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

Franca Podo
Istituto Superiore di Sanità, Rome, Italy

Roberto Strom and Carlo Crifò
Institutes of Biochemistry, Universities of Rome and L'Aquila, Italy

Carmen Berthet and Martin Zulauf
European Molecular Biology Laboratory, Grenoble Outstation, Grenoble, France

Giuseppe Zaccai
Institut Laue-Langevin, Grenoble, France

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

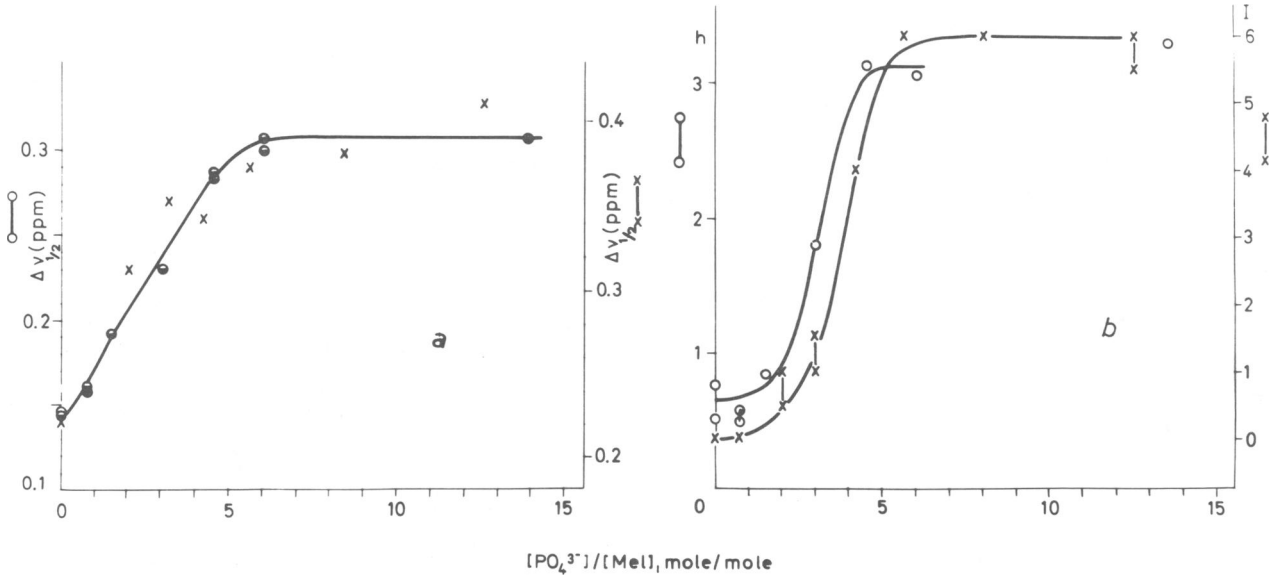

FIGURE 1 Variations of melittin methyl ^{1}H-NMR signal as a function of phosphate:melittin molar ratio. *a*, width at half-height, $\Delta\nu_{1/2}$, of the main -CH_3 band centered at ~0.9 ppm (200 MHz, 24°C: O—O; 100 MHz, 29°C: x—x); *b* relative height (at 200 MHz: O—O) and intensity (at 100 MHz: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

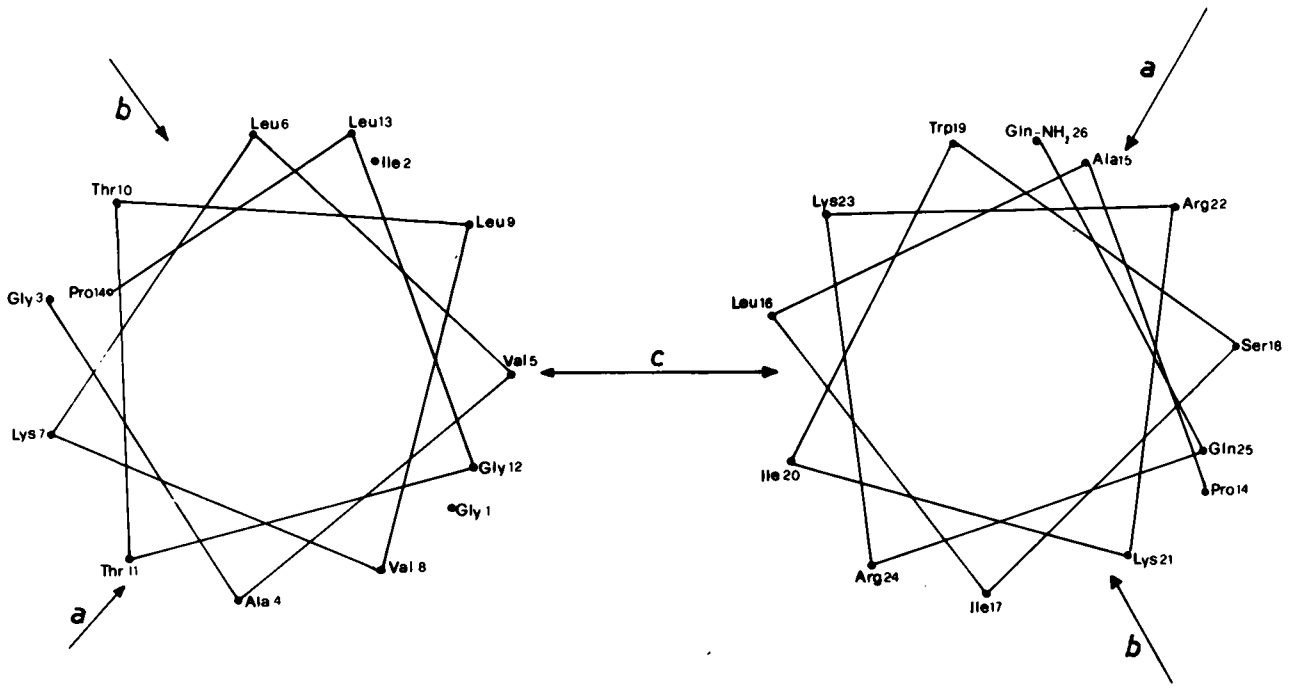

FIGURE 2 Projection along the central axis of the N-terminal (left) and *C*-terminal (right) segments of melittin in right-handed α-helical conformation. Arrows, *a*, *b*, and *c* divide the surface of each segment into three 120° sectors with different characters of polarity.

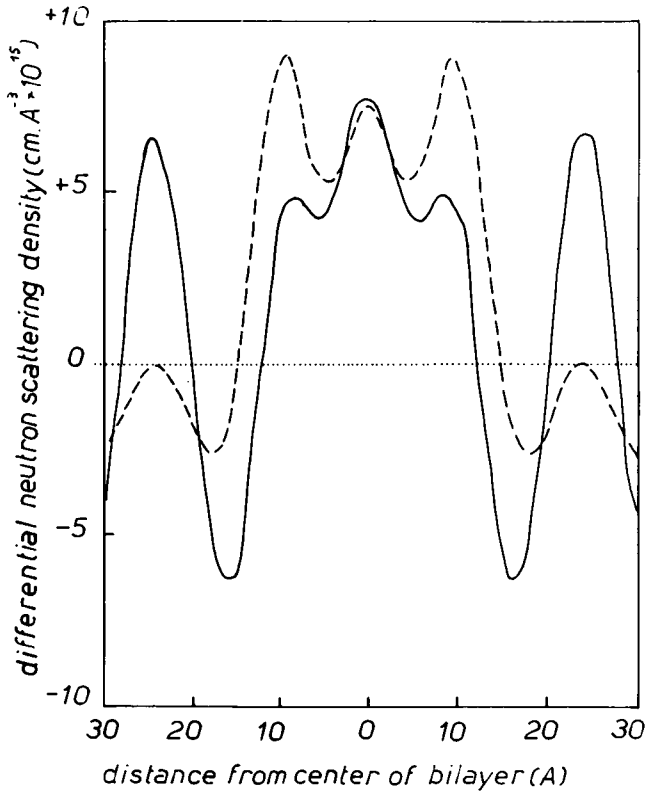

FIGURE 3 Differential neutron scattering density profile giving the position of melittin incorporated in egg lecithin multilayers in the presence of H_2O (solid line) or of a 45% D_2O - 55% H_2O mixture (dashed line).

states, depending on anion concentration and charge and on pH. In pure water at neutral pH, melittin appeared to exist as a flexible random-coil monomer; in dilute NaCl it was still monomeric and essentially random-coil, but presented a pronounced rigidity of structure, and could be approximated to a prolate ellipsoid. When divalent anions were present (or when high ionic strengths were reached even with monovalent anions) melittin molecules associated into compact disk-like tetramers, where each protomer consisted essentially of two right-handed helical stretches, connected presumably by a 135° bend at the level of Pro14. Correlations could be established between the binding of phosphate ions, evaluated by ^{31}P-NMR, and the structural variations undergone by the peptide. In particular, ^{1}H-NMR spectral changes indicative of interchain interactions required the binding of a larger number of phosphate ions (Fig. 1 *b*) than those corresponding to the overall ordering of the single peptide protomer (Fig. 1 *a*) or to changes in the local conformation around Pro14. At alkaline pH a helical tetramer was also present, which was different from that formed in the presence of divalent anions at neutral pH.

Upon binding to phospholipids, the conformation of each protomer was similar to that occurring in aqueous phosphate solutions, with a grouping of polar residues along one face of the molecule (Fig. 2). The Gln and Lys residues were more strongly immobilized, and there was no NMR evidence for any self-aggregation of the peptide; the ability of melittin to induce dichromate efflux from

phospholipid vesicles was in fact higher when the peptide was in the monomeric state rather than in the tetrameric one.

Despite the dramatic effects of melittin on the permeability of phospholipid vesicles to water and to water solutes, the bilayer structure was preserved, as evidenced by Pr(III) permeation experiments followed by ^{1}H-NMR and by low-angle x-ray and neutron diffraction analyses. Although the lamellar pattern was not grossly perturbed, melittin could be shown, by comparing the neutron scattering profiles on absolute scale, to be present both at the center of the bilayers and in the aqueous region separating them, the peptide spanning therefore at least half of each bilayer (Fig. 3). The increase of permeability to water solutes caused by melittin was reflected, in H_2O/D_2O exchange experiments, by a deeper penetration of water into the lipid region of the bilayers (up to 4 Å from the center, at 95% relative humidity). Below T_m, the diffraction pattern seemed to be consistent with a lamellar array of bilayers, together with an in-plane ordering of melittin-phospholipid complexes. The presence of water appeared in all cases to be essential for the incorporation of melittin in the bilayer.

Received for publication 6 May 1981.

STUDIES ON THE INTERACTION OF GLUCAGON WITH PHOSPHOLIPIDS

R. M. EPAND AND J. M. STURTEVANT
Department of Chemistry, Yale University, New Haven, Connecticut 06511 U.S.A.

The formation of an amphipathic helix, with segregation of hydrophobic and hydrophilic residues on opposite sides of the helix, is one mechanism by which proteins are believed to interact with phospholipid bilayers (1). Glucagon, a 29-amino acid polypeptide hormone, contains two segments of amphipathic helix in its crystal structure (2). It is capable of forming stable complexes with a number of synthetic phospholipids including diheptanoyl (3); dicapryl;[1] dilauroyl (4); dimyristoyl (5); and dipalmitoyl (5) phosphatidylcholine, as well as sphingomyelins (6),[2] dimyristoyl phosphatidylglycerol;[3] and dipalmitoyl phosphatidylserine;[3] but not with dimyristoyl phosphatidylethanolamine[3] or diacyl phosphatidylcholines in the presence of more than 20% cholesterol (6). Glucagon has also been shown to interact with the detergent lysolecithin (3, 7).

RESULTS AND DISCUSSION

The interaction of glucagon with dimyristoyl phosphatidylcholine (DMPC) occurs rapidly around the phase transition temperature of the phospholipid (4) and leads to the formation of a discoidal particle of 1.4×10^6 mol wt, 250 $\times$ 70 Å (8). In this complex the glucagon has an increased helical content (9) and is believed to be located, at least in part, around the rim of this disk. There are 55 mol of DMPC/mol glucagon in the particle. The interaction of glucagon with DMPC results in a marked broadening of its phase transition and a shift in its temperature from 23.8° to 26.1°C. There is also an additional small transition at 23° in presence of glucagon whose enthalpy is one-quarter that for the 26.1°C transition (10). The total enthalpy change for the transition is reduced by glucagon from 4.8 to 3.2 kcal/mol DMPC. This reduction may be caused by the perturbation of the gel-state lipid in the presence of the hormone as has been observed by Raman spectroscopy (11). As a result of the change in the phase transition behavior caused by glucagon there is a much larger apparent heat capacity of the pure DMPC between 23° and 25°C, where it is completely converted from solid to liquid phase, than is the case for the glucagon-DMPC complex which undergoes only a relatively small change in phase in this temperature span (10). Thus there is a large apparent ΔCp of reaction leading to a marked temperature dependence of the isothermal enthalpy of reaction. Expressed per mole of glucagon, the reaction enthalpy changes from endothermic (+ 80 kcal/mol) at 23°C to a highly exothermic (−150 kcal/mol) at 25°C. In the case of glucagon the major contribution to the isothermal enthalpy of reaction is found to be the glucagon-induced phase change in the lipid (10). An analogous situation should occur for any substance that causes a change in the phase transition properties of a phospholipid.

The nature of the interaction between glucagon and phospholipids is similiar to that between serum apolipoproteins and DMPC. In both cases discoidal particles containing a single phospholipid bilayer are formed (12–14) with

---

Dr. Epand's permanent address is the Department of Biochemistry, McMaster University, Hamilton, Ontario L8N 3Z5, Canada.

[1]Ernandes, J. R., S. Schreier, and R. M. Epand. Interaction between the peptide hormone glucagon and dicapryl phosphatidylcholine. Spectroscopic studies and characterization of the lipoprotein complex. Submitted for publication.

[2]Epand, R. M., L. Bond, and T. S. Hui. 1981. Interaction of glucagon with natural and with synthetic sphingomyelius. Manuscript submitted for publication.

[3]Epand, R. M., and J. M. Sturtevant. Unpublished observations.