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INFLUENCE OF BASIC POLYPEPTIDES ON THE PHASE TRANSITION OF PHOSPHOLIPID LIPOSOMES

D. BACH and I. R. MILLER

Department of Membranes and Bioregulation, The Weizmann Institute of Science, Rehovot (Israel)

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SUMMARY

Interaction of basic polypeptides (copolymers of lysine with phenylalanine or tyrosine) with phosphatidylserine or dipalmitoyl phosphatidylcholine liposomes was investigated by differential scanning calorimetry. The polypeptides cause a decrease in enthalpy of melting of the phospholipids almost without affecting the midpoint melting temperature.

INTRODUCTION

The fatty acid composition of biological membranes varies considerably, allowing different regulatory functions. Lipids and phospholipids can undergo a phase transition from crystalline to liquid crystalline states. The temperature of phase transition depends upon chain length, degree of unsaturation of the hydrocarbon chains [1] and the head groups of the phospholipids [2].

Since the biological membranes also contain proteins, the interactions between the phospholipids and proteins should influence the phase transition of lipids, namely the enthalpy and the transition temperature. Steim et al. [3] were the first to show that the enthalpy of transition of *Mycoplasma laidlawii* membranes was approx. 75 % of that of the extracted lipids, concluding that approx. 25 % of the phospholipids are directly bound to proteins. Similar results were reported by Martonosi [4] on sarcoplasmic reticulum membranes and extracted lipids, where the enthalpy of membrane melting was only a fraction (about half) of that of the extracted lipids. Biological membranes are very complicated structures, usually containing many proteins, so an attempt was made to study the influence of lipid-protein interactions on the thermotropic behavior by using model systems. Verkleij et al. [5] reported from differential scanning calorimetric measurements that A_1 basic protein from myelin raises the transition temperature of synthetic phosphatidylglycerol in a way similar to Ca^{2+}.

Chapman et al. [6] found from differential scanning calorimetric measurements that binding of basic proteins or polylysine to phosphatidylserine causes a decrease in transition temperature of the phospholipid. On the other hand, Träuble and Eibl [7] reported that addition of polylysine to phosphatidic acid increases the melting temperature as revealed by fluorescence measurements.

It has been shown that the interaction between basic polypeptides and the phospholipid bilayers affects the permeability of the bilayers [8, 9] and the conformation of the polypeptides [10, 11]. These effects depend very strongly on the composition of the basic polypeptides, e.g. the copolypeptide lysine-phenylalanine increases strongly the conductance of phosphatidylserine bilayers [8] and cause destabilization of phosphatidylcholine bilayers. The copolymer turns into an α-helix upon interaction with phosphatidylserine vesicles and stays as the random coil when interacting with phosphatidylcholine vesicles. The copolypeptide lysine-tyrosine has only a small effect on the conductance and it gives a β structure upon interaction with phosphatidylserine or phosphatidylcholine vesicles [10]. To obtain additional information on the phospholipid-peptide interactions, we decided to investigate the effect of these two polypeptides on the phase transition of phosphatidylserine liposomes and of the copolymer lysine-phenylalanine on the phase transition of zwitterionic dipalmitoyl phosphatidylcholine by employing differential scanning calorimetry.

After this work was completed, papers by Papahadjopoulos et al. [12, 13] appeared showing that apoprotein from myelin and other proteins interacting with phospholipids cause a decrease in the enthalpy of melting of the phospholipids.

MATERIALS AND METHODS

Phosphatidylserine monosodium salt grade I from bovine spinal cord was purchased from Lipid Products, Nutfield, England. No impurity was found by thin-layer chromatography. It was dispersed in 10^{-2} M NaBr/10^{-3} M Tris buffer (pH 7.2)/ 15 % (v/v) ethyleneglycol after removal of the organic solvents by evacuation. NaBr has been used because the lysine in the polypeptides employed was in the HBr salt form. Ethyleneglycol was added to reduce the freezing point of water and to eliminate overlap of its melting peak with the phospholipid transition. The dispersion was sonicated in de-aerated closed ampoules (at concentration approx. 100 mg/ml) in a bath type sonicator (Lab. Supplies, Hicksville, N.Y.) for 1.5 h at 45–55 °C.

[^{3}H]Dioleoyl phosphatidylcholine was a gift from Dr. R. Pagano of Carnegie Institution of Washington, Baltimore, Md., U.S.A. and was sometimes added as a marker to the above-mentioned lipids at concentration of about 1 % of the total phospholipids.

The random copolypeptides lysine-phenylalanine (1.4 : 1, molecular weight 30 000) and lysine-tyrosine molecular weight (1 : 1, 82 000) were obtained from Miles-Yeda, Kiryat Weizmann, Rehovot. Their concentrations were determined in an amino acid analyzer. Addition of 15 % (v/v) ethyleneglycol to the peptides did not introduce a conformational transition as judged by circular dichroism measurements.

The interaction products were prepared by shaking the phospholipid liposomes with the polypeptide at different ratios for 5 min on the Vortex mixer at room temperature for phosphatidylserine and at approx. 45 °C for dipalmitoyl phosphatidylcholine liposomes. In ratios other than 1 : 1 of lysine to phospholipid, small volumes of 1 M NaBr were added to maintain a ratio 1 : 1 of inorganic salt to phospholipid.

The samples with dipalmitoyl phosphatidylcholine were incubated for 1 h at 45–50 °C and then all the samples were left overnight. Subsequently two types of procedure were undertaken. (1) The samples were concentrated by evaporating

part of the water by a stream of N_2 and the slurry was transferred to aluminium pans (for volatile substances) of the instrument and sealed. (2) The material was centrifuged in a Beckmann 152 microfuge for 10 min, and the supernatant pulled off. Its radioactivity was counted in a scintillation counter and its serine and phenylalanine content determined in an amino acid analyzer. The wet precipitate was transferred into aluminium pans. Phosphatidylserine and dipalmitoyl phosphatidylcholine were treated in the first way after adding NaBr to give a ratio of phospholipid/Na^+ of 1 : 1. The measurements were performed on the Perkin Elmer DSC-1B apparatus at a scanning rate of 8 °C/min and at ranges of 2 or 4 mcal/s. To ensure the presence of water (in the first case) the scanning was started at approx. −50 °C, ice melting started at about −40 °C. The temperature and heat flow of the instrument were calibrated with pure water and oleic acid for the lower range and with stearic acid and indium for the upper range.

The concentration of phosphorus in the pans was obtained in two ways: (i) by weighing the slurry and calculating the concentration of the phospholipid from the known initial amount and (ii) by direct phosphorus determination [14] after digesting the content of the whole pan with perchloric acid. ΔH of the phospholipids was calculated from the area of the transition peak and the known heat flow. The percent of phospholipids undergoing melting was given by the ratio ΔH/mg phospholipid in the presence and absence of the polypeptides.

RESULTS

Fig. 1 shows the differential scanning calorimetric thermograms of phosphatidylserine interacting with the polypeptides. As is possible to see from the figure, the interaction changes the shape of the peaks and their size without affecting the onset temperature of melting, T_c .The value of the enthalpy of melting of pure phosphatidylserine is 5 kcal/mol, similar to that reported by Jacobson and Papahadjopoulos [15] for beef brain phosphatidylserine.

Fig. 2A presents the percentage of phosphatidylserine undergoing melting as a function of the ratio of lysine residues of the copolypeptides to phosphatidylserine. In Figs 1 and 2A, the ratios of lysine/phospholipid indicated are the final ratios in the aluminium pans. For phosphatidylserine interacting with the copolymer phenylalanine-lysine the ratios in the precipitate are the same as those in the solution. This is brought about by the positively charged polypeptide forming crosslinking bridges between the negatively charged vesicles.

By measuring the radioactivity (phospholipid) and phenylalanine in the supernatant after separation of the precipitate by centrifugation, it was found (for a ratio of N^+/P^- of 0.36 : 1) that less than 10 % of the phospholipid and less than 2 % of the polypeptide remained in the supernatant. For higher ratios of N^+/P^- the remaining amount was even smaller. At the highest experimental ratio of polypeptide to phospholipid ($N^+/P^- = 1.3 : 1$) about 12 % of the polypeptide and about 3 % of the phospholipid remained in the supernatant. Even in this case the ratio in the precipitate does not differ by more than 8 % from the initial ratio.

As seen from the figure, the fraction of phosphatidylserine in the product undergoing melting decreases almost linearly with the amount of polypeptide added and reaches a value of approx. 50 % at the molar ratio lysine/phosphatidylserine =

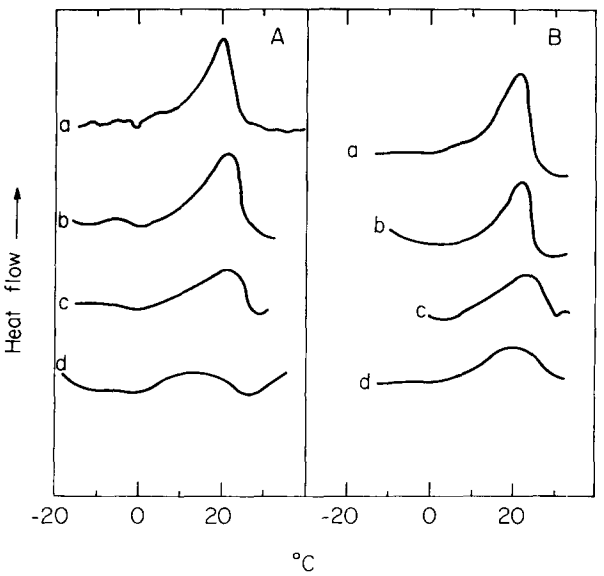

Fig. 1. Differential scanning calorimetric thermograms of phosphatidylserine liposomes interacting with basic polypeptides. Instrument sensitivity 2 mcal/s; full scale, scan rate 8 °C/min. (A) phosphatidylserine interacting with lysine-phenylalanine copolypeptide: a, phosphatidylserine alone, 2.7 mg phospholipid; b, phosphatidylserine/lysine (3.6 : 1 molar ratio), 3.3 mg phospholipid; c, phosphatidylserine/lysine (1.8 : 1 molar ratio), 2.6 mg phospholipid; d, phosphatidylserine/lysine (1 : 1 molar ratio), 2.7 mg phospholipid. (B) phosphatidylserine interacting with lysine-tyrosine copolypeptide: a, phosphatidylserine alone, 2.9 mg phospholipid; b, phosphatidylserine/lysine (3.4 : 1 molar ratio), 3.1 mg phospholipid; c, phosphatidylserine (1.7 : 1 molar ratio), 2.7 mg phospholipid; d, phosphatidylserine/lysine (1 : 1 molar ratio), 2.8 mg phospholipid.

1 : 1. In the present case about every four amino acid residues seem to eliminate melting of one phospholipid molecule. For technical reasons it was difficult to carry out the measurements in the presence of a large excess of polypeptide. The polypeptides cause a widening of the transition peak and a decrease in ΔH of the phospholipids as obtained from the peak area of the differential scanning calorimetric curves. These results are similar to those reported for biological membranes [3, 4], where the enthalpies of bound lipids are smaller than those of extracted ones and are in agreement with those reported by Papahadjopoulos et al. [12].

Fig. 2B presents the percentage of diplamitoyl phosphatidylcholine undergoing melting as a function of the ratios N^+/dipalmitoyl phosphatidylcholine. The samples for differential scanning calorimetry were obtained by water evaporation. Here, also about four amino acid residues fluidize about one phospholipid molecule. When the same experiments were performed by separating the precipitate by centrifugation, very little precipitate was obtained. The supernatant contained about 80 % of the total phospholipid and practically all the polypeptide. The ΔH of the precipitated dipalmitoyl phosphatidylcholine corresponds within experimental error to that of the noninteracting one.

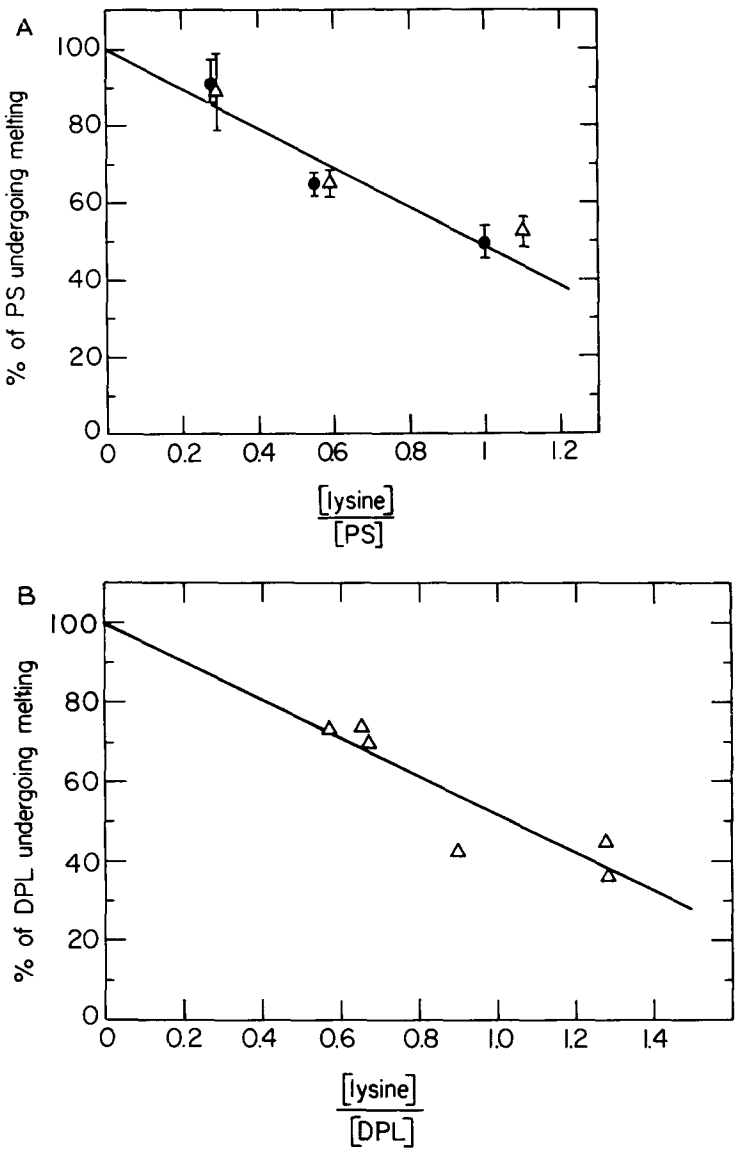

Fig. 2. (A) The percentage of phosphatidylserine (PS) in the interaction products undergoing melting as function of the molar ratio of lysine/phospholipid: (●) lysine-phenylalanine copolypeptide, (△) lysine-tyrosine copolypeptide. The bars denote the mean error and the number of experiments for each point was from three to seven. (B) The percentage of dipalmitoyl phosphatidylcholine (DPL) undergoing melting as a function of the molar ratio of lysine/phospholipid.

DISCUSSION

An interacting substance may either penetrate and dissolve in the liquid crystal, and thus reduce the melting temperature, or interact with the outer surface of the bilayer, lowering the potential of the electrical double layer or introducing a

condensing effect by dipole-dipole interaction with ultimate increase in the melting temperature. Indeed, interaction with multivalent cations usually results in increase in the melting temperature [5, 6, 15]. Interaction of negatively charged bilayers with different basic proteins and polypeptides affects the melting temperature along these two lines causing either a decrease or an increase in melting temperature [5–7]. One can imagine that in some cases the effects of the two types of interaction may balance, resulting in no shift in melting temperature. However, a more plausible possibility is a phase separation between the pure lipid bilayer melting with the original ΔH and the lipid-polypeptide interaction products. Either the interacting lipids in the mixed polypeptide-lipid domain melt outside the region of the measured temperatures (20 °C below and 30 °C above T_c) or the melting extends over such a wide temperature region that no additional peak can be discerned. The conformation of phenylalanine-lysine copolymer has not been affected by phosphatidylcholine vesicles when measured at low concentrations of both [10]. Centrifugation of dipalmitoyl phosphatidylcholine liposome suspensions at high concentrations in the presence of phenylalanine-lysine copolymer produced only negligible coprecipitation, indicating weak interaction. The interaction between dipalmitoyl phosphatidylcholine and phenylalanine-lysine copolymer is nonionic only and much weaker than between phenylalanine-lysine copolymer and phosphatidylserine liposomes. At low centrifugal forces the product does not precipitate, but when the suspension was concentrated for differential scanning calorimetry by water evaporation it was found that the polypeptide has the same effect on melting of dipalmitoyl phosphatidylcholine liposomes as on phosphatidylserine.

Gramicidin A interacting with dipalmitoyl phosphatidylcholine shows a similar thermotropic behaviour [6]. It lowers the transition peaks and at the same time widens them to such an extent that the peaks merge together. The lowering of the peaks increases with the gramicidin to phospholipid ratio. Similarly, it was reported recently that apolipoprotein eliminates the lipid phase transition of dimyristoyl phosphatidylcholine as found from light scattering [16] and broadens it (fluorescence and ESR measurements) [17]. Recently, Papahadjopoulos [12, 13] reported that myelin protein, cytochrome *c* and A_1 protein interacting with phospholipids decrease the enthalpy of melting, while polylysine increases ΔH and T_m.

The difference in behaviour of phenylalanine-lysine and tyrosine-lysine copolymers (Fig. 2) is very small, in spite of the fact that our previous work [10] shows that the two copolymers behave differently with respect to conformational transitions induced by interaction with lipid vesicles or penetrability into black lipid films. Presumably the different concentrations are responsible for the apparently different behavior. Evidently at high concentrations both copolypeptides may induce a phase separation, causing a similar melting pattern. One cannot conclude from this whether the two copolypeptides have identical conformation when interacting with phospholipid liposomes at the concentrations required for differential scanning calorimetry experiments.

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