Interaction of Synthetic Polypeptides with Lipids in Monolayers

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(Received October 3, 1977)

The interaction of polypeptide (benzyloxycarbonyl derivatives of basic poly(α-amino acid), benzyl esters of acidic poly(α-amino acid), and poly(L-leucine)) and lipid (fatty acids, fatty alcohols and cholesterol) at the air–water interface was investigated. From the additivity rule of mean molecular areas and the work of collapse of the monolayers, it was found that the characteristic interaction occurs in the polymer rich region (less than 0.2 lipid mol fraction), and that the interaction is stronger for the polymer with higher helical content. The interaction was found to take place between side chains of polypeptides and lipids, and to be mainly of hydrophobic nature. The components in mixed polypeptide–cholesterol monolayers exhibit characteristic miscibilities depending upon the chemical composition of polymer side chains. The polarized infrared spectra of collapsed films consisting of single or mixed components indicate that the polymer component is aligned in a direction parallel to the compressing barrier.

Lipid-protein interactions are of great interest for understanding the structure and function of biological membranes. Spread monolayers provide one of the most promising means for studying molecules in fixed orientations, constituting an important model system for many naturally-occurring phenomena which involve surfaces or oriented arrays of molecules. The monolayer approach has been found very useful for understanding molecular aspects of lipid-protein interactions presumably occurring in biomembranes.[1–3] Synthetic polypeptides are useful as a simple model of proteins. A number of investigations on the physicochemical and biological properties have been carried out.[4]

Recently, the interactions of lipid monolayers spread at the air–water interface with synthetic polypeptides dissolved in the subsolution have been investigated.[5–8] For poly(L-lysine) and stearic acid system, the maximum interaction occurs at a pH value where polypeptide is in the helical conformation, and the helical conformation of the poly(L-lysine) molecule can be stabilized by electrostatic interaction with the ionized stearic acid monolayer.[7] On the other hand, from studies on various combinations of lipid monolayers (phosphatidyl serine, DL-α-dipalmitoyllecithin) and synthetic polypeptides-(poly(L-aspartic acid), poly(L-glutamic acid), poly(L-lysine)), in the subsolution, it has been found that the interaction between lipids and polypeptides at the interface is essentially hydrophobic.[8] The interactions have also been studied by using mixed monolayers of synthetic polypeptides and lipids. The mixed monolayer approach is particularly useful for studying the surface compatibility of polypeptides and lipids.[9–11] No interactions are detected probably owing to the quite different steric arrangement of the two components at the interface.[9,10] On the other hand, the formation of 5:1 and 1:1 (residue: molecule) complexes for poly-(DL-leucine) and sodium octadecyl sulfate system and 1:1 complex for poly(DL-phenylalanine) and sodium octadecyl sulfate system has been also reported.[11]

The purpose of this investigation is to establish the existence or nonexistence of interactions between synthetic polypeptides and lipids, and to clarify the nature of the interaction at the interface.

Experimental

Poly(δ-benzyloxycarbonyl-L-ornithine) (PLO(Z)), poly-(ε-benzyloxycarbonyl-L-lysine) (PLL(Z)), poly(L-leucine) (PLLeu), poly(β-benzyl-L-aspartate) (PBLA), poly(γ-benzyl-L-glutamate) (PBLG), and poly(γ-benzyl-DL-glutamate) (PBDLG-1, PBDLG-2) were prepared by the polymerization of the *N*-carboxy anhydrides of respective amino acids. The molecular weights determined from the viscosities at 25 °C in *N*,*N*-dimethyl-formamide (DMF)[12] or dichloroacetic acid (DCA)[13] are given in Table 1. Infrared spectra indicate that, with the exception of PBDLG-1 and PBDLG-2, these polymers are in the α-helical conformation in the solid state. The helical content of poly(γ-benzyl-DL-glutamates) was more than 90% regular α-helix for PBDLG-1, and 13% regular α-helix and 60% perturbed α-helix for PBDLG-2. No β-conformation was found for PBDLG-1 and PBDLG-2. The regular α-helix and perturbed α-helix have been designated by Tsuboi *et al.*[14] The perturbed α-helix portion in a polypeptide molecule contains an antipode residue, the sequence length being relatively short.

TABLE 1. MOLECULAR WEIGHTS OF POLYPEPTIDE SAMPLES

Polymer	[η]	*MW*
PLO(Z)	2.17(DMF)	3.5×10^5
PLL(Z)	2.40(DMF)	3.8×10^5
PBLA	0.28(DCA)	4.0×10^4
PBLG	1.67(DCA)	3.1×10^5
PBDLG-1	0.34(DCA)	5.0×10^4
PBDLG-2	0.40(DCA)	6.0×10^4
PLLeu: $\eta_{sp}/c=4.56$ ($c=0.2\%$ TFA)		

Lipids employed were myristic acid (C_{14} acid), stearic acid (C_{18} acid), 1-tetradecanol (C_{14} alcohol), 1-octadecanol (C_{18} alcohol), and cholesterol. These materials were purified either by fractional distillation or by recrystallization, and were chromatographycally pure.

The spreading solvents (DMF, dichloromethane (DCM) and trifluoroacetic acid (TFA)) were distilled under nitrogen atmosphere. The subsolution, 0.01 M HCl, was made up from twice distilled water and distilled 6 M HCl.

The spreading solvents were DCM for PBLA- and PBG (PBLG, PBDLG-1 and PBDLG-2)–lipid systems, a 9:1 (v/v) mixture of DCM and DMF for PLO(Z)- and PLL(Z)–lipid

systems, and a 9: 1 (v/v) mixture of DCM and TFA for PLLeu. When a 0.3 cm^3 solvent was deposited on water surface, a 10 to 1 compression of the surface produced less than 0.1 dyn/cm film pressure. The solutions of polypeptide and lipid were prepared separately, and mixed in the desired ratio immediately before spreading. The spreading solution was deposited from a micrometer syringe onto the surface of subsolution, and left to stand for 15 min. Polypeptide or lipid concentration in the spreading solution was 4—6 mg/10 cm^3. Unless otherwise stated, the initial spreading-area was 50 $Å^2$ (0.5 nm^2)/residue or molecule. The trough (65×15×1 cm) and compressing barriers were made of Teflon. The film was compressed at a rate of 10 mm/min (1 $Å^2$/residue or molecule/min). The surface pressure was measured by the Wilhelmy method. Measurements were carried out at 25 °C.

The polarized infrared spectra of collapsed films were measured by a JASCO DS-701G spectrometer. The monolayer was spread and compressed until collapse under the same conditions as in the case of surface pressure measurement. In order to remove the monolayer, it was compressed between two Teflon barriers, until the separation was about 1.5 cm. The polymer was then removed by drawing a stainless steel net across the trough between the barriers. The transferred collapsed film was dried at room temperature.

Results

Figure 1 shows the surface pressure-area (π-A) curves for PLO(Z), PLL(Z), PLLeu, PBLA, and PBG's. Plateaux or inflections are seen in the curves. The plateau or inflection which is characteristic of a number of synthetic polypeptide monolayers is associated with the collapse or transition of the monolayer in the α-helical conformation from a two-dimensional oriented state to a three-dimensional disoriented state.[4,15] The limiting areas extrapolated to zero surface pressure for the steep region are 22.5 $Å^2$/residue for PLO(Z), 23.8 $Å^2$/residue for PLL(Z) and 20.7 $Å^2$/residue for PBLA. When the monolayers were formed at the various spreading areas (40—60 $Å^2$/residue), the π-A curves were reproducible to $\pm$0.2 $Å^2$/residue in repeated experiments. The areas are in good agreement with those reported by other workers.[4,16-19] The limiting area for PLLeu is 17.4$\pm$0.3 $Å^2$/residue which is smaller than that reported by Yamashita (19 $Å^2$/residue).[20] According to Malcolm,[21] the result may be associated with the difficult spreading of PLLeu.

The π-A curves of PBLG, PBDLG-1 and PBDLG-2 are shown in Fig. 1-b. The limiting area and monolayer transition pressure are 21.6 $Å^2$/residue and about 5.3 dyn/cm for PBLG, 21.0 $Å^2$/residue and 7.8 dyn/cm for PBDLG-1, and 20.8 $Å^2$/residue and 8.1 dyn/cm for PBDLG-2. The transition pressure of PBLG is much lower than that of PBDLG-1 and PBDLG-2. This might be related to the conformational difference between monolayers of PBLG and PBDLG's at the air–water interface. The difference in transition pressures of PBDLG-1 and PBDLG-2 is quite small. For PBG's, it has been reported that the temperature coefficient of transition pressure is dependent upon the regular α-helix content: the negative value of temperature coefficient is smaller for PBDLG-1 than for PBDLG-2 (regular α-helix content; PBDLG-1>PBDLG-2).[22] The height of transition pressure may be reversed at higher temperature. The monolayer of PBLG is more expanded than that of PBDLG-1 and PBDLG-2 (Fig. 1-b). The compressibility at close packed area is 0.027 for PBLG, 0.014 for PBDLG-1, and 0.015 for PBDLG-2.

The π-A curves of various lipids are shown in Fig. 2. C_{14} acid gives an expanded monolayer. On the other hand, the monolayers of C_{18} acid, C_{14} alcohol, C_{18} alcohol, and cholesterol are of condensed type. The film of cholesterol is highly incompressible.

As typical examples, the π-A curves of mixed PLO(Z)–C_{14} acid and mixed PLO(Z)–cholesterol monolayers are shown in Fig. 3. Two transition points are shown to

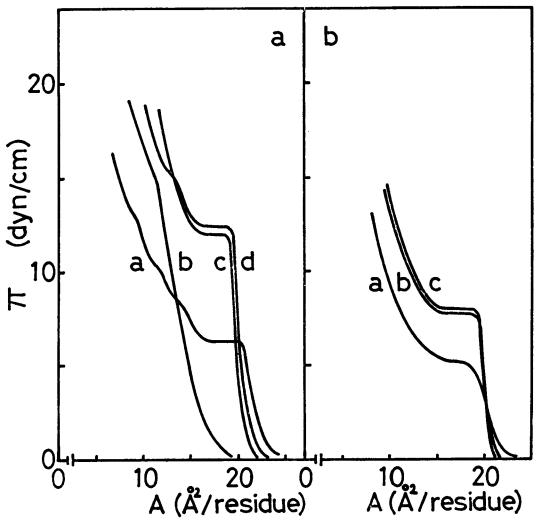

Fig. 1. Surface pressure-area curves of polypeptide monolayers on 0.01 M HCl at 25 °C. 1 dyn=10^{-5} N, 1 Å=0.1 nm. a) a: PLL(Z), b: PLLeu, c: PBLA, d: PLO(Z). b) a: PBLG, b: PBDLG-1, c: PBDLG-2.

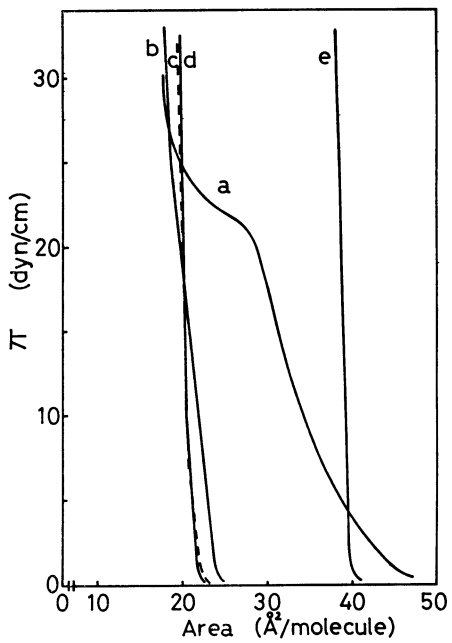

Fig. 2. Surface pressure-area curves of lipid monolayers on 0.01 M HCl at 25 °C. a: C_{14} acid, b: C_{18} acid, c: C_{14} alcohol, d: C_{18} alcohol, e: cholesterol.

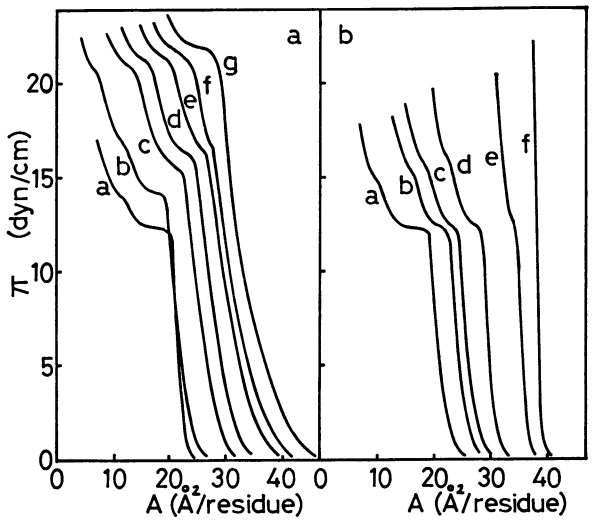

Fig. 3. Surface pressure-area curves of mixed monolyers on 0.01 M HCl at 25 °C. a) PLO(Z)–C_{14} acid, a: 1: 0, b: 4: 1, c: 7: 3, d: 1: 1, e: 3: 7, f: 1: 4, g: 0: 1, b) PLO(Z)–cholesterol, a: 1: 0, b: 4: 1, c: 7: 3, d: 1: 1, d: 1: 1, e: 1: 4, f: 0.: 1 (residue mol: mol).

exist (Fig. 3-a). The surface pressure at the lower point increases with increase in C_{14} acid mol fraction, while the higher point (about 22 dyn/cm) is independent of the mol fraction of C_{14} acid. The lower transition point is evidently caused by the polymer component. Discussion will be limited to this lower transition point or pressure. For mixed PLO(Z)–C_{14} acid system, up to C_{14} acid mole fraction of 0.5, the transition pressure caused by the polymer component increases steeply, the increase becoming gradual beyond this mole fraction. No significant change in transition pressure is observed for the mixed PLO(Z)–cholesterol system (Fig. 3-b).

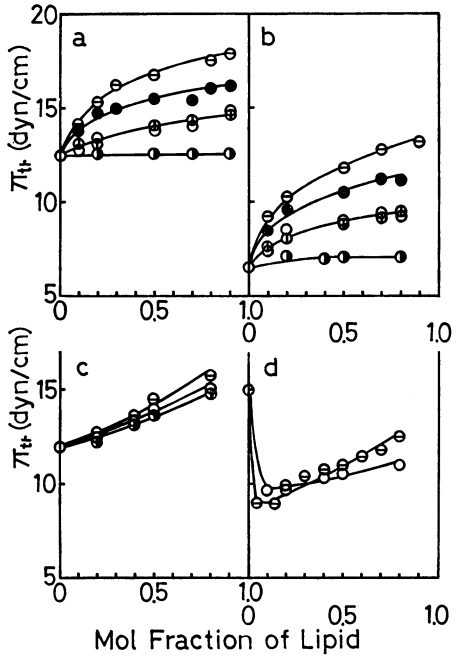

Fig. 4. Transition pressure of mixed monolayers as a function of lipid mole fraction at 25 °C.
a) PLO(Z)–lipid, b) PLL(Z)–lipid, c) PBLA–lipid, d) PLLeu–lipid, ⊖: polypeptide–C_{14} acid. ○: polypeptide–C_{18} acid, ●: polypeptide–C_{14} alcohol, ⓘ: polypeptide–C_{18} alcohol, ◑: polypeptide–cholesterol.

Figure 4 shows the relationship between the transition pressure and the composition of lipid in mixed monolayers for PLO(Z)–lipid, PLL(Z)–lipid, PBLA–lipid and PLLeu–lipid systems. From Figs. 4-a and 4-b, we see that no difference in transition pressures between PLO(Z)–C_{18} acid and PLO(Z)–C_{18} alcohol molayers and between PLL(Z)–C_{18} acid and PLL(Z)–C_{18} alcohol monolayers. In addition, the transition pressures for mixed PLL(Z)–lipid systems are larger than those for mixed PLO(Z)–lipid systems. PLO(Z) has less methylene group as compared with PLL(Z).

The transition pressure for PBLA–lipid systems (Fig. 4-c) differs remarkably from PLO(Z)–lipid and PLL(Z)–lipid systems. The difference in transition pressure among PBLA–C_{14} acid, PBLA–C_{18} acid and PBLA–cholesterol is small. There is an inflection point at about 15 dyn/cm for a monolayer of PLLeu (Fig. 1). For mixed monolayers of PLLeu and either C_{14} acid or C_{18} acid (Fig. 4-d), we see that the transition pressure decreases remarkably with the addition of a small amount of lipid, becoming minimum at about 0.2 lipid mole fraction.

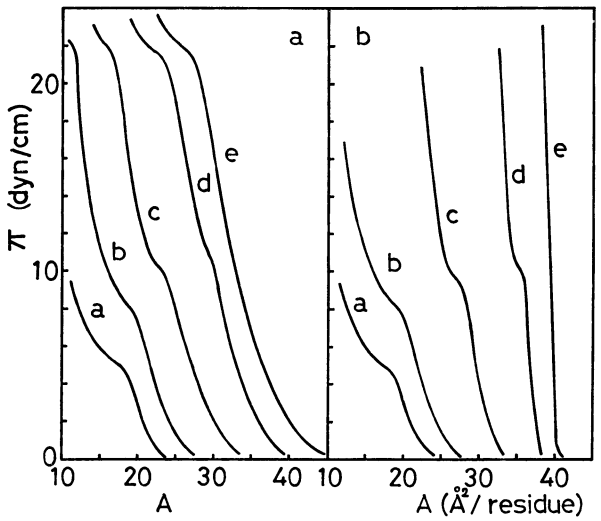

Fig. 5. Surface pressure-area curves of mixed monolayers on 0.01 M HCl at 25 °C. a) PBLG–C_{14} acid, b) PBLG–cholesterol. a: 1: 0, b: 4: 1, c: 1: 1, d: 1: 4, e: 0: 1 (residue mol: mol).

Figure 5 shows the π-A curves of mixed PBLG–C_{14} acid and mixed PBLG–cholesterol monolayers. It was found that the π-A curves for mixed monolayers of C_{14} acid, and either PBDLG-1 or PBDLG-2 also shows a similar tendency to that for mixed PBLG–C_{14} acid monolayers.

Figure 6 shows the relationship between the transition pressure and the composition of lipid for mixed monolayer of PBG with C_{14} acid, C_{18} acid or cholesterol. The increment of the transition pressure is in the order of α-helical content of polymers in solid state. With exception of mixed PBLG–cholesterol monolayers, the change in transition pressure is considerably small

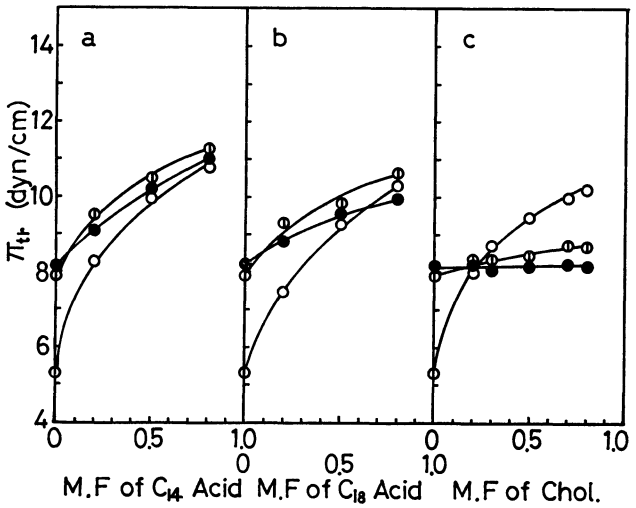

Fig. 6. Transition pressure of mixed monolayers as a function of lipid mole fraction at 25 °C.
a) PBG–C_{14} acid, b) PBG–C_{18} acid, c) PBG–cholesterol. ○: PBLG–lipid, ◑: PBDLG-1–lipid, ●: PBDLG-2–lipid.

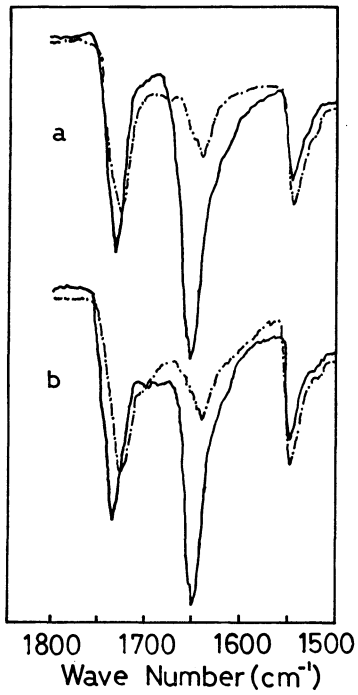

Fig. 7. Polarized IR spectra of collapsed films transferred onto a stainless steel net. a) PBLG, b) PBLG–C_{14} acid mixture (4: 1 (residue mol: mol)). ——: Electric vector parallel to the barrier used to collapes the film, –•–•: electric vector perpendicular.

(PBDLG-1) or practically absent (PBDLG-2).

As a typical example, the polarized infrared spectra for collapsed films of PBLG and a 4: 1 (residue mol: mol) mixture of PBLG and C_{14} acid are shown in Fig. 7. The amide I and amide II bands (about 1650 cm^{-1} and about 1550 cm^{-1}) suggest that the polymer is in α-helical conformation. Dichroism is also observed at about 1730 cm^{-1} (assignable to stretching of the ester C=O bond of the side chain). A similar tendency was observed for other single and mixed films.

Discussion

Polypeptide–Lipid Interaction. One way to explain the interaction occurring in mixed monolayers is the application of the surface phase rule.[23,24] If a polypeptide and a lipid are completely miscible in the monolayer and the new three-dimensional phase begins to appear at the transition point, the transition pressure should vary with composition. If, on the other hand, the monolayer components are immiscible, the transition pressure is independent of composition. As can be seen in Figs. 4 and 6, the mixed systems of cholesterol with PLO(Z), PLL(Z), PBDLG-1 or PBDLG-2 are immiscible, other systems forming homogeneous miscible monolayers.

In general, the interaction between two different lipid components results in a deviation from the additivity rule of molecular areas only if one or both components form expanded monolayer. The additivity rule is also satisfied if two components are completely immiscible.

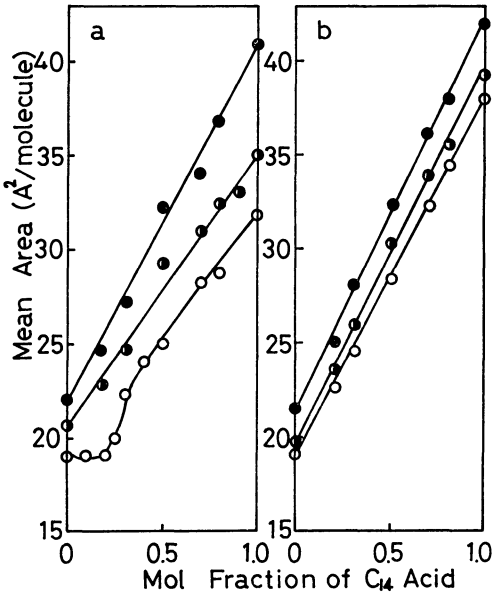

Fig. 8. Mean molecular areas of mixed monolayers as a function of lipid mole fraction at 25 °C.
a) PLO(Z)–C_{14} acid (●: 3 dyn/cm, ◑: 7 dyn/cm, ○: 10 dyn/cm), b) PBLG–C_{14} acid (●: 2 dyn/cm, ◑: 4 dyn/cm, ○: 5 dyn/cm).

Figure 8 shows the plots of mean molecular (residual) areas of mixed PLO(Z)–C_{14} acid and PBLG–C_{14} acid monolayers as a function of the composition under constant pressure. The maximum deviation can be seen at about 0.2 mole fraction of C_{14} acid at the pressure near the transition point of PLO(Z) (Fig. 8-a). A similar maximum deviation was observed for mixed PLL(Z)–C_{14} acid monolayer. No deviation can be seen for mixed PBLG–C_{14} acid monolayer (Fig. 8-b). No deviation was found for other mixed polypeptide–lipid systems (PLO-(Z)– and PLL(Z)–C_{18} acid, PLO(Z)– and PLL(Z)–cholesterol, PBDLG-1– and PBDLG-2–C_{14} acid, PBDLG-1– and PBDLG-2–cholesterol). From the results of two-

dimentional phase rule and additivity rule, it was found that for the miscibility between two component the side chain composition of polypeptide is important.

The results given in Fig. 4 indicate that the hydrophobic portion of lipid and polypeptide side chains is much more important for the interaction than the hydrophilic portion. The transition pressures of mixed PLO(Z)–C_{14} acid and PLL(Z)–C_{14} acid monolayers are higher than those of PLO(Z)–C_{14} alcohol and PLL(Z)–C_{14} alcohol monolayers, respectively. This suggests that the state of monolayers of lipid is also important; C_{14} acid and C_{14} alcohol form a expanded monolayer and a condensed monolayer, respectively, at 25 °C. There is a considerable difference in the transition pressure between PBLA–lipid and PLO(Z)–lipid (or PLL(Z)–lipid) systems. It is of interest that in mixed PBLA–cholesterol system the transition pressure increase with lipid composition. On the contrary, in the case of mixed PLO(Z)–cholesterol and PLL(Z)–cholesterol systems, the change in transition pressure is practically absent. This suggests that for interaction the size and shape of lipid molecules (fatty acids, fatty alcohols and cholesterol) are important.

The work of collapse of mixed monolayers may provide a further information concerning the polypeptide-lipid interaction. The work of collapse, W, is given by

$$W = \pi_{tr} \cdot \Delta A,$$

where π_{tr} is the transition pressure, and ΔA is the difference between initial and final areas of collapse.

Figure 9 shows the work of collapse as a function of lipid mole fraction. Corresponding to the results shown in Fig. 4-c, the PBLA–lipid systems differ a great deal from other mixed systems. The work of collapse decreases with lipid mole fraction. As shown in Fig. 9-b, there is a remarkable difference in W values between PBLA and PBLG. The difference in side chain length would mainly affect the work of adhesion to water.[19] A significant difference in solution and solid properties has been reported between PBLA and PBLG.[25] It can be seen that with exception of PBLA–lipid systems, the maximum work of collapse is found at about 0.2 lipid mol fraction in agreement with the maximum deviation from the additivity rule of molecular areas for PLO(Z) (or PLL(Z))–C_{14} acid system at the pressure near the transition point of polypeptides. At about 0.2 lipid mole fraction, the cohesive force might be reinforced as a result of the formation of the 4: 1 complex (residue: molecule) of polypeptide and lipid in monolayers. Although no deviation from the molecular areas is found for PLLeu–C_{14} acid and PLLeu–C_{18} acid systems, the relationship between the transition pressure and the mol fraction of lipid suggests that the 4: 1 complex is also formed in these systems.

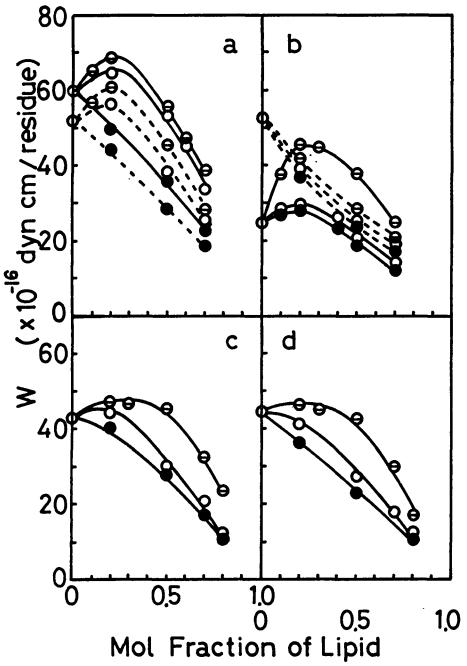

Fig. 9. Work of collapse for mixed monolayers as a function of lipid mole fraction at 25 °C.
a) PLO(Z)–lipid(——), PLL(Z)–lipid(----), b) PBLG–lipid(——), PBLA–lipid(----), c) PBDLG-1–lipid, d) PBDLG-2–lipid. ⊖: polypeptide–C_{14} acid, ○: polypeptide–C_{18} acid, ●: polypeptide–cholesterol.

The drastic decrease in transition pressure of PLLeu with the incorporation of small amounts of C_{14} acid and C_{18} acid also suggests that the hydrophobic interaction is important (Fig. 4-d). As a result of hydrophobic interaction between polypeptide side chains and lipids, the adhesive force of PLLeu decreases remarkably, the film collapsing more easily than in the case of PLLeu alone.

The dichroism (amide I band) shown in Fig. 7 indicates that the horizontally oriented polypeptide molecules at the interface are aligned in a direction parallel to the compressing barrier. The dichroism at about 1730 cm^{-1} suggests that the side chain is considerably ordered at the interface. This might favor the polypeptide–lipid interaction at close packed state in the monolayer.

The discussion given above is summarized as follows: 1) the interaction between polypeptide and lipid is mainly of hydrophobic nature, 2) the interaction is considered to depend on the orientation and the chemical composition of polypeptide side chains and on the size, shape and state of lipid molecules in the monolayer.[26] In connection with the number of residue in a turn of the α-helix (3.6 residues), the intermolecular complex between polypeptide and lipid is formed at a molar ratio of 4: 1 (0.2 lipid mole fraction).

Effect of Conformation on Interactions. The effect of polypeptide conformation on the polypeptide–lipid interaction was investigated by using PBG's of different α-helical content in solid state.

As shown in Fig. 6, the change in transition pressure of mixed PBG–fatty acid monolayer is in order PBLG> PBDLG-1>PBDLG-2. Thus, it is assumed that PBLG interacts most strongly with the lipids. The interaction is the weakest for PBLG-2. It is of interest that this order corresponds to the helical content in the solid state. The maximum interaction of lipid monolayers with polypeptides occurs when the polypeptides dissolved in the subsolution in the α-helical conformation.[5,6,8]

If we assume that a PBLG monolayer is in the α-helical

conformation[15,27] and a perturbed helix portion of PBDLG is unfolded at the interface owing to relatively weak stability of the helix.[22,28] PBLG might become more hydrophobic than PBDLG's, since the polypeptide main chain is considerably shielded from the interaction with water molecules. This can be seen from the fact that the transition pressure of a monolayer of PBLG is much lower than that of PBDLG (Fig. 1-b).

The PBLG monolayer is more compressible than the PBDLG monolayers. Because of a highly rigid nature of the polypeptide main chain of the α-helix compared with other conformations. The high compressibility of a PBLG monolayer can be ascribed to the flexibility of side chains. The flexible side chains of PBLG might undergo rearrangement more easily into position favorable to the interaction with normal alkyl chain of a fatty acid. The state of a lipid monolayer and the flexibility of side chains of a polypeptide seem to affect the degree of interaction. C_{14} acid interacts more strongly than C_{18} acid with PBG.

The influence of cholesterol on the PBG monolayers is much more pronounced than that of fatty acids. PBLG interacts characteristically with cholesterol. However, PBDLG-1 and PBDLG-2 interact with considerable difficulty. Cholesterol does not interact with benzyloxycarbonyl derivatives of basic poly(α-amino acid). The interaction of cholesterol with synthetic polypeptides in monolayers depends upon the chemical composition of side chains of a polymer. The present study indicates that the conformation of a polymer is also important for the interaction. The change in transition pressure is in the order PBLG–cholesterol > PBDLG-1–cholesterol > PBDLG-2–cholesterol. This corresponds to the helical content of respective polymers.

From the work of collapse shown in Figs. 9-b, -c, and -d, it can be seen that the strong interaction between two components takes place at about 0.2 lipid mole fraction for PBLG–fatty acid and PBLG–cholesterol systems. For PBDLG–lipid systems except PBDLG–cholesterol systems, a specific interaction is found in a polymer rich region (less than 0.2 mole fraction of a lipid). This might indicate that side chains in the helical region in a polymer molecule is an interacting site.

The above arguments are summerized as follows: 1) the flexibility of polypeptide side chains is related to the conformation of a polypeptide at the interface, 2) the hydrophobic interaction of PBG's and lipids in monolayers is considerably affected by the conformation of a polymer, and the interaction is stronger when the α-helical content in the film is higher. This seems to be important in relation to the nature of lipid-protein interactions in biomembranes.

This research was supported by a Scientific Research Grant from the Ministry of Education.

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