Effect of Water Structure on Mixed Synthetic Polypeptide-Lipid Monolayers

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The effect of water structure on the interaction of synthetic polypeptides with fatty acids in monolayers at the air–water interface was investigated. It was found that SCN^-, F^-, and urea exert a remarkable effect upon the hydrophobic interaction in monolayers. SCN^- and urea disrupt the hydrated structure biult up around the hydrocarbon moieties of polypeptides and lipids at the interface. On the other hand, the presence of F^- as a structure maker tends to stabilize the hydrophobic bonding. The infrared spectra of polymer components were practically identical for the collapsed films in the absence and presence of SCN^-, F^-, and urea.

The hydrophobic bonding is important in stabilizing macromolecular structures such as proteins and biological membranes.[1] Water is considered to affect the properties and structure of biopolymers at the interface. Recent evidence suggests that the water adjacent to interfaces significantly differs from bulk water.[2]

In relation to the structure of water at the interfacial region, we have reported the effects of monovalent anions on the surface pressure-area (π-A) curves of poly(ε-benzyloxycarbonyl-L-lysine).[3] The effects of anions on increasing the surface pressure were in the order $SCN^->Br^->Cl^->F^-$ in line with that of these anions as breakers of water structure.[4]

The action of urea on macromolecular structures closely resembles that of chaotropic ions in which both unfolding and denaturation are strongly favored. It is not clear if the action of urea is similarly mediated by a modification in the structure of water. However, there is widespread belief that in the presence of urea, hydrophobic bonding is weakened.[5]

The purpose of this work is to clarify the effect of SCN^-, F^-, and urea on the interaction of polypeptides and lipids in relation to the structure of water at the interfacial region.

Experimental

Synthetic polypeptides used in the present study were poly(δ-benzyloxycarbonyl-L-ornithine) (PLO(Z)), poly(ε-benzyloxylcarbonyl-L-lysine) (PLL(Z)), poly(γ-benzyl-L-glutamate) (PBLG), and poly(γ-benzyl-DL-glutamate) (PBDLG) (13% regular α-helix and 60% perturbed α-helix). Lipids used were myristic acid (C_{14} acid) and stearic acid (C_{18} acid). Polypeptides, lipids, and spreading solvents were the same as those used in the preceding work.[6] Salts and urea solutions 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 salt or urea subsolutions, after being left to stand for 10 min, produced less than 0.1 dyn/cm film pressure.

The surface pressure was measured by the Wilhelmy method at 25±0.5 °C. The films were compressed at a rate of 1 Å²/residue/min.

The polarized infrared spectra of collapsed films were measured with a JASCO DS-701G instrument.[6]

Results

Figure 1 shows the π-A curves for PBLG, PBDLG, PBLG–C_{14} acid and PBDLG–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. The transition pressures for PBLG and PBDLG monolayers are much higher on the subsolution containing KSCN than on 0.01 M HCl. On the other hand, in the presence of KF, the film transition pressure becomes slightly lower. In the presence of KSCN, film expansion is observed for PBLG and PBDLG. The effect of KF on exapansion is quite small. Similar tendencies were observed for the mixed monolayers.

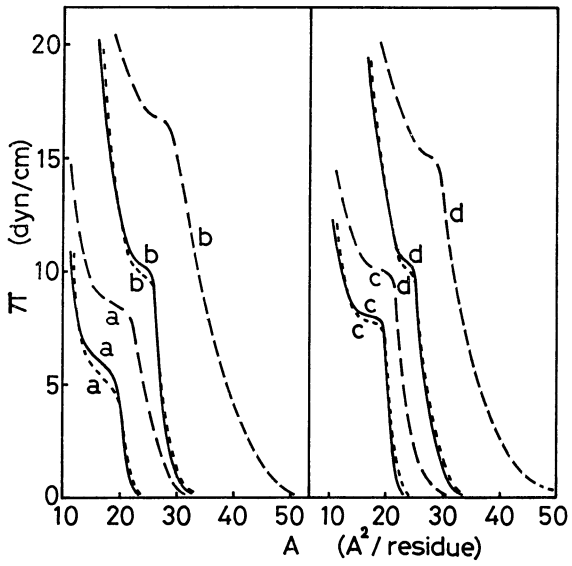

Fig. 1. Surface pressure-area curves of single and mixed monolayers on 0.01 M HCl(——), 2 M KSCN(— —) and 2 M KF(----) at 25 °C. 1 dyn=10^{-5} N, 1 Å=0.1 nm. a: PBLG, b: PBLG–C_{14} acid (1: 1(residue mol: mol)), c: PBDLG, d: PBDLG–C_{14} acid (1: 1(residue mol: mol)).

Figure 2 shows the π-A curves for PLO(Z), PLL(Z), PBLG and PBDLG spread on 0.01 M HCl and 2 M urea subsolutions together with those for C_{14} acid and C_{18} acid monolayers. The transition pressure of polypeptide monolayers increases in the presence of urea. Expansion of polypeptide and lipid monolayers is also observed. The monolayer behavior in the presence of urea is similar to that of KSCN.

Figure 3 shows the film transition pressure, π_{tr}, and the limiting area extrapolated to zero surface pressure for the steep region, A_0, against urea and KSCN concentrations. Up to the concentration of 6 M urea

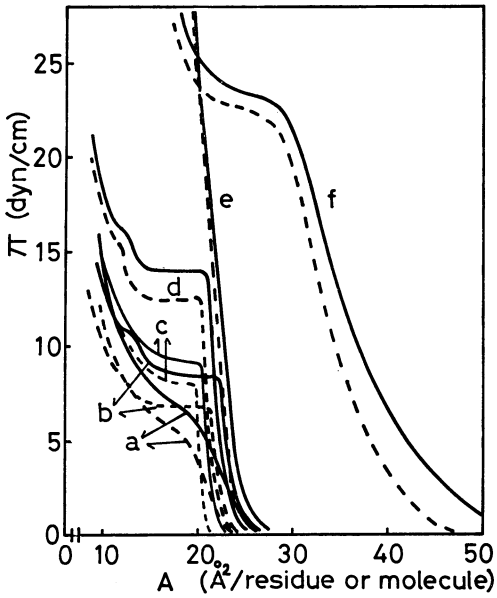

Fig. 2. Surface pressure-area curves of polypeptide and lipid monolayers on 0.01 M HCl(-----) and 2 M urea (——) at 25 °C. a: PBLG, b: PLL(Z), c: PBDLG, d: PLO(Z), e: C_{18} acid, f: C_{14} acid.

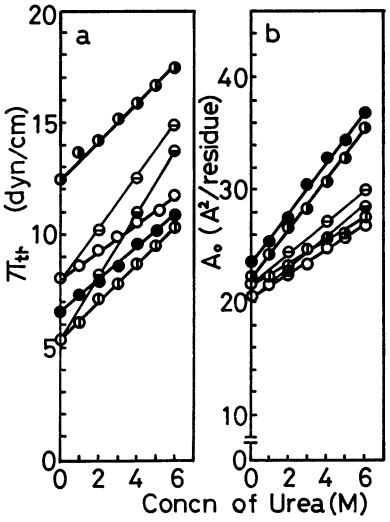

Fig. 3. Transition pressure, a, and limiting area, b, of polypeptide monolayers as a function of urea and KSCN concentration at 25 °C. Urea; ◑: PLO(Z), ●: PLL(Z), ⦶: PBLG, ○: PBDLG. KSCN; ◒: PBLG, ⊖: PBDLG.

and KSCN, π_{tr} and A_0 increase linearly. The effect of KSCN on π_{tr} and A_0 is much larger than that of urea.

As a typical example of mixed monolayers, Fig. 4 shows the π-A curves for mixed PLO(Z)-C_{14} acid monolayer on 0.01 M HCl and 2 M urea subsolutions. The transition pressures caused by the polymer components increase with increase in lipid mol fraction irrespective of the absence and presence of urea.

Figure 5 shows the polarized infrared spectra of collapsed films of PBLG formed on the subsolutions in the absence and presence of urea, KSCN and KF. In the α-helical amide I (about 1650 cm^{-1}) and amide

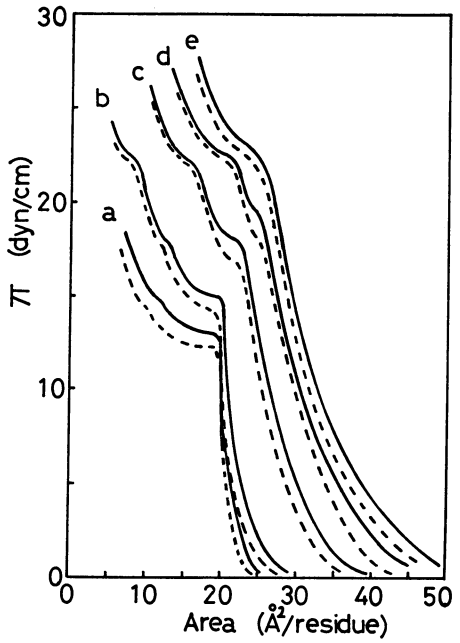

Fig. 4. Surface pressure-area curves of mixed monolayers of PLO(Z) with C_{14} acid on 0.01 M HCl(-----) and 2 M urea(——) at 25 °C. a: 1: 0, b: 4: 1, c: 1: 1, d: 1: 4, e: 0: 1 (residue mol: mol).

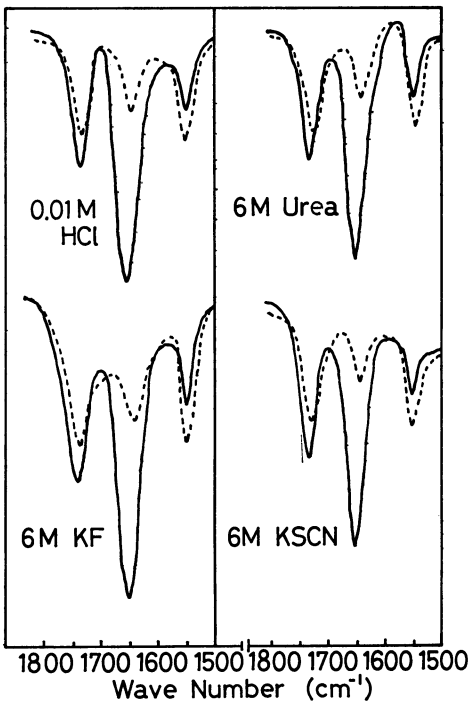

Fig. 5. Polarized IR spectra of collapsed films of PBLG formed on 0.01 M HCl, 6 M urea, 6 M KF and 6 M KSCN subsolutions. ——: Electric vector parallel to the barrier used to collapse the film, -----: electric vector perpendicular.

II (about 1550 cm^{-1}) regions, the spectra are practically identical with each other. Dichromism is also observed at about 1730 cm^{-1} (assignable to stretching of the ester C=O bond of the side chain). Similar results were

obtained for the other single and mixed monolayers. This suggests that polypeptide molecules are aligned in a parallel direction to the compressing barrier, irrespective of the kind of subsolution. The highly ordered alignment of polypeptide molecules may be favorable for the interaction between polypeptide and lipid.

Discussion

Effect of Salts. The order of film expansion is KSCN>KF≥0.01 M HCl (Fig. 1). This suggests that salts are transferred into the interface relative to the surface without monolayer. Fatty acid and fatty alcohol monolayers expand in the presence of salts[7] and urea.[8] Ralston and Healy gave an expansion for fatty alcohol monolayer in terms of interfacial water structure.[7] The film transition pressure is related to the collapse of monolayers from a two-dimensional oriented state to a three-dimensional disoriented state.[9,10]

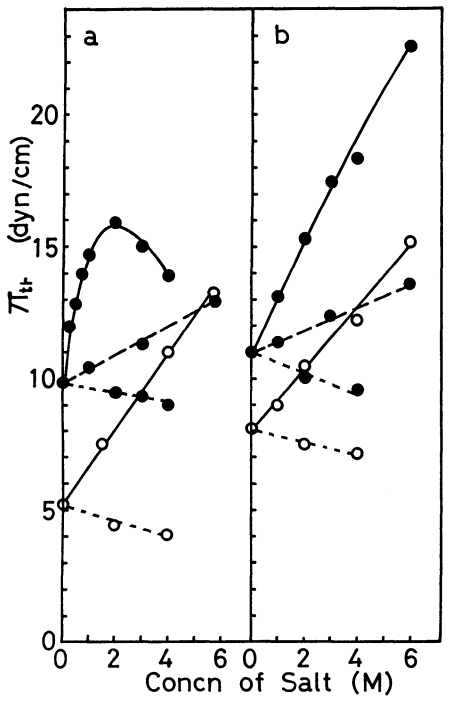

Fig. 6. Transition pressure of single and mixed monolayers as a function of concentration of KSCN(——), KF(----) and urea(——) at 25 °C. a) ○: PBLG, ●: PBLG–C_{14} acid (1: 1 (residue mol: mol)). b) ○: PBDLG, ●: PBDLG–C_{14} acid(1: 1(residue mol: mol)).

Figure 6 shows the transition pressure as a function of salts and urea concentrations for PBLG, PBDLG together with PBLG– and PBDLG–C_{14} acid mixtures (1: 1 (residue mol: mol)) spread on KSCN or KF subsolution. The transition pressures of PBLG and PBDLG increase linearly with increase in KSCN concentration. The hydrated structure around the hydrocarbon moieties of the side chains of polypeptides disrupted by SCN^- ion preferentially accumulated on the surface. As a result of the disruption, the affinity of the hydrocarbon moieties to water might increase.[1,11,12] The increment of transition pressure appears to be caused by destabilized hydrophobic bonding as a result of breakdown of the water structure. From the relationship between the transition pressure and the KSCN concentration in Fig. 3, the value of $d\pi_{tr}/dC$ for PBLG is larger than that of PBDLG. This can be attributed to the difference in the conformation in monolayer states between PBLG and PBDLG. In the conformation of synthetic polypeptides in monolayers, the α-helical conformation is reported to be one of the stable conformations.[9] Other conformations can also exist depending upon conformation in the solid state[13] or solution[14] and stability of the helix.[15,16] In the presence of KF, contrary to the case of KSCN, the transition pressure decreases linearly with the concentration for polypeptide monolayers. The decrease of transition pressure (Fig. 6) indicates that the adhesive force of polypeptide to water becomes weak with the formation of hydrated structure at the interface by the action of F^- as a maker of water structure.[4]

For mixed PBLG–C_{14} acid monolayer, there is a maximum value in transition pressure at 2 M KSCN. On the other hand, for mixed PBDLG–C_{14} acid monolayer, the transition pressure increases linearly. This can be associated with the difference in the stability of the hydrophobic bonding between two mixed systems. In analogy with SCN^-, the presence of urea also gives rise to the increase of transition pressure and the expansion in single and mixed monolayers. For PBLG– and PBDLG–C_{14} acid mixtures (1: 1 (residue mol: mol)), the effect of SCN^- on transition pressure is more remarkable than that of urea (Fig. 6). For PBLG–C_{14} acid mixture in particular, the transition pressure gives a maximum value at 2 M KSCN, while in the presence of urea, the pressure increases linearly with concentration. A remarkable difference in the increment of transition pressure is also observed for PBDLG–C_{14} acid mixture, suggesting that the effect of SCN^- on the hydrophobic interaction is much larger than that of urea. In the presence of KF, the decrease in transition pressure is small (about 1 dyn/cm at 4 M KF), little difference between PBLG and PBDLG monolayers, and between mixed PBLG–C_{14} acid and mixed PBDLG–C_{14} acid monolayers being observed. This suggests that the hydrophobic bonding tends to be stabilized by the action of F^-.

The results suggest that the action of ions (SCN^- and F^-) takes place on water and not primarily on the monolayer components itself. The monolayer behavior on salt subsolutions is closely related to the interfacial water structure.

Effect of Urea. It would be enlightening to elucidate the effect of urea in the polypeptide monolayers.

Film characteristics of synthetic polypeptides are given in Table 1. Film compressibility extrapolated to zero surface pressure, δ, is shown in the absence and presence of urea, together with the increment of limiting area and transition pressure against the urea concentration (dA_0/dC and $d\pi_{tr}/dC$). The change in limiting area of polypeptide with increase in the concentration of urea can be attributed to the penetration of a polypeptide monolayer by urea in the subsolution. The

TABLE 1. FILM CHARACTERISTICS OF SYNTHETIC POLYPEPTIDES

Polypeptide	dA_0/dC (Å²/residue/M)	$d\pi_{tr}/dC$ (dyn/cm/M)	δ ((dyn/cm)$^{-1}$) 0.01 M HCl	δ ((dyn/cm)$^{-1}$) 6 M urea
PLO(Z)	2.47	0.83	0.0131	0.0202
PLL(Z)	2.42	0.72	0.0144	0.0205
PBLG	1.12	0.81	0.0270	0.0333
PBDLG-2	1.18	0.60	0.0151	0.0284

increments of the limiting areas of PBLG and PBDLG against urea concentration differ a great deal from those of PLO(Z) and PLL(Z). This suggests that the surface activity of urea depends on the difference in the composition and geometrical arrangement of side chains between benzyloxycarbonyl derivatives of basic poly(amino acid) and benzyl esters of acidic poly(amino acid).

In the presence of 6 M urea, the film compressibility of a polypeptide increases considerably. The film transition pressure increases with urea concentration. This indicates that the affinity of hydrophobic moieties of polypeptide side chains to water and the side chain flexibility increase with the penetration by urea as a breaker of water structure.[4,17,18] The value of $d\pi_{tr}/dC$ for PBLG (0.81) is larger than that for PBDLG (0.60). This suggests that urea molecules mainly affect the side chains in the α-helical region of a polypeptide molecule.

Any deviation of mixed monolayer behavior from the additivity rule of molecular area might give useful information as regards the miscibility and interaction in the film. Figure 7 shows the mean molecular areas of mixed PLL(Z)–C_{14} acid and mixed PLL(Z)–C_{18} acid monolayers as a function of lipid mole fraction in the

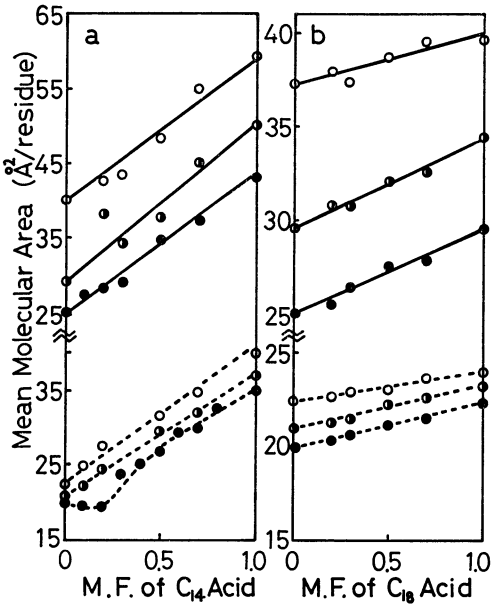

Fig. 7. Mean molecular area of mixed monolayers as a function of lipid mole fraction at 25 °C. ----: 0.01 M HCl, ——: 6 M urea. a) PLL(Z)–C_{14} acid (○: 2 dyn/cm, ◑: 4 dyn/cm, ●: 6 dyn/cm). b) PLL(Z)–C_{18} acid(○: 2 dyn/cm, ◑: 4 dyn/cm, ●: 6 dyn/cm).

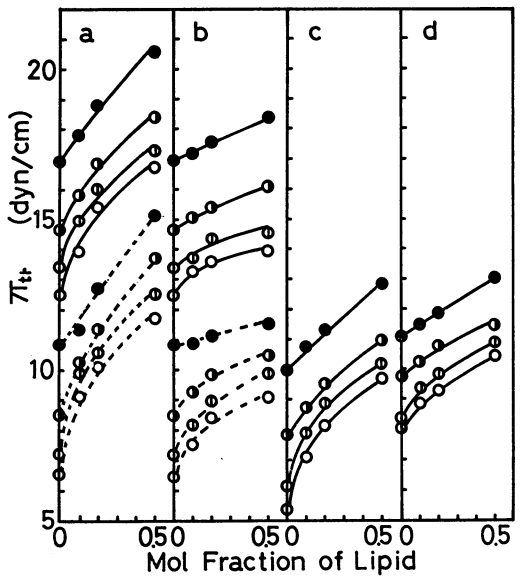

Fig. 8. Transition pressure of mixed monolayers as a function of lipid mole fraction in various concentration of urea at 25 °C. a) PLO(Z)–C_{14} acid (——), PLL(Z)–C_{14} acid(-----). b) PLO(Z)–C_{18} acid (——), PLL(Z)–C_{18} acid(-----). c) PBLG–C_{14} acid. d) PBDLG–C_{14} acid. ●: 6 M urea, ◑: 3 M urea, ⓘ: 1 M urea, ○: 0.01 M HCl.

absence and presence of 6 M urea. When PLL(Z) is mixed with C_{14} acid which forms an expanded monolayer, the maximum negative deviation is observed at about 0.2 mole fraction of the acid in the absence of urea. However, no deviation from the additivity rule takes place in the presence of 6 M urea. A similar tendency was observed for mixed PLO(Z)–C_{14} acid monolayers.

When there is no deviation from the additivity rule of mean molecular areas, it is difficult to determine the miscibility in the film. Figure 8 shows the transition pressure as a function of lipid mole fraction for mixed monolayers of fatty acid with PLO(Z), PLL(Z), PBLG or PBDLG for various concentrations of urea. The film transition pressure caused by the polymer component increases with C_{14} acid mole fraction (Fig. 8-a). The surface phase rule[19,20] predicts that two film components are miscible in the presence and absence of urea in the subsolutions. In the presence of 6 M urea, the transition pressure increases linearly with lipid mole fraction. This suggests that an ideal surface mixture of PLO(Z) (or PLL(Z)) and C_{14} acid is formed in the film at high concentration of urea.

The molecular area additivity rule[6] holds in other mixed monolayers such as PLO(Z) with C_{18} acid, PLL(Z) with C_{18} acid, PBLG with C_{14} acid, and PBDLG with C_{14} acid. On the other hand, the film transition pressure against lipid mole fraction for these mixed monolayers indicates that the film components are miscible, irrespective of urea concentration (Figs. 8-b, -c, and -d).

The formation of an ideal surface mixture can be ascribed to the breaking of hydrated structure around the hydrophobic moieties of polypeptide and lipid by the action of urea. As a result, the affinity of polypeptide

side chains and a nonpolar portion of the lipid to water[21] and the flexibility of polypeptide side chains increase. This might be supported by the film expansion (Figs. 2 and 4) and the increased compressibility of polypeptide monolayers in the presence of urea (Table 1).

From the conformational aspects of polypeptide monolayers, the action of urea on the interactions of PBLG–lipid and of PBDLG–lipid in monolayers are of interest. The perturbed α-helix portion of PBDLG molecule might be unfolded in the monolayer state owing to the relatively weak stability of the helix.[15,16] The interaction of PBDLG and lipid is weak as compared with that of PBLG and lipid.[5] The results shown in Figs. 8-c and -d indicate that the change in transition pressure against C_{14} acid mole fraction is smaller for PBDLG than for PBLG. This supports the view that the perturbed helix is unstable in monolayers. Urea molecules predominantly attack the hydrophobic moieties around the regular helix.

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