodium phosphate. Surprisingly, phosphate at this relatively high concentration triggers a structural change in melittin, inducing a predominantly α-helical structure (Fig. 1). NaCl (0.15 M) fails to induce such a change [1].

In view of this strong perturbation of melittin by inorganic phosphate, it was decided to study the incorporation of melittin into egg yolk phosphatidylcholine vesicles by CD in the absence of high phosphate concentrations. The results shown in Fig. 1 clearly demonstrate that the α-helix-rich form of melittin is favoured when the peptide interacts with phosphatidylcholine vesicles. As melittin is strongly surface active [6] a likely conformation is that proposed by Dawson et al. [1] on the basis of SDS-melittin interactions. A similar transformation of protein structure on the surface of phospholipid vesicles has been reported for lipophilin, a hydrophobic protein isolated from myelin [7].

The observation that inorganic phosphate induces an increase in the α-helix content of melittin may well have some relevance to the mode of transformation of the melittin tetramer [6] to the α-helical form of melittin bound at water-phospholipid interfaces.

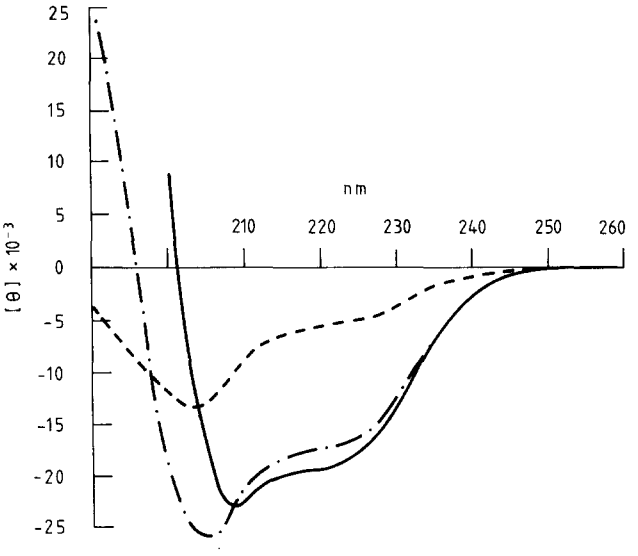

Fig. 1. The CD of melittin (0.5 mg•ml^{-1}, Sigma Chemical Co.) dissolved in sodium phosphate (0.15 M, pH 7.4), ——— ; dissolved Tris-HCl (10 mM, pH 7.4), — — — and melittin (0.4 mg•ml^{-1}) dissolved in Tris-HCl (10 mM, pH 7.4) containing liposomes prepared from egg yolk phosphatidylcholine (1.3 mg•ml^{-1}), —•—•—. The liposome preparation was sonicated for 5 min under N_2 and centrifuged at 10 000×*g* for 10 min prior to the addition of melittin. This centrifugation step ensured that only small vesicles were present in the preparation, thus distortion due to scattering over the 205—260 nm range was minimal [8]. In the absence of melittin the base line was not significantly changed by the presence of liposomes in the 0.1 mm pathlength cell. The number of membrane shells, per liposome, was in the range 5—10. The melittin:phospholipid ratio was 1:13. The spectra were obtained on a Jasco J40CS using 0.1 mm and 1.0 mm cells at 20°C. At this temperature melittin did not cause liposome fusion. The results are expressed in terms of molar ellipticity based on an average monomer molecular weight of 110, the units are degree•cm^2•mol^{-1}.

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