Calorimetric Studies of the Interaction between the Lecithin and Copolymers of L-Lysine and L-Leucine

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(Received June 29, 1984)

The interaction between the dimyristoyl phosphatidylcholine (DMPC) bilayers and aqueous copolymers of L-lysine and L-leucine was investigated by means of differential scanning calorimetry. None of the copolymers had any effects on the transition temperature of DMPC, irrespective of the lysine/leucine ratio, although the pretransition peak disappeared. The transition enthalpy did not appreciably change upon the addition of copolymers if the leucine fraction was small (<0.25), but it remarkably decreased upon the addition of a copolymer with a large leucine content (>0.3). The latter case suggests the penetration of copolymers into the hydrophobic region (hydrocarbon-chain region) of DMPC as a result of the hydrophobic effect of leucine residues. On the contrary, copolymers of a small leucine content can penetrate at most into the polar region of DMPC because of the electric charge derived from lysine residues, indicating the effect of the copolymer composition on the penetrability of a copolymer into lipid layers.

The interaction between proteins or polypeptides and lipid membranes has been studied by means of various kinds of physicochemical techniques. It is considered that calorimetric studies are useful in investigating the effect of these proteins or polypeptides on the state of lipid membranes, particularly on the fluidity of hydrocarbon chains or on the phase transition of these membranes. Many calorimetric studies have thus been carried out of the systems of various lipids and proteins or polypeptides,[1-6] and different effects of proteins on the lipid phase transition have been proposed.[7]

The surface pressures of lecithin monolayers spread on lysine-leucine copolymer solutions were measured, and the penetration of these copolymers into lecithin monolayers was investigated in our previous study.[8] It was found, in that study, that the extent of penetration was dependent on the copolymer composition: The penetration was less when the leucine fraction in the copolymer, X_{Leu}, was smaller than 0.25, while it was remarkable at a X_{Leu} value larger than 0.3. Under our experimental conditions, however, all the copolymers are randomly coiled, and the conformational changes are not induced in the bulk solution.[8] Therefore, some kinds of substantial changes in lipid membranes should be regarded as possible reasons for the above penetration.

In the present study, differential scanning calorimetry (DSC) has been carried out in order to investigate the interaction between these copolymers and lecithin bilayers, and to discuss the characteristic properties of lecithin-copolymer interaction in some detail.

Experimental

Materials. As lecithin, L-α-dimyristoyl phosphatidylcholine (DMPC) from the Sigma Chemical Company was used without further purification. The random copolymers of lysine and leucine were the same as those previously used.[8] These copolymers were dissolved in redistilled water.

Differential Scanning Calorimetry. The differential scanning calorimetry was carried out on a Shimadzu DSC-30 calorimeter. The samples were prepared as follows: A 2—5 mg portion of the lipid was weighed in an aluminium sample pan. A copolymer solution of known concentration (0—20.3 mg/0.1 ml) was then added by capillary. After the lipid and the solution had been mixed with a pin at 30—40 °C and dispersed uniformly, the pan was hermetically sealed. The samples were heated and cooled at a constant rate of 5 °C/min. All scans were repeated for at least three cycles in order to confirm that the observed transition profile was reproducible. The water/lipid ratio was high enough to form multibilayers or lamellar phases of DMPC. Palmitic acid (mp 62.65 °C; enthalpy of melting, 10.0 kcal mol^{-1}) was used to standardize the apparatus for a quantitative heat determination. The peak areas were measured by weighing paper cutouts of the peaks.

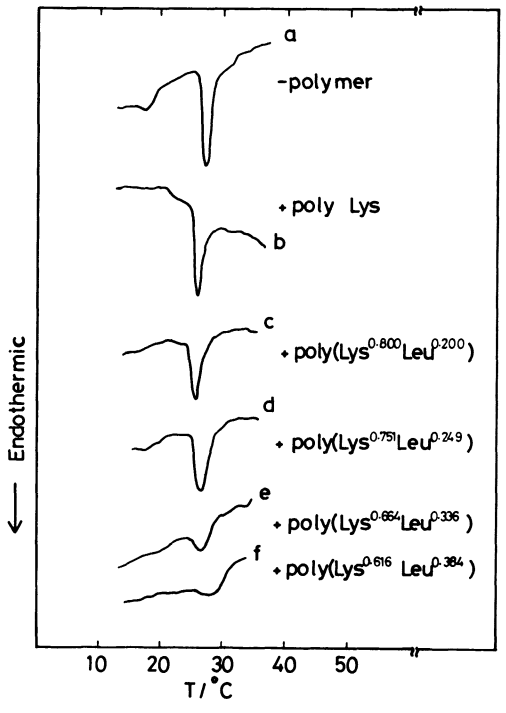

Fig. 1. DSC thermograms of DMPC dispersed in aqueous polymer solution.
(a): Without polymer, (b): with poly Lys, (c): with poly($Lys^{0.800}Leu^{0.200}$) (d): with poly($Lys^{0.751}Leu^{0.249}$) (e): with poly($Lys^{0.664}Leu^{0.336}$) and (f): with poly-($Lys^{0.616}Leu^{0.384}$). Heating rate: 5 °C/min.

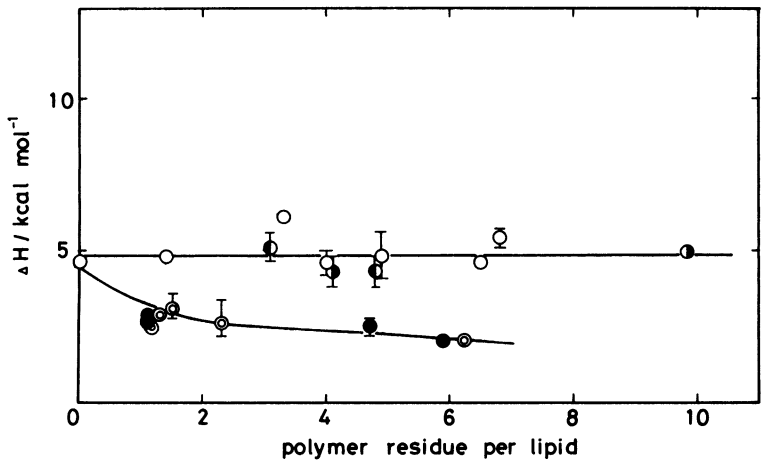

Fig. 2. The dependence of the calorimetric enthalpy of transition (ΔH) of DMPC on the polymer content in DMPC-polymer system: poly Lys (○), poly($Lys^{0.800}Leu^{0.200}$) (◑), poly($Lys^{0.751}Leu^{0.249}$) (◐) poly($Lys^{0.664}Leu^{0.336}$) (●), and poly-($Lys^{0.616}Leu^{0.384}$) (◎).

Results and Discussion

DSC Curves of DMPC Multilayers in Copolymer Solutions. Figure 1 shows the DSC curves upon the heating of DMPC dispersed in water containing various copolymers. It is known that phospholipids dispersed in excess water form lamellar phases.[9] The heating curve accompanied by the gel-to-liquid crystalline transition of pure DMPC is shown in Fig. 1-(a); it indicates that the main transition is at 24°C, and the pretransition, at 15°C. The calculated value of the calorimetric enthalpy change, ΔH, was 4.6 kcal mol^{-1}. Figures 1-(b)—(f) give the DSC heating curves of DMPC dispersed in aqueous copolymer solutions of various lysine-leucine ratios. The copolymer concentration was 10 mg/0.1 ml. In each case, the pretransition peak disappeared upon the addition of a copolymer. However, the transition temperature (the onset of main transition), T_c, did not change.

Calorimetric Enthalpy Change Accompanied by Phase Transition. The calorimetric enthalpy change, ΔH, accompanied by the gel-to-liquid crystalline transition of DMPC, is calculated from the peak area, and the values are plotted against the polymer residue/lipid ratio in Fig. 2. In the case of polylysine or the copolymers containing a small leucine fraction (<0.25), the ΔH values did not appreciably change compared with the case without copolymers (pure DMPC alone), irrespective of the value of the polymer/lipid ratio. On the other hand, the ΔH values are decreased by the addition of copolymers with large leucine fraction (>0.3). The values of ΔH are decreased with an increase in the polymer/lipid ratio and asymptotically converge, similarly in the case of the lecithin-gramicidin A system previously reported.[10]

The Effect of the Copolymer Composition. The dependencies of the transition temperature (T_c) and the enthalpy change (ΔH) of DMPC on the copolymer composition were established. The results when the polymer residue/lipid ratio is 5 are shown in Fig. 3. A similar tendency was found in the case of other polymer residue/lipid ratios (not shown). As is shown in Fig. 3-(a), the transition temperature was almost constant and was not appreciably different from that of pure DMPC (24°C), irrespective of the copolymer composition. The values of ΔH were, however, dependent on the copolymer composition: (1) if the leucine fraction (X_{Leu}) was smaller than 0.25, the ΔH values were almost constant (4.3—4.8 kcal mol^{-1}) and not appreciably different from that of pure DMPC (4.6 kcal mol^{-1}), irrespective of the composition of the added copolymers, and (2) in the cases of X_{Leu} values larger than 0.34, the ΔH's were decreased remarkably to 2.3—2.5 kcal mol^{-1}.

According to the differential scanning calorimetry studied by Susi *et al.*,[11] T_c is increased and ΔH is

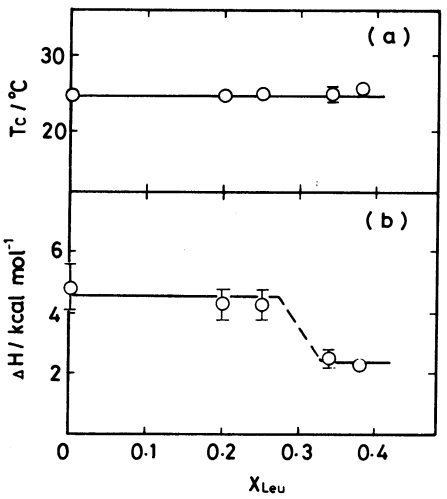

Fig. 3. The dependence of (a) the transition temperature (T_c) and (b) the enthalpy of transition (ΔH) of DMPC, on the leucine fraction of copolymers (X_{Leu}), at the polymer residue/lipid ratio of 5.

decreased in the case of polylysine-DMPC liposomal systems. This seems to contradict our present results. However, this apparent contradiction may be ascribed to the difference in experimental conditions, such as the method of preparation or the polymer/lipid ratio. Under the present conditions, no appreciable effects on T_c and ΔH were observed upon the addition of polylysine except for the disappearance of the pretransition, while the ΔH values were decreased by the addition of copolymers with a large leucine fraction, suggesting the effect of the leucine fraction of the copolymer on the membrane behavior.

The Mechanism of the Interaction between Lecithin and Lysine-Leucine Copolymers. The enthalpy change in the lecithin bilayer membrane accompanied by a phase transition, ΔH, was dependent on the leucine fraction of the copolymer, X_{Leu}, as has been described above. Further, the ΔH values are remarkably decreased around a X_{Leu} value of 0.3, which corresponds to the region with a sharp increase in the surface pressure of a lecithin monolayer upon the addition of copolymers.[8] On the other hand, copolymers had no effects on the transition temperature of the lecithin. It is, therefore, ascertained that the remarkable surface pressure increase of a lecithin monolayer in the presence of copolymers[8] was not due to the conformational change of copolymers.

Papahadjopoulos *et al.* have suggested three categories of protein-lipid interactions according to the correlation in calorimetric studies between the membrane permeability and the monolayer-area expansion.[7] Our results for the lysine-leucine copolymers will be discussed in comparison with their suggestion, and a plausible mechanism for the interaction between lecithin and lysine-leucine copolymers will be considered.

First, the interaction between lecithin and copolymers with a leucine fraction smaller than 0.25 may correspond to the Group 1 interaction in Ref. 7. According to the Papahadjopoulos postulate, no effects on monolayer expansion have been observed at a high value of the initial surface pressure (25 mN m^{-1}). In our results, a small increase in the area per molecule was observed in the low-surface-pressure region, while the extent of the increase becomes smaller at a higher surface pressure. Further, as has been described in Ref. 7, the enthalpy change of the transition, ΔH, of neutral phospholipids such as lecithin did not appreciably change; this also coincides with our present result. According to the results of Papahadjopoulos *et al.*,[7] this interaction is ascribable to the binding of the protein to the surface of lipid bilayers, without any penetration into the hydrocarbon region.

On the other hand, the interaction between lecithin and copolymers of a leucine fraction larger than 0.34 may correspond to the Group 3 interaction in Ref. 7. In this case, the surface pressure of a lipid monolayer increases as has previously been reported in Ref. 8, while the enthalpy change in the transition(ΔH) decreases. The transition temperature (T_c), however, does not change appreciably, These phenomena are attributable to the fact that proteins or polypeptides penetrate deeply into the hydrophobic domain. The deep penetration of proteins or polypeptides enhances the monolayer expansion.[8] By this hydrophobic interaction between lipids and proteins, the number of free lipid molecules decreases, and finally the ΔH value decreases. Since only a few lipid molecules participate in this interaction, and the bilayer is almost unperturbed,[7] no appreciable effects are observed on the transition temperature of a lipid, T_c.

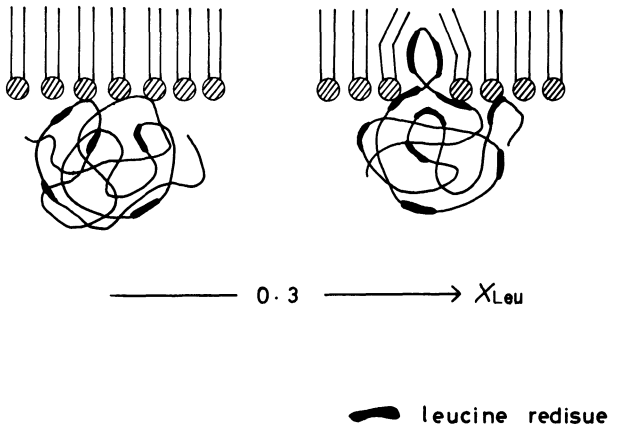

Fig. 4. A schematic model of the possible interaction between lecithin and lysine-leucine copolymers.

In the interaction between lecithin and lysine-leucine copolymers, the copolymers with a leucine fraction,X_{Leu}, smaller than 0.3, at most, interact only with the polar region of the lipid membrane because of the large electric charge derived from the lysine residues of the copolymer. On the other hand, the copolymers with X_{Leu} values larger than 0.3 penetrate into the hydrocarbon region of bilayers because of the hydrophobicity of the leucine residues. Figure 4 shows a schematic model of the possible interaction between lecithin and copolymers.

It has been accepted that the penetration ability of proteins into lipid membranes depends mainly on both the hydrophobicity and the electrostatic character of such proteins and polypeptides. Furthermore, it is of great interest that a change in the amino-acid composition of a copolymer also induces a remarkable increase in the penetration ability into lipid layers.

We are grateful to Professor Dr. J. T. Yang and Dr. Shigeo Kubota for the gift of the lysine-leucine copolymers.

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