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.

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STUDIES ON THE INTERACTION OF GLUCAGON WITH PHOSPHOLIPIDS

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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

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 Unpublished observations.

similar phase transition properties (15, 16) and large exothermic heats of reaction per mole of protein at 25°C (17, 18). It has also been recently reported that the enthalpy of reaction of apolipoprotein A-II with DMPC is markedly temperature dependent, with the enthalpy change becoming positive below the phase transition of the phospholipid (19). In the case of the serum apolipoproteins, each protein molecule contains several segments of amphipathic helix which may interact differently with phospholipids, leading to the formation of a variety of final products (20). However, with the small peptide glucagon the lipoprotein particle appears to be quite uniform (8). The main difference between glucagon and the serum apolipoproteins in their interaction with DMPC is that in the case of glucagon the complex dissociates at temperatures above the phase transition region (5) while the serum lipoprotein remains stable. In this regard glucagon is similiar to a synthetic 20-residue model peptide (21). This peptide as well as glucagon and the serum apolipoproteins have increased helical content in the presence of phospholipid. However, only in the cases of glucagon and the model synthetic peptide is the structure of the peptide in the absence of lipid almost completely devoid of helix content. The larger negative free energy contribution resulting from the denaturation of small peptides upon dissociating from a lipid bilayer may be the driving force leading to their instability at higher temperatures. The maximal thermodynamic stability of these small peptide-lipid complexes occurs in the region of the phase transition of the phospholipid where boundaries between lipid phases may accommodate the peptide. At lower temperatures these complexes have kinetic (22) but not thermodynamic (6) stability.

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