Specific Anion Effects on Water Structure at Polypeptide Monolayer-Water Interface

Akira SHIBATA,* Shinsuke YAMASHITA, and Takuya YAMASHITA
Faculty of Pharmaceutical Sciences, Tokushima University, Shomachi, Tokushima 770
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The specific anion effects on water structure at the polypeptide monolayer–water interface were investigated. It was found that the effects of anions on increasing the film transition pressure were in the order of $SCN^->Br^->Cl^->F^-$ in line with that of those anions as breakers of water structure. The SCN^- ion disrupts the hydrophobic hydration surrounding the hydrocarbon moieties of polypeptides and lipids at the interface. On the other hand, the presence of F^- ion as a structure maker tends to stabilize the hydrophobic bonding. The increase of the film transition pressure on KSCN subsolution was associated with a difference in the conformation between poly(γ-benzyl L-glutamate) and poly(γ-benzyl DL-glutamate). The infrared spectra of polymers obtained from single and mixed films were practically identical with the collapsed films in the presence and the absence of SCN^- and F^- ions.

In recent years, a number of attentions have been focused on the structure of water in living cells and the role of hydration of water around protein molecules in life processes. It is currently believed that the structure of water at biosurface is significantly influenced and ordered by constituent macromolecules.[1] In other words, water adjacent to biosurface differs appreciably from bulk water.[2]

A wide variety of electrolyte effects on biological systems has been found to give rise to the same sequence observed by Hofmeister.[3] The unifying principle underlying the effects of electrolytes on macromolecules and on water itself is that the order of effectiveness of anions is nearly the same for all. The anions are considered to exert their effects by altering the structure of water, thus weakening the tendency toward hydrophobic bonding. Recent evidence suggests that the electrolyte effects are associated with the structure of water at an interface.[2]

The structure breaking and structure making effects of electrolytes on the water structure should have application to interfacial water structure. One way of enhancing water structure at the interface is the application of monolayer technique. Weil[4] and Goddard *et al.*[5] reported on the effects of electrolytes on the interfacial water structure at "ionized" monolayers. It is necessary to use "un-ionized monolayer to emphasize the specific electrolyte effects on water structure at the interfaces. Ralston and Healy[6] have studied the effects of cations on 1-octadecanol monolayers in connection with the interfacial water structure.

In relation to the structure of water in the surface region, we have reported the effects of monovalent anions on the surface pressure-area(π-A) curves of poly(N^ε-benzyloxycarbonyl-L-lysine) monolayer.[7] The effects of anions on increasing the surface pressure were in the order of $SCN^->Br^->Cl^->F^-$ in line with that of these anions as breakers of water structure.[8]

The purpose of this work is to clarify the effects of anions on the conformation of polypeptide and on the interaction of polypeptide and lipid in relation to the structure of water in the interfacial region.

Experimental

Poly(N^δ-benzyloxycarbonyl-L-ornithine) (PLO(Z)), poly(N^ε-benzyloxycarbonyl-L-lysine) (PLL(Z)), poly(γ-benzyl L-glutamate) (PBLG), and poly(γ-benzyl DL-glutamate) (PBDLG) were prepared by the polymerization of the *N*-carboxy anhydrides of respective amino acids. The molecular weights determined from the viscosities in *N*,*N*-dimethylformamide (DMF)[9] or dichloroacetic acid[10] are 3.5×10^5 for PLO(Z), 3.8×10^5 for PLL(Z), 3.1×10^5 for PBLG, and 6.0×10^4 for PBDLG, respectively. Infrared spectra indicated that PLO(Z), PLL(Z) and PBLG are in the α-helical conformation in the solid state. The helical content of PBDLG was 13% regular α-helix and 60% perturbed α-helix. Lipids used were myristic acid(C_{14} acid) and stearic acid(C_{18} acid). These materials were purified by fractional distillation, and were chromatographycally pure.

The spreading solvents (DMF, dichloromethane (DCM)) were distilled under nitrogen atmosphere. The subsolution, 0.01 M HCl, was made up from twice distilled water and distilled 6 M HCl (1 M = 1 mol dm^{-3}). The spreading solvents were DCM for PBLG– and PBDLG–lipid systems and a 9:1 (v/v) mixture of DCM and DMF for PLO(Z)– and PLL(Z)–lipid systems. 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. The initial spreading-area was 0.5 nm^2/residue or molecule. The trough (60 cm × 15 cm × 1 cm) and compressing barrier were made of Teflon.

Electrolyte solutions (KSCN, KBr, KCl, and KF) were treated with activated charcoal in order to remove surface active contaminants, and were used as a subsolution after being adjusted to contain 0.01 M HCl. A 10 to 1 compression of the surface of the electrolyte subsolutions, after standing for 10 min, produced less than 0.1 mN m^{-1} film pressure. The surface pressure was measured by the Wilhelmy method at 25±0.5 °C. The film was compressed at a rate of 10 mm/min (0.01 nm^2 min^{-1} per residue or molecule).

The polarized infrared spectra of collapsed films were measured by JASCO-701G instrument. The monolayer was spread and compressed until collapse under the same conditions as in the case of surface pressure measurements. 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.

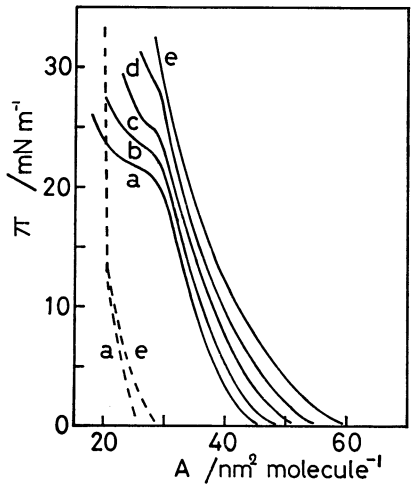


Fig. 1. Surface pressure-area curves of C_{14} acid (——) and C_{18} acid (- - -) on HCl and KSCN subsolutions. 1 dyn=10^{-5} N, 1 Å=0.1 nm. a: 0.01 M HCl, b: 1 M KSCN, c: 2 M KSCN, d: 3 M KSCN, e: 4 M KSCN.

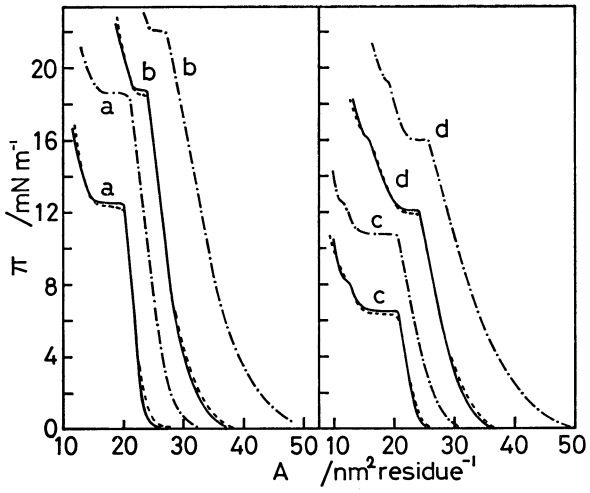


Fig. 2. Surface pressure-area curves of single and mixed monolayers on 0.01 M HCl(——), 2 M KSCN (—·—) and 2 M KF(- - -) subsolutions.
a: PLO(Z), b: PLO(Z)–C_{14} acid(1:1(residue mol: mol)), c: PLL(Z), d: PLL(Z)–C_{14} acid(1:1(residue mol:mol)).

The transferred collapsed films were dried at a room temperature.

Results

Figure 1 shows the π-A curves for C_{14} acid and C_{18} acid monolayers on 0.01 M HCl and KSCN subsolutions containing 0.01 M HCl. The increasing concentration of KSCN causes the increase of transition pressure and the expansion of C_{14} acid monolayer. The transition point of C_{14} acid monolayer tends to disappear with increasing concentration of KSCN, and is no longer detectable at 4 M KSCN. The expansion of C_{18} acid monolayer on KSCN subsolution is considerably small compared with C_{14} acid monolayer.

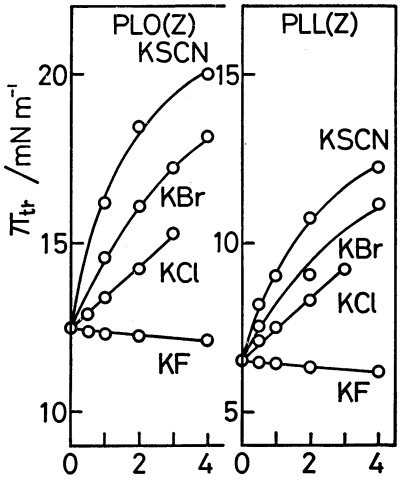


Fig. 3. Transition pressures of PLO(Z) and PLL(Z) monolayers as a function of electrolyte concentration.

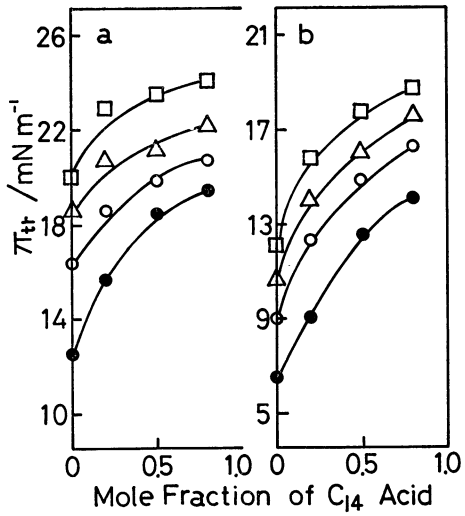


Fig. 4. Transition pressures of mixed monolayers of C_{14} acid with PLO(Z) (a) or PLL(Z) (b) as a function of C_{14} acid mole fraction on HCl and KSCN subsolutions.
●: 0.01 M HCl, ○: 1 M KSCN, △: 2 M KSCN, □: 4 M KSCN.

Figure 2 shows the π-A curves for PLO(Z), PLL(Z), PLO(Z)–C_{14} acid and PLL(Z)–C_{14} acid mixtures(1:1 (residue mol:mol)) spread on 0.01 M HCl, and on 2 M KSCN and 2 M KF subsolutions containing 0.01 M HCl. In the π-A curves of PLO(Z) and PLL(Z) monolayers, the height of plateau which is associated with the transition pressures is much higher on the subsolution containing KSCN than 0.01 M HCl. On the other hand, in the presence of KF, the film transition pressure becomes slightly lower. The film expansion is observed for PLO(Z) and PLL(Z) in the presence of KSCN. The effect of KF on expansion is quite small.

Figure 3 shows the transition pressures of PLO(Z) and PLL(Z) monolayers as a function of electrolyte concentration. The transition pressures of PLO(Z) and PLL(Z) increase with electrolyte concentration

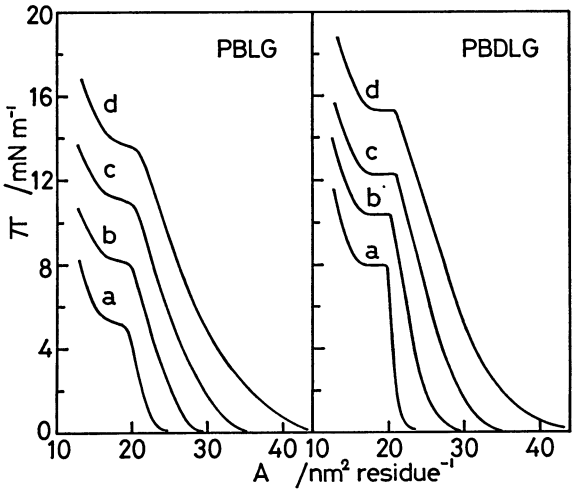


Fig. 5. Surface pressure-area curves of PBLG and PBDLG monolayers on HCl and KSCN subsolutions. a: 0.01 M HCl, b: 2 M KSCN, c: 4 M KSCN, d: 6 M KSCN.

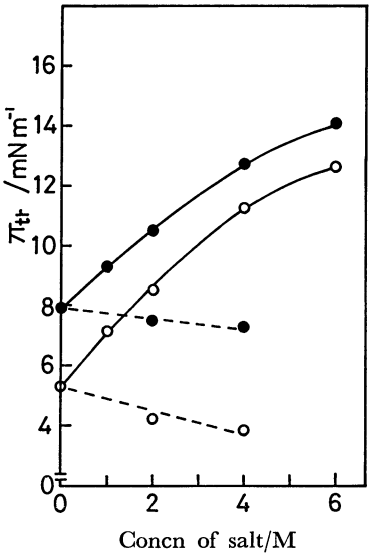


Fig. 6. Transition pressures of PBLG(○) and PBDLG (●) as a function of electrolyte concentration. ——: KSCN, - - -: KF.

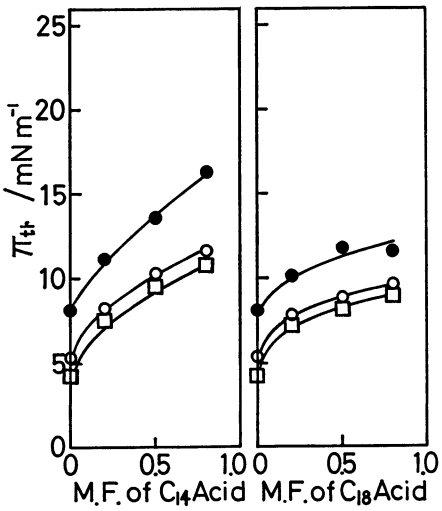


Fig. 7. Transition pressures of mixed monolayers of PBLG with C_{14} acid or C_{18} acid as a function of lipid mole fraction on 0.01 M HCl(○), 2 M KSCN(●), and 2 M KF(□) subsolutions.

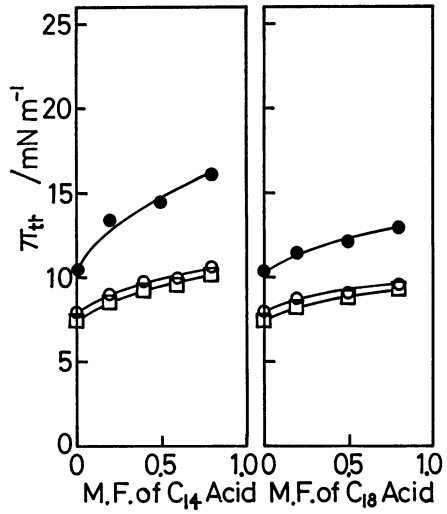


Fig. 8. Transition pressures of mixed monolayers of PBDLG with C_{14} acid or C_{18} acid as a function of lipid mole fraction on 0.01 M HCl(○), 2 M KSCN (●), and 2 M KF(□) subsolutions.

except for KF. In the lower concentration of KSCN or KBr, in particular, the transition pressures show the marked increase.

Figure 4 shows the transition pressures of mixed monolayers of C_{14} acid with PLO(Z) or PLL(Z) on 0.01 M HCl and KSCN subsolutions as a function of the mole fraction of C_{14} acid. The phase rule of the surface predicts that two film components are miscible in the presence and the absence of KSCN in the subsolution.[11,12] The increasing concentration of KSCN causes the increase of transition pressure. The effect of KSCN on the transition pressure for mixed PLO(Z)–C_{14} acid monolayer is smaller than for mixed PLL(Z)–C_{14} acid monolayer.

Figure 5 shows the π-A curves for PBLG and PBDLG monolayers on 0.01 M HCl and KSCN subsolutions containing 0.01 M HCl. As such case of benzyloxycarbonyl derivatives of basic poly(α-amino acid), PLO(Z) and PLL(Z), the film expansion is also observed on KSCN subsolution.

The transition pressures of PBLG and PBDLG monolayers as a function of KSCN and KF concentration is shown in Fig. 6. The transition pressures of PBLG and PBDLG monolayers increase with KSCN concentration, and in the presence of KF, decrease linearly.

Figures 7 and 8 show the relationship between transition pressure and lipid mole fraction for mixed PBLG–fatty acid and PBDLG–fatty acid monolayers on 0.01 M HCl, and 2 M KSCN and 2 M KF subsolutions, respectively. The transition pressures for both mixed PBLG–fatty acid and PBDLG–fatty acid systems increase with the mole fraction of C_{14} acid or C_{18} acid. The increments of transition pressure for mixed PBLG–fatty acid systems are larger than for mixed PBDLG–fatty acid systems. The effect of

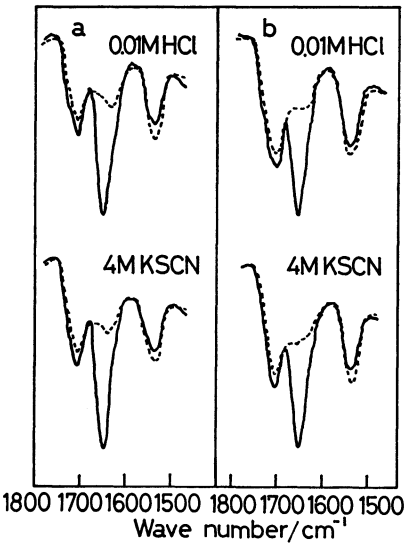


Fig. 9. Polarized IR spectra of collapsed films of PLL-(Z) (a) and 4:1(residue mol:mol) mixture of PLL(Z) and C_{14} acid(b) formed on 0.01 M HCl and 4 M KSCN subsolutions.
——: Electric vector parallel to the barrier used to collapse the film, - - -: electric vector perpendicular.

C_{14} acid on the transition pressure is larger compared with C_{18} acid.

As typical examples, the polarized infrared spectra for collapsed films of PLL(Z) and a 4:1(residue mol: mol) mixture of PLL(Z) and C_{14} acid are shown in Fig. 9. The spectra which are characteristic of polypeptide designated as a α-helix form have major diagnostic peaks at about 1650 cm^{-1} (amide I band) and 1550 cm^{-1} (amide II band). Dichroism is also observed at about 1700 cm^{-1}(assignable to streching of C=O bond of the side chain). A similar tendency was observed for other single and mixed films.

Discussion

Mixed PLO(Z)–C_{14} Acid and PLL(Z)–C_{14} Acid Systems. C_{14} acid and C_{18} acid monolayers are of expanded and condensed type at 25 °C, respectively (Fig. 1).[13] The transition is seen in the π-A curves of C_{14} acid. The transition region becomes short with increasing KSCN concentration, and ultimately disappears at 4 M. A similar tendency is also recognized for the effect of temperature on transition pressure of C_{14} acid.[14] If the monolayer expansion is due to the flexibility of the hydrophobic moieties of C_{14} acid, the rotation of the flexible hydrophobic moieties may be hindered with compressing monolayer. The hindered molecular rotation may lead to the discontinuity of compressibility associated with the onset of the transition.[15] In analogy with the effect of temperature, the addition of SCN^- ion as a breaker of water structure may favor the molecular rotation of C_{14} acid at the interface.

A complex polymer like a protein will be sheathed in a complex hydration envelope consisting of regions of differing local water structure at the interface.[16] The polypeptides used in this work will also have two types of water structure arising from the hydrophobic and Coulombic hydrations. The nonpolar moieties will be encased by the same hydrophobic hydration that one associates with nonpolar solutes and neutral interface, whereas polar regions will be surrounded by the same Coulombic hydration that surrounds ion in solution.

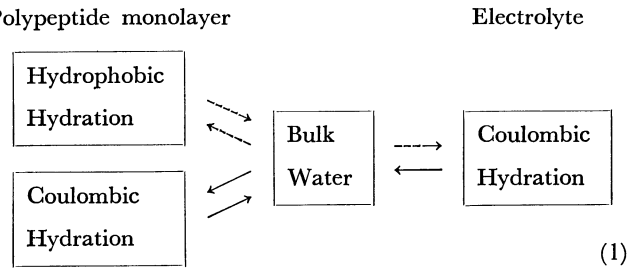
There exist plateaux in the π-A curves for PLO(Z) and PLL(Z) monolayers(Fig. 2). The plateau was found for a number of synthetic polypeptide monolayers, and ascribed to the transition from a two-dimensional ordered state to a three-dimensional disordered state.[17,18] The height of plateau can be regarded as a measure of the adhesion force of monolayer to water. As shown in Fig. 3, the transition pressures increase with concentration of electrolyte, and the effects of anions on the transition pressure are in the order of $SCN^->Br^->Cl^->F^-$ in accordance with that of these anions as breakers of water structure.[8] A structure breaker like SCN^- is more easily accomodated in the interface than F^-, a structure maker, due to the fact that it introduces more disorder in the structure region at the air-water interface.[19] The hydration atmosphere of SCN^- ion is compatible with the Coulombic hydration surrounding the polar group of polypeptide and enable SCN^- ion to penetrate, and profoundly alter the hydration. In addition, the hydrophobic hydration surrounding the polypeptide side chain is disrupted by the action of SCN^- ion. This will be accompanied by a decrease in adhesion of the hydrophobic moieties of the side chains to water, followed by an increase in the surface pressure.[20]

Comparing the π-A curves of PLO(Z) with PLL(Z) monolayers, the difference in height of the transition pressures is accounted for as arising essentially from the additional methylene group (Fig. 2). The effects of anions on the transition pressure of PLO(Z) are larger than PLL(Z) (Fig. 3). If the α-helix form can be stable at the air–water interface, the geometry of the α-helix is such that the side chains of adjacent helices can interpenetrate to form good hydrophobic contacts.[18]

It can be assumed that the hydrophobic and Coulombic hydrations surrounding polypeptide monolayers are in equilibrium with the Coulombic hydration for electrolyte in the presence of electrolyte. As the electrolyte is added. In other words, hydrophobic hydration is disrupted, the equilibrium indicated in the scheme (1) is shifted to right along the dotted line, and after that, shifted to left along the full line. Therefore, the compatibility of Coulombic hydration between polypeptide and electrolyte may cause the increase of transition pressure.

In the presence of F^- ion, the transition pressure decreases slightly with increasing concentration(Fig. 3). Increasing concentration of F^- ion as a maker of water structure, the equilibrium in the scheme (1) will be shifted to the direction of the stabilization of hydrophobic hydration. In the present case, the effect of F^- ion on the transition pressure may imply the stabilization of hydrophobic hydration surrounding the monolayers.

For mixed monolayers of C_{14} acid and either PLO(Z) or PLL(Z) on 0.01 M HCl subsolution, the increase of transition pressure is due to the hydrophobic bonding between polypeptide side chain and C_{14} acid (Fig. 4).[21] The effect of C_{14} acid on the transition pressure caused by PLL(Z) is larger than that of PLO(Z). This is caused by the additional methylene group for PLL(Z).[21] A similar tendency was also recognized on KSCN subsolutions (Fig. 4).



(1)

In the presence of KSCN, the relationship between transition pressure and the mole fraction of C_{14} acid gives a parallel shift to the direction of the increase of transition pressure in the range of 1 to 4 M KSCN concentration. This parallel shift suggests that the hydrophobic hydration surrounding polypeptide–C_{14} acid complex is "partially" disrupted with increasing concentration of SCN^- ion. If, however, the hydrophobic bonding between polypeptide and C_{14} acid is "essentially" disrupted by the action of SCN^-, the value of transition pressure caused by polymer will have of coming close to that of polypeptide itself or the lower value compared with that of mixed monolayer different from our results.

Mixed PBLG–Fatty Acid and PBDLG–Fatty Acid Systems. The perturbed helix portion of PBDLG is unfolded at the interface owing to relatively weak stability of the helix.[22] As is shown in Fig. 5, the difference in shape of the π-A curves between PBLG and PBDLG is accounted for as arising essentially from the conformation of these polymers at the interface.[21] The more increase of the transition pressure of PBDLG compared with PBLG may be caused by the hydration surrounding the perturbed helix portion of PBDLG. The water structure beneath the monolayer must be also reflected to the monolayer compressibility. We have reported that the compressibilities at closed packed area are 0.027 for PBLG and 0.015 for PBDLG.[21] The film expansion on KSCN subsolution suggests that SCN^- ion is transferred into the interface relative to the surface without monolayer(Fig. 5).

The change in the transition pressures of mixed monolayers is larger for PBLG than for PBDLG(Figs. 7 and 8). The flexible side chains of PBLG in α-helical conformation might undergo rearrangement more easily into the position favorable to the interaction with hydrophobic moieties of fatty acids. Chatelain *et al.*[23] reported that the maximum interaction of lipid monolayer with polypeptides occurs when the polypeptides dissolved in the subsolution in α-helical conformation.

Comparing the transition pressures of mixed PBLG–C_{14} acid and PBLG–C_{18} acid monolayers, the state of fatty acid monolayers(expanded and condensed films) seems to affect the degree of interaction (Fig. 7). The C_{14} acid interacts more strongly than C_{18} acid with PBLG.

The transition pressures of mixed PBLG– and PBDLG–fatty acid monolayers on 2 M KSCN subsolution become higher compared with those on KSCN free subsolution (Figs. 7 and 8). The increase of transition pressure is larger for mixed PBDLG–fatty acid systems than for mixed PBLG–fatty acid systems. In the presence of SCN^- ion, the hydrophobic hydration surrounding mixed monolayer is disrupted, and then, the increase of the compatibility of Coulombic hydration around unfolded PBDLG may be caused. Therefore, this may be associated with a difference in the conformation between PBLG and PBDLG in mixed monolayers.

As shown in Figs. 7 and 8, the transition pressures of mixed monolayers on 2 M KF subsolution become low compared with those on KF free subsolution. In the presence of F^- ion as a maker of water structure, the adhesion force of polypeptide to water becomes weak with the formation of hydrated structure surrounding the mixed films. The effect of F^- ion on the transition pressure of mixed monolayer is slightly large for PBLG compared to PBDLG. This suggests that the hydrophobic hydration around PBLG–C_{14} acid complex tends to be stabilized by the action of F^-. PBLG is expected to be more hydrophobic than PBDLG since the polypeptide main chain is considerably shielded from the interaction with water molecules.

Infrared Spectra of Collapsed Films. The parallel dichroism of the amide I band and the perpendicular dichroism of the amide II are those known to be characteristic of the α-helix, with the molecules showing alignment parallel to the barrier used to collapse the monolayer (Fig. 9). Similar spectra of the collapsed films were obtained in the presence and the absence of electrolyte. This is an evidence that as mentioned above, SCN^- ion disrupts partially hydrophobic hydration surrounding the monolayers, and that, in other words, the monolayers are stabilized by the hydrophobic bonding. The dichroism for the side chains suggests that the side chains are considerably ordered at the interface. This might favor the polypeptide-lipid interaction at close packed state in monolayers.

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