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Effect of Polar Group on the Polypeptide-Lipid Interaction at the Air-Water Interface

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The effect of polar group on the interaction of synthetic polypeptides with lipids at the air-water interface was investigated. Methyl stearate interacts with benzyloxycarbonyl derivative of basic poly(α-amino acid) or benzyl ester of acidic poly(α-amino acid) on distilled water and KF, KSCN, and urea subsolutions. No interaction between polypeptide and stearamide on various subsolutions was observed except for the mixed system of stearamide with benzyl ester of acidic poly(α-amino acid) in α-helical conformation. The components in mixed monolayers exhibit characteristic miscibilities depending upon the flexibility of polymer side chain, the conformation of polymer and the steric factor of head group in lipid molecules. All of the mixed polypeptide–lipid monolayers spread on the subsolution containing SCN^- as a breaker of water structure become miscible. The dipole-dipole interaction between stearamide and benzyl ester of acidic poly(α-amino acid) in α-helical conformation is weakened by the action of urea.

The hydrophobic bonding is important in stabilizing macromolecular structures such as proteins and biological membranes.1) Water is considered to affect the stability of macromolecular structure and the interaction of macromolecule with lipid at the interface.2) In relation to the structure of water at the interfacial region, we have reported the effects of salts and urea on the interaction of polypeptide with fatty acid in monolayers.3,4) 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.5) KSCN, urea, and KF are known as breakers and a maker of water structure, respectively.

We have found that 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 acid, fatty alcohol and cholesterol) at the air-water interface is mainly of hydrophobic nature.6) The interaction was found to take place between side chain of polypeptide and lipid. The interaction of polypeptide with myristic acid was stronger than that between polypeptide and myristyl alcohol. This suggests that weak interactions may exist between the polar group of lipid and polypeptide side chain. These polymer side chains consist of polar group and methylene groups. The polar groups in polymer side chain, as well as polar group in stearamide and methyl stearate, are ROCONHR′ for benzyloxycarbonyl derivative of basic poly(α-amino acid) and RCOOR′ for benzyl ester of acidic poly(α-amino acid).

In this study, by the use of methyl stearate and stearamide, the effect of the polar groups on the interaction of polypeptide with lipid was investigated in relation to the structure of water at the interfacial region.

Experimental

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 viscometry at 25 °C were 3.8×10^5, 3.0×10^5, and 6.4×10^4, respectively. Infrared spectra indicate that 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. No β-conformation was found for PBDLG. The regular α-helix and perturbed α-helix have been designated by Tsuboi *et al.*7) The perturbed α-helix portion in a polypeptide molecule contains an antipode residue, the sequence length being relatively short. Lipids used were methyl stearate and stearamide. These materials were chromatographically pure.

The spreading solvents (*N,N*-dimethylformamide (DMF), dichloromethane (DCM)) were distilled under nitrogen atmosphere. The spreading solvents were DCM for mixed PBLG– and PBDLG–lipid systems, a 9:1(v/v) mixture of DCM and DMF for mixed PLL(Z)–lipid systems. The solutions of polypeptide and lipid were prepared separately, and mixed in the desired ratio immediately before spreading.

Salt and urea solutions were treated with activated charcoal in order to remove surface active contaminants. A 10 to 1 compression of the surface of the salt or urea subsolutions, after standing 10 min, produced less than 0.1 dyn/cm film pressure.

The initial spreading-area was 50 Å²/residue or molecule. The trough (65 cm × 15 cm × 1 cm) and compressing barriers were made of Teflon. The surface pressure was measured by Whilhelmy method at 20 °C. The film was compressed at a rate of 0.5 mm/min (1 Å²/residue or molecule).

Results

Figure 1 shows the surface pressure-area(π-*A*) curves for PLL(Z), PBLG, and PBDLG. Plateau which is characteristic of a number of synthetic polypeptide in α-helical conformation in the monolayer state are seen in the curves. The plateau is associated with the collapse or the transition of monolayer from a two-dimensional oriented state to a three-dimensional disoriented state.6,8,9)

Figures 2 and 3 show the π-*A* curves of mixed PLL(Z)–methyl stearate and mixed PLL(Z)–stearamide monolayers. The π-A curves of methyl stearate and stearamide are also shown in Figs. 2 and 3. These lipids form condensed films with the cross sectional area of about 20 Å² on distilled water. The areas are in agreement with those reported by other workers.10,11) The transition pressure for mixed PLL(Z)–methyl stearate monolayer increases with methyl stea-

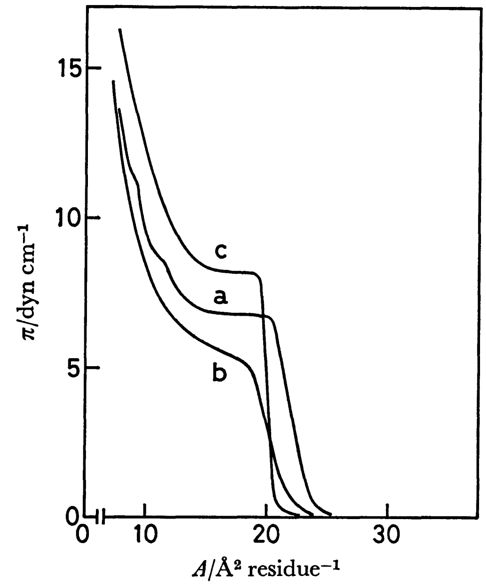

Fig. 1. Surface pressure-area curves of polypeptide monolayers on distilled water.
1 dyn=10^{-5} N, 1 Å=0.1 nm. a: PLL(Z), b: PBLG, c: PBDLG.

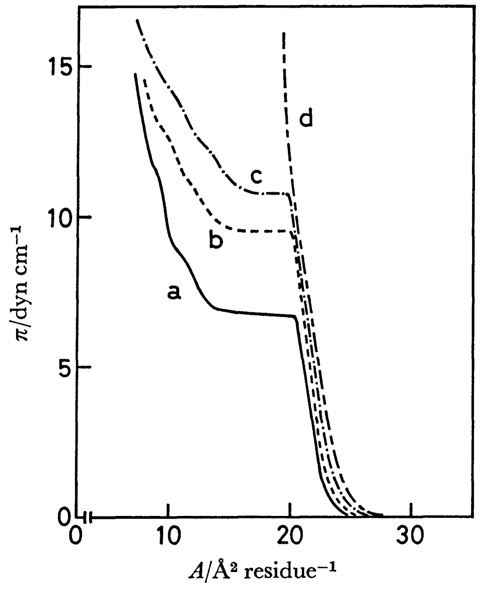

Fig. 2. Surface pressure-area curves of mixed PLL(Z)–methyl stearate monolayers on distilled water.
a; PLL(Z), b; 9:1(residue mol:mol), c; 1:1(residue mol:mol), d; methyl stearate.

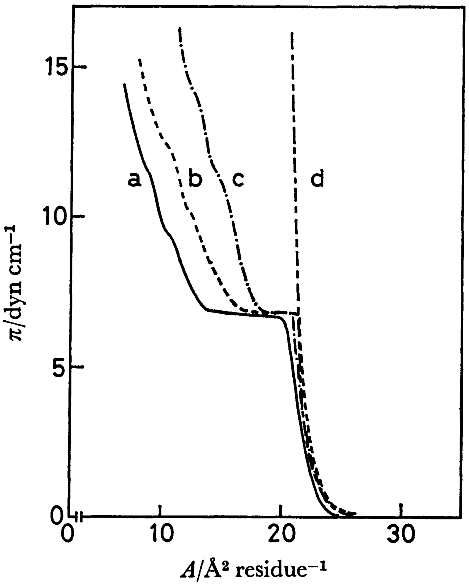

Fig. 3. Surface pressure-area curves of mixed PLL(Z)–stearamide monolayers on distilled water.
a; PLL(Z), b; 4:1(residue mol:mol), c; 1:1(residue mol:mol), d; stearamide.

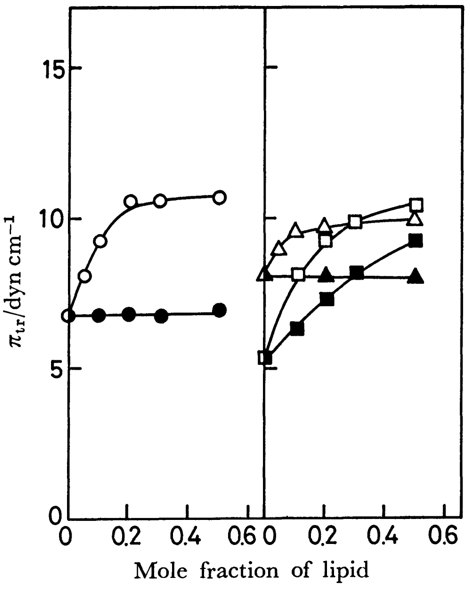

Fig. 4. Transition pressures of single and mixed monolayers as a function of lipid mole fraction.
○: PLL(Z)–methyl stearate, ●: PLL(Z)–stearamide, □: PBLG–methyl stearate, ■: PBLG–stearamide, △: PBDLG–methyl stearate, ▲: PBDLG–stearamide.

rate mole fraction, while for mixed PLL(Z)–stearamide monolayer is independent of stearamide mol fraction.

Figure 4 shows the relationship between the transition pressure and the composition of lipid in mixed monolayers for PLL(Z)–lipid, PBLG–lipid, and PBDLG–lipid systems on distilled water. For mixed polypeptide–methyl stearate systems, up to methyl stearate mol fraction of about 0.2, the transition pressures caused by the polymer components increase steeply, the increase becoming gradual beyond this mole fraction. On the other hand, no significant change in transition pressure is observed for mixed monolayer of stearamide and either PLL(Z) or PBDLG. The transition pressures for mixed PBLG–stearamide system increase with stearamide mol fraction different from those for mixed systems of stearamide and either PLL(Z) or PBDLG.

As a example of mixed monolayers on salt subsolutions, Fig. 5 shows the π-A curves for mixed PLL(Z)–methyl stearate (1:1 residue mol: mol) spread on distilled water, 6 M urea, 3 M KSCN and 3 M KF subsolutions (1 M=1 mol dm^{-3}). The transition pressures caused by PLL(Z) are much higher on the subsolution containing KSCN or urea than on distilled water. In the presence of KF, on the other hand,

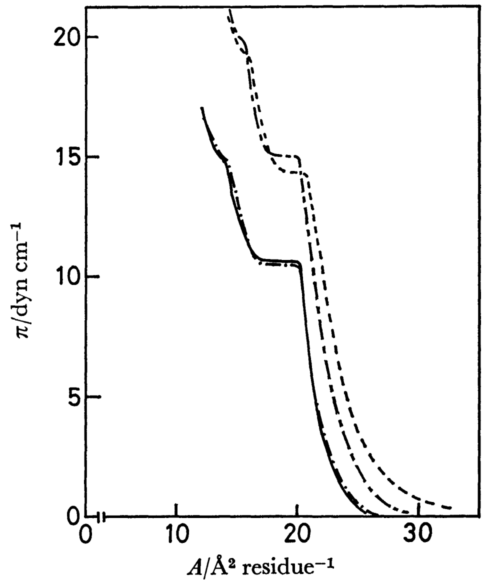

Fig. 5. Surface pressure-area curves of mixed PLL(Z)-methyl stearate (1:1(residue mol : mol)) monolayers on different subsolutions.
——: Distilled water, —— 3 M KSCN, —·—: 3 M KF, ----: 6 M urea.

the film transition pressure becomes slightly lower. The film expansion is observed in the presence of KSCN or urea. The effect of KF on expansion is quite small. Similar tendencies were observed for the other mixed monolayers on salt and urea subsolutions.

Table 1. Miscibility between polypeptide and lipid

Mixed System	Miscibility			
	Water	KF	KSCN	Urea
Methyl stearate-PLL(Z)	+	+	+	+
Methyl stearate-PBLG	+	+	+	+
Methyl stearate-PBDLG	+	+	+	+
Stearamide-PLL(Z)	−	−	+	−
Stearamide-PBLG	+	+	+	−
Stearamide-PBDLG	−	−	+	−

+: Miscible, −: immiscible.

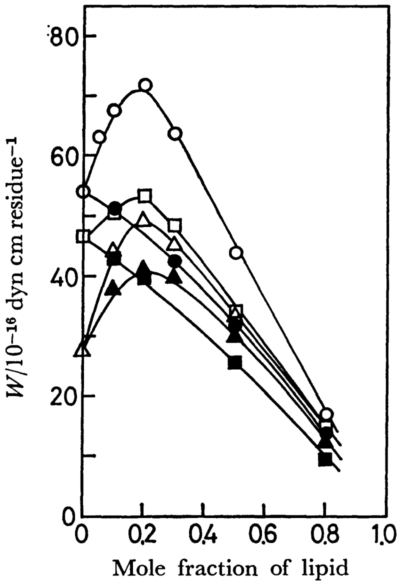

Fig. 6. Work of collapse for mixed monolayers as a function of lipid mole fraction.
○: PLL(Z)-methyl stearate, ●: PLL(Z)-stearamide, △: PBLG-methyl stearate, ▲: PBLG-stearamide, □: PBDLG-methyl stearate, ■: PBDLG-stearamide.

Discussion

Interaction on Distilled Water. The application of the two-dimensional phase rule provides an important tool for detecting miscibility in two-component monolayers.[12] If a polypeptide and a lipid are 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 Fig. 4, the mixed systems of methyl stearate with PLL(Z), PBLG, or PBDLG are miscible. Stearamide is immiscible with PLL(Z) or PBDLG except PBLG. Both monolayers of methyl stearate and stearamide are the condensed type. Because of the similarity of cross sectional area between polypeptide and lipid monolayers, the deviation from the additivity rule was difficult to detect.

To obtain a further information concerning polypeptide-lipid interaction, the work of collapse of mixed monolayers was estimated. The work of collapse, W, is given by[6]

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

where π_{tr} is the transition pressure, and ΔA is the difference between initial and final areas of collapse. Figure 6 shows the work of collapse as a function of lipid mole fraction. It can be seen that with the exception of mixed PLL(Z)-stearamide and PBDLG-stearamide systems, the maximum work of collapse is found at about 0.2 lipid mol fraction. At this fraction, the surface adhesive forces might be reinforced as a result of the formation of the 4:1 complex (residue:molecule) of polypeptide and lipid as reported previously for mixed polypeptide-fatty acid and polypeptide-fatty alcohol systems.[6] In the lipid rich region, the adhesive force of polypeptide decrease steeply, the film collapsing more easily in the case of polypeptide alone. There is a remarkable difference in W values between mixed PLL(Z)-stearamide and mixed PBLG-stearamide systems (Fig. 6). This would be due to the difference between the polar groups, RCOOR′, in PBLG side chain and ROCONHR′ in PLL(Z) side chain.

Effect of Salts. The effect of polar group on interaction could be discussed in more detail by the use of subsolutions containing either KSCN or urea as a breaker and KF as a maker of water structure.

The polypeptide and mixed monolayers of polypeptide with methyl stearate or stearamide on salt subsolutions expand with salt concentration. The order of film expansion is KSCN>KF>distilled water, and independent of the difference in miscibility between polypeptide and either methyl stearate or ste-

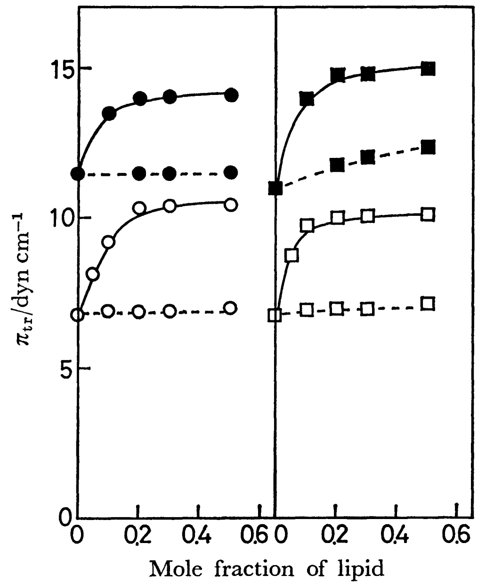

Fig. 7. Transition pressures of single and mixed monolayers as a function of lipid mole fraction on different subsolutions.
——: PLL(Z)-methyl stearate, ----: PLL(Z)-stearamide, ○: distilled water, ●: 6 M urea, ■: 3 M KSCN, □: 3 M KF.

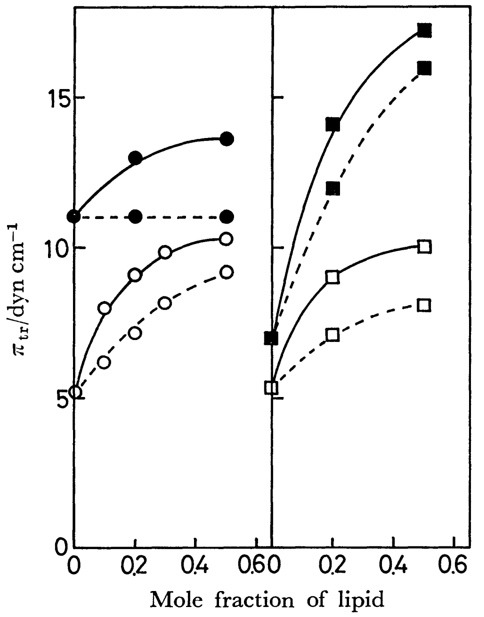

Fig. 8. Transition pressures of single and mixed monolayers as a function of lipid mole fraction on different subsolutions.
——: PBLG-methyl stearate, ----: PBLG-stearamide, ○: distilled water, ●: 6 M urea, ■: 3 M KSCN, □: 3 M KF.

aramide. This order was essentially the same as obtained for mixed polypeptide-fatty acid systems.[6] Salts may be transferred into the interface relative to the monolayer free surface. Ralston and Healy[13] reported that salts are transferred into fatty alcohol monolayer in terms of interfacial water structure and this process continued until the surface and bulk concentration become equal.

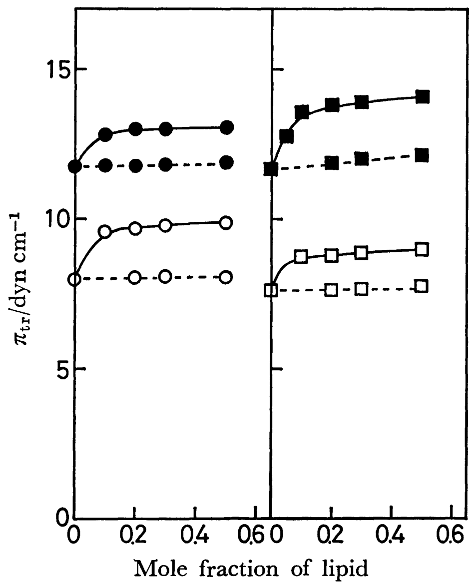

Fig. 9. Transition pressures of single and mixed monolayers as a function of lipid mole fraction on different subsolutions.
——: PBDLG-methyl stearate, ----: PBDLG-stearamide, ○: distilled water, ●: 6 M urea, ■: 3 M KSCN, □: 3 M KF.

The general tendencies relating to the miscibility of polypeptide with lipid on various subsolutions was summarized in Table 1. The miscibilities between polypeptide and stearamide are extremely poor compared with polypeptide-methyl stearate systems.

Figures 7, 8, and 9 show the relationship between the transition pressure and the composition of lipid in mixed PLL(Z)-lipid, PBLG-lipid, and PBDLG-lipid monolayers spread on distilled water, 6 M urea, 3 M KSCN and 3 M KF subsolutions, respectively. The transition pressures for single and mixed monolayers spread on KSCN subsolution are higher than those on distilled water, and is independent of miscibility between polypeptide and lipid. For mixed polypeptide-methyl stearate monolayers on KSCN or urea subsolution, up to 0.2 methyl stearate mole fraction, the transition pressure caused by the polymer component increases steeply, the increase becoming almost constant beyond this mol fraction. The hydrated structure around the hydrocarbon moieties of the side chains of polypeptide is disrupted by SCN$^-$ ion preferentially accumulated on the surface. As a result of the disruption, the affinity of the hydrocarbon moieties to water may increase.

The transition pressures for both miscible and immiscible mixed systems on KF subsolution becomes slightly low compared with that on distilled water (Figs. 7, 8, and 9). In the presence of F$^-$ ion as a maker of water structure, the adhesive force of polypeptide to water become weak with the formation of hydrated structure around the mixed films.

The molecular structure of urea is similar to the polar group in stearamide molecule and PLL(Z) side chain, and urea acts as a breaker of water structure.[14] The expansion of single and mixed films may be ascribed to the penetration of polypeptide monolayer by urea in the subsolution (Fig. 5).[15] As shown in Figs. 7 and 8, the transition pressures of mixed PLL-(Z)–lipid and PBLG–lipid monolayers on urea subsolution are higher than those on distilled water. The hydrated structure around hydrophobic moieties of polypeptide side chain would be disrupted by the action of urea.

It is noteworthy that stearamide becomes practically immiscible with PBLG on urea subsolution (Table 1 and Fig. 8). In the presence of urea, the dipole-dipole interaction between $RCONH_2$ in stearamide molecule and $RCOOR'$ in PBLG side chain may be weakened by urea because of the similarity of polar group in stearamide and urea molecule.

Effect of Polypeptide Conformation. The perturbed helix portion of PBDLG is unfolded at the interface owing to relatively weak stability of the helix.[6,7] As shown in Fig. 1, the difference in shape of the π-A curves between PBLG and PBDLG is accounted for as arising essentially from the conformation of these polymer at the interface.[6,16] The change in transition pressure for mixed PBLG–lipid monolayer is larger than that of mixed PBDLG–lipid monolayer (Fig. 4). The PBLG monolayer is more compressible than the PBDLG monolayer.[4] The flexible side chains of PBLG might undergo a rearrangement more easily into position favorable to the interaction with alkyl chain of methyl stearate or stearamide The strength of van der Waals forces of lipid molecules in condensed monolayers and the flexibility of polypeptide side chain seem to affect the degree of interaction as in the case of mixed systems of polypeptide and either fatty acid or fatty alcohol.[4]

The effect of stearamide on interaction in mixed monolayers is much more pronounced than that of methyl stearate. PBLG interacts characteristically with stearamide. However, PBDLG interacts with considerable difficulty (Fig. 4). The conformation of a polymer may also be important for the interaction at the interface.

The transition pressures of mixed PBDLG–methyl stearate monolayer spread on KF subsolution are smaller than those on distilled water. For mixed PBLG–methyl stearate system, on the other hand, there is little difference between KF and KF free subsolutions. The formation of hydrated structure around PBDLG molecule unfolded considerably at the interface may be caused by F^- ion as a maker of water structure.

The transition pressures of mixed monolayers of lipid and either PBLG or PBDLG spread on KSCN subsolution become higher than those on distilled water (Figs. 8 and 9). In the presence of KSCN, the increment of transition pressure for mixed PBLG–lipid systems is larger than that of mixed PBDLG–lipid systems. The geometry of the α-helix in the monolayer state is such that the side chains of adjacent helices can interpenetrate to form good hydrophobic contacts.[16] PBLG might become more hydrophobic than PBDLG unfolded considerably at the interface since the polypeptide main chain is appreciably shielded from the interaction with water molecules. Therefore, the interaction of the amide group in the main chain with SCN^- ion would accompany the difficulty. SCN^- ion is considered to act primarily as a breaker of water structure around the hydrophobic moieties in mixed PBLG–lipid film.

Irrespective of the composition of subsolution, methyl stearate is miscible with PBLG or PBDLG. On the other hand, mixed PBLG–stearamide and PBDLG–stearamide monolayers are miscible and immiscible on distilled water, respectively (Table 1 and Figs. 8 and 9). As such the case of mixed PBLG–cholesterol system,[6] PBLG interacts characteristically with stearamide on distilled water. In the presence of urea, however, both mixed monolayers become immiscible through the interaction between the polar group, $RCONH_2$, in stearamide and urea molecule.

PBDLG is miscible for methyl stearate though immiscible for stearamide on distilled water and urea subsolution. The head group, $RCOOCH_3$, of methyl stearate in monolayer state exists under the surface. The methyl group under the surface shields the ester group from attack by polar group of polypeptide side chains,[17] and in addition, is able to interact with hydrophobic moieties of polypeptide side chains. On the other hand, stearamide monolayer on distilled water or urea subsolution may be stabilized by the intermolecular hydrogen bonding between C=O and NH_2 groups in stearamide molecules or by the interaction with urea. Therefore, it seems that the miscibility between PBDLG and stearamide becomes less.

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