Penetration of Lysine-Leucine Copolymers into Lecithin Monolayers from Underlying Aqueous Solutions

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The penetration of water soluble polymers into lecithin monolayers was investigated for the random copolymers of L-lysine and L-leucine at various compositions in order to discuss the effect of hydrophobicity and charge density of the copolymers on their interaction with lipid monolayers. Compared to the penetration of the amino acids(L-lysine and L-leucine), the copolymer penetrated at very low concentration whereas the monomer (amino acid) did not. The amount of copolymer penetration increased significantly when the leucine fraction of a copolymer, X_{Leu}, was larger than 0.3, while little penetration occurred when X_{Leu} was smaller than 0.25. From these facts, it has been concluded that the penetration of lysine–leucine copolymers was not only attributed to the hydrophobicity or the charge density of copolymer molecules, but also to specific surface effects such as (1) the enhanced penetration of macromolecules by the preceded penetration of polymer segments of the same molecule, or (2) the formation of a compact α-helical structure as the result of the restricted motion of the segment near the lipid surface.

It has been widely accepted that biomembranes essentially consist of lipid bilayers in which proteins are dissolved.[1] Although the composition of membrane lipids depends on the kind of membranes, one of the typical constituents may be phospholipids.[2] From this point of view, the interaction between phospholipids and proteins has been studied by the use of phospholipid monolayers as a model of lipid membranes.[3,4]

Since the structure of a protein is complex, synthetic polypeptides have been used in many studies as models for proteins.[5-9] Polylysine is one of the basic polypeptides, and the dissociation of the ε–amino group in a lysine residue is known to vary with pH or on the addition of alcohols or electrolytes to the solution.[10-16] In solutions of neutral pH, polylysine has a positive charge due to the protonation of ε-amino groups. It is found that a strong interaction occurs between polylysine and lipids and causes aggregation by forming lipid–peptide complexes or clusters if the phospholipids are acidic such as phosphatidylserine or phosphatidic acid.[17,18] It is considered that this is due to electrostatic interaction occuring at the negatively charged lipid monolayer surface because polylysine does not interact with the neutral phospholipids like lecithin. However, electrostatic interaction between polylysine and zwitterionic phospholipids such as lecithin has been reported in recent liposomal studies, being weak but not negligible.[19] A similar interaction also occurs between neutral phospholipids and acidic polypeptides like polyglutamic acid.[20] Moreover, Campbell *et al.* have also indicated the existence of hydrophobic interaction between polylysine and lecithin.[19] From this standpoint, the author's interest is focussed on the penetration of polylysine into lecithin monolayers.

Many apolar amino acids as well as polar exist in a protein molecule. Not only homopolymers such as polylysine but also copolymers which involve both ionic and apolar chains should be regarded as models for the actual protein molecules. Leucine is an apolar amino acid since it does not have any ε-amino groups in the hydrocarbon chain, although it has six carbon atoms in the monomer residue, which is the same as lysine. Therefore, lysine–leucine copolymers of varying hydrophobicity or charge density are obtained by changing the lysine–leucine ratio.

The penetration of amino acids like lysine, leucine and norleucine into lecithin monolayers was discussed in our previous work.[21-23] In this paper, random copolymers consisting of lysine and leucine were used and the penetration of these copolymers into lecithin monolayers was investigated in order to study the effect of the hydrophobicity and the charge density of the copolymer on its penetration into lipid monolayers.

Experimental

Materials. Table 1 shows the lysine–leucine copolymers used in this experiment. Polylysine (poly-L-Lys·HCl) was purchased from the Protein Research Foundation, and the molecular weight was estimated from the viscosity measurement according to Yaron and Berger.[24] The other copolymers [poly(Lys^{x}Leu^y)$_n$ (HCl)$_{xn}$] in Table 1 were supplied from the Cardiovascular Research Institute, University of California. These copolymers were all water soluble. L-α-Dimyristoyl phosphatidylcholine (DMPC) from the Sigma Chemical Company was used without further purification. Chloroform was used as the spreading solvent.

Surface-tension Measurement. The surface tension of the aqueous copolymer solution, γ_{cop}, was measured by the capillary-rise method, and the surface pressure of copolymers, F_{cop}, was obtained by means of Eq. 1:

$$F_{cop} = \gamma_0 - \gamma_{cop}, \quad (1)$$

where γ_0 is the surface tension of pure water. The temperature

Table 1. Characterization of copolymers of L-lysine and L-leucine

Composition		MW	Degree of polymerization
Lys	Leu		($\overline{DP}$)
1.0	0	66000	359
0.859	0.141	94000	534
0.829	0.171	102000	586
0.800	0.200	85000	491
0.751	0.249	88000	518
0.664	0.336	56000	337
0.616	0.384	43000	264

of the solution was kept at 25±0.5 °C.

Surface-pressure Measurement. The surface pressure of the DMPC monolayer was measured by Wilhelmy's plate method. Since the surface tension of a solution is not always equal to that of pure water, the surface pressure, F, was calculated by means of Eq. 2:[25]

$$F = \gamma_0 - \gamma = F' + F_{cop}, \quad (2)$$

where γ is the surface tension of a copolymer solution covered with a lipid monolayer, while F' is the practical surface pressure defined by:

$$F' = \gamma_{cop} - \gamma, \quad (3)$$

and is measured directly by Wilhelmy's plate method as the decrease in the surface tension of a copolymer solution upon the spreading of a lecithin monolayer. That is:

$$F' = (T_{cop} - T)/l, \quad (4)$$

where l is the length around the horizontal cross section of the plate. The forces acting on the Wilhelmy plate, T_{cop} and T, were measured as follows. A copolymer solution of 57 cm³ was poured into a trough (5.0×20.0×0.4 cm³) coated with Teflon, and was kept in thermostatted air at 25±0.5 °C. After the surface of the copolymer solution was swept by a barrier covered with a Teflon sheet, the lower end of a ground glass plate, vertically suspended, was touched to the liquid surface and the vertical pull force acting on the plate, T_{cop}, was measured by means of a Shimadzu T-NR torsion balance. A lipid monolayer was then spread from an Agla micrometer syringe (accuracy ±0.0002 cm³) and left for 5 minutes. The force acting on the plate, T, was then measured by compressing the monolayer with a barrier. The values of T_{cop} and T were measured several times, respectively, until the equilibrium values were obtained. The experimental error of the measurement was within ±0.1 mN m⁻¹.

Results

Surface Tension of a Copolymer Solution. Figure 1 shows the surface tension of polylysine and various copolymer solutions. It is seen that the surface tension decreases with the increase in the concentration, C_2, for all copolymer solutions, and it also decreases with the leucine content of copolymer. This is attributable to the fact that the higher the leucine content, the more hydrophobic and less electrically charged the molecule becomes. The surface concentration of the copolymer is, therefore, higher at higher content of leucine or lower content of lysine, since lysine is more hydrophilic and positively charged due to its ε-ammonium group.

Surface Pressure-Area Curves of the DMPC Monolayers. The relation between the surface pressure, F, and the area per molecule of lecithin, A_3, spread on polylysine solutions is shown in Fig. 2. The curve shifted to a higher surface pressure region at higher C_2, indicating that the penetration of polylysine into the lecithin

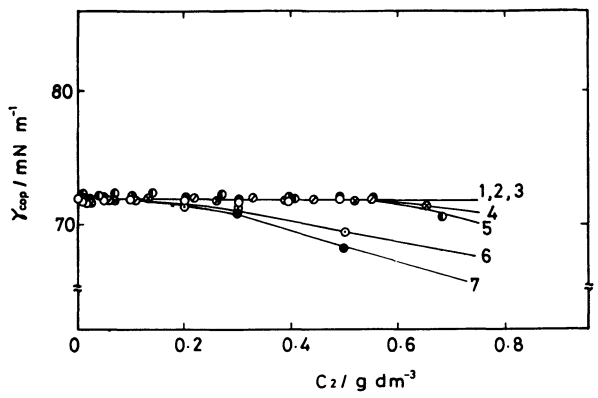

Fig. 1. The dependence of surface tension, γ_{cop}, on the concentration of lysine–leucine copolymers, C_2.
Leucine fraction: 1. ○ 0; 2. ◑ 0.141; 3. ⊘ 0.171; 4. ⊗ 0.200; 5. ◐ 0.249; 6. ⊙ 0.336; 7. ● 0.384.

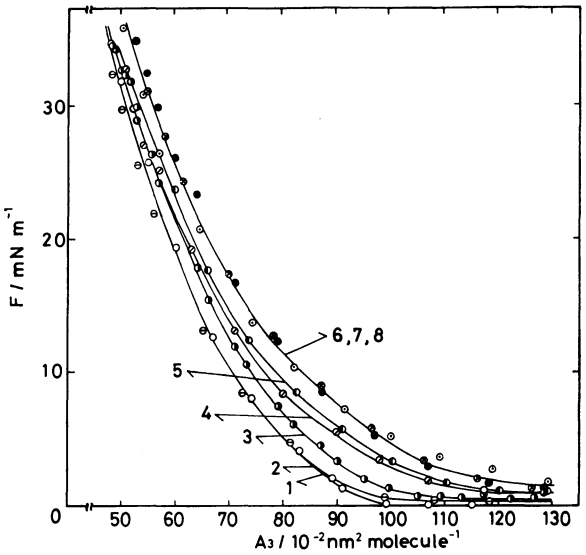

Fig. 2. The relation between the surface pressure, F, and the area per molecule of lecithin, A_3, spread on polylysine solutions. pH range 4.8—5.0.
C_2/g dm⁻³: 1. ○ 0; 2. ⊖ 0.020; 3. ◑ 0.067; 4. ⊘ 0.164; 5. ◐ 0.226; 6. ⊗ 0.343; 7. ⊙ 0.504; 8. ● 0.623.

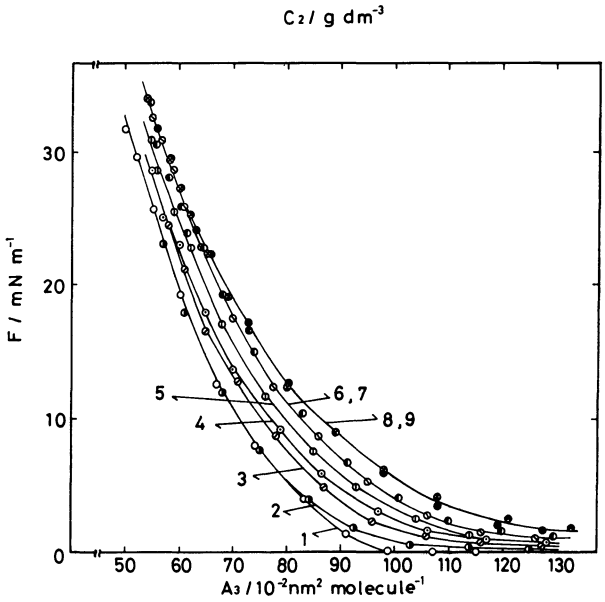

Fig. 3. The surface pressure (F)-area (A_3) curves of lecithin monolayers spread on poly($Lys^{0.800}Leu^{0.200}$)$_n$ solutions. pH range 5.2—5.4.
C_2/g dm⁻³: 1. ○ 0; 2. ◑ 0.146; 3. ⊘ 0.220; 4. ⊙ 0.292; 5. ⓘ 0.355; 6. ◐ 0.440; 7. ⊘ 0.492; 8. ⊗ 0.635; 9. ● 0.709.

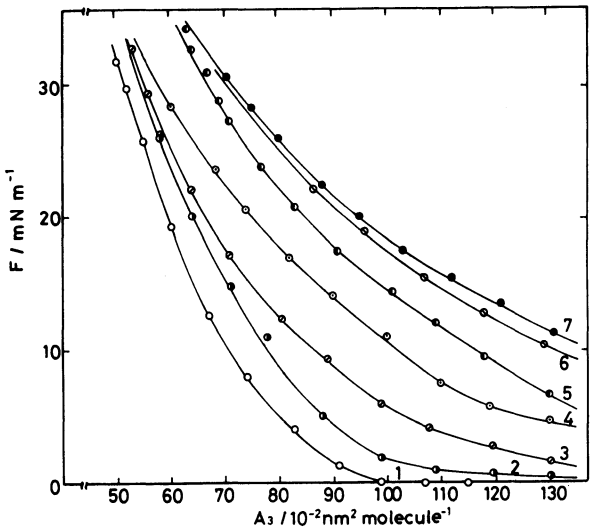

Fig. 4. The surface pressure (F)-area (A_3) curves of lecithin monolayers spread on poly($Lys^{0.616}$ $Leu^{0.384}$)$_n$ solutions. pH range 4.5—5.0.
C_2/g dm^{-3}: 1. ○ 0; 2. ◑ 0.112; 3. ⊘ 0.176; 4. ⊙ 0.224; 5. ◐ 0.322; 6. ⊘ 0.449; 7. ● 0.500.

monolayers occurred. Figures 3 and 4 show the surface pressure-area curves of lecithin monolayers spread on leucine-containing copolymer solution, namely, poly-($Lys^{0.800}Leu^{0.200}$)$_n$ in Fig. 3 and poly($Lys^{0.616}Leu^{0.384}$)$_n$ in Fig. 4, respectively. The curve also shifts to a higher surface pressure region with higher C_2, similar to the case of polylysine (Fig. 2), although the extent of the shift differs in each case. The shifts were also observed for other lysine–leucine copolymers [poly($Lys^{0.857}Leu^{0.143}$)$_n$, poly ($Lys^{0.751}Leu^{0.249}$)$_n$, and poly ($Lys^{0.664}Leu^{0.336}$)$_n$], though not shown in the figure. Our previous studies of the penetration of amino acids[21-23] also showed similar shift of the curves to a higher surface pressure region, however, the shift was not appreciable unless the concentration of the amino acids was about ten to one hundred times higher than that of the polypeptides. This may be attributable to the fact that the penetration of one part of the polymer residues restricts the other part of the polymer molecule in the vicinity of the surface. The penetration of polymer molecules is therefore found at very low concentrations where the monomer penetration does not occur.

Effect of the Copolymer Composition on Its Penetration into Lecithin Monolayer. The increase in the surface pressure, ΔF, caused by the penetration of polymer molecules into lipid monolayer,

$$\Delta F = F - F_0, \tag{5}$$

is considered to be a measure of the interaction of the polymer with membranes,[26] where F and F_0 are the surface pressure at a given area per molecule of lecithin respectively with and without polymer in the aqueous solution. The typical results of ΔF are shown in Fig. 5 against the polymer concentration, C_2, where the area per molecule of lecithin, A_3, is 90×10^{-2} nm^2. It is seen from this figure that the values of ΔF increases with the polymer concentration, C_2, and that the shape of the ΔF *vs.* C_2 relations are independent of the mole

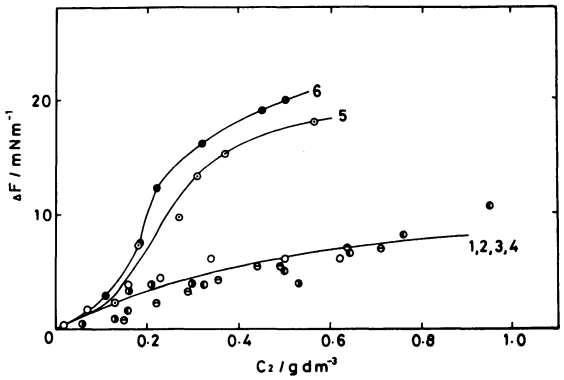

Fig. 5. The dependence of the increase in surface pressure, ΔF, on the concentration of copolymer, C_2, where the area per molecule of lecithin is 90×10^{-2} nm^2.
Leucine fraction, X_{Leu}: 1. ○ 0; 2. ◑ 0.141; 3. ⊖ 0.200; 4. ◐: 0.249; 5. ⊙ 0.336; 6. ● 0.384.

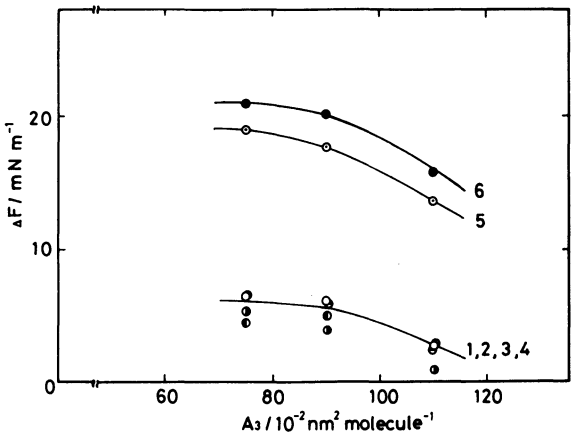

Fig. 6. The dependence of the increase in surface pressure, ΔF, on the area per molecule of lecithin, A_3, where copolymer concentration, C_2, is 0.5 g dm^{-3}.
Leucine fraction, X_{Leu}: 1. ○ 0; 2. ◑ 0.141; 3. ⊖ 0.200; 4. ◐ 0.249; 5. ⊙ 0.336; 6. ● 0.384.

fraction of leucine in the copolymer, X_{Leu}, provided X_{Leu} is smaller than 0.25. However, ΔF becomes large when X_{Leu} is larger than 0.34. This behavior was also observed at other values of A_3, although the value of ΔF was smaller at larger A_3 value, as is shown in Fig. 6 when C_2 is 0.5 g dm^{-3}.

Discussion

It is considered that polypeptides with electric charge, such as polylysine, are more hydrophilic, and, therefore, the interaction of these polypeptides with phospholipids like lecithin is weaker than for polypeptides without net charge. The charge density or hydrophilicity of polylysine decreases at a higher pH (about 10) since the ε-ammonium groups are deprotonated ($-NH_3^+ \rightarrow -NH_2$). It also decreases on the addition of perchlorate (ClO_4^-) due to the specific shielding effect on the positive charge ($-NH_3^+$).[12,15] In fact, the increase in the surface pressure of the lecithin monolayer, ΔF, was enhanced under these conditions as established in our work (unpublished data).

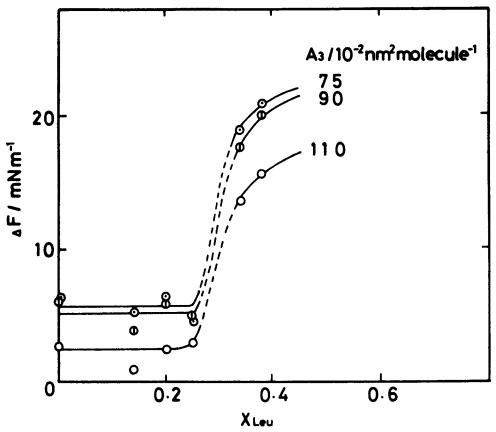

Fig. 7. The increase in surface pressure, ΔF, against the leucine fraction, X_{Leu}, where copolymer concentration, C_2, is 0.5 g dm^{-3}.

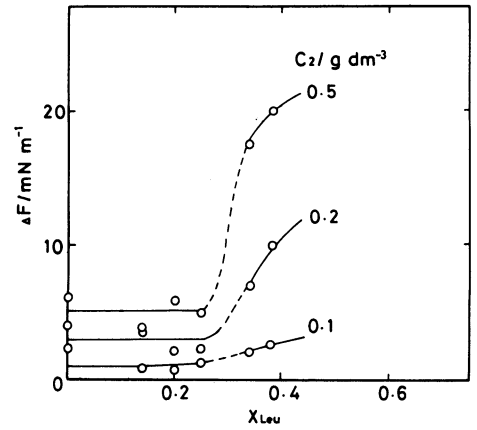

Fig. 8. The increase in surface pressure, ΔF, against the leucine fraction, X_{Leu}, at constant area per molecule of lecithin ($A_3=90\times10^{-2}$ nm^2).

The surface pressure increase, ΔF, due to the penetration was plotted against the leucine fraction, X_{Leu}, of the copolymer in Fig. 7 at a constant concentration of 0.5 g dm^{-3}. The ΔF values did not appreciably change when the leucine fraction, X_{Leu}, is from 0 to 0.25, irrespective of the area per molecule of lecithin, A_3. However, a remarkable increase was found in the vicinity of 0.3 of X_{Leu}, and the increase was again restrained when X_{Leu} exceeded 0.34. This remarkable increase in ΔF, however, does not occur at a small polymer concentration (0.1 g dm^{-3}), but it occurs when polymer concentration, C_2, is larger than 0.2 g dm^{-3}, as is shown in Fig. 8 for the area per molecule of lecithin of 90×10^{-2} nm^2.

In the copolymers of lysine and leucine, the charge density and hydrophilicity decrease with the increase in leucine fraction, and the penetration of these polymers into lecithin monolayers is expected to occur more easily. The amount of penetration of lysine–leucine copolymers into lecithin monolayers, however, did not change appreciably when the leucine fraction, X_{Leu}, is less than 0.25, but increased remarkably when X_{Leu} exceeded 0.3, as was described before. Accordingly, the penetration of these polymers is not only enhanced by the increase in hydrophobicity, the decrease in the charge density.

It is claimed that an α-helical structure is formed in lysine–leucine copolymers when the leucine fraction is large, because of the α-helix stabilizing effect of leucine.[27] A possible reason for the characteristic penetration as described above is that the transition from a coiled form to an α-helical structure occurs at a leucine fraction larger than about 0.3. According to circular dichroism, all the copolymers (leucine fraction, from 0 to 0.384) are in coiled forms in aqueous solution under our experimental conditions (pH≈5, in salt-free water). The remarkable increase in surface pressure is, therefore, not attributable to the conformational change of the polymers in the bulk solution.

The effect of the interface on the penetrability of these lysine–leucine copolymers into lecithin monolayer should be finally considered. D. Bach *et al.* have investigated the interaction of basic polypeptides consisting of two different amino acids with phospholipid vesicles by circular dichroism measurements and have reported the occurrence of a conformational change of these copolymers at a lipid surface.[9] In our experiment, the penetration of a part of the polymer segments to the lecithin monolayers restricts the other segments of the same macromolecule to a limited thermal motion in the vicinity of the surface. It is possible, therefore, that these restricted segments penetrate into lecithin monolayers more easily than when these segments are free in the bulk solution. It is, therefore, reasonable that the penetration of these copolymers occurs at such a low concentration where the monomers (residue amino acids) cannot penetrate appreciably. Moreover, the α-helical structure of the polypeptide is considered to be easily formed under the limited motion on the lipid surface, in comparison with the case where they are free in the bulk solution. More than one leucine residue are contained in one period of an α-helical spiral when the leucine fraction, X_{Leu}, is larger than 0.3, since there are 3.6 residues per α-helical unit. It is expected, therefore, that the α-helical structure is stabilized by the hydrophobic binding between leucine residues, which induces the contraction of polymer molecules, and the increase in penetration into the monolayer.

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