On the Differences in Monolayer Properties of Poly-γ-benzyl-L-glutamate and Poly-β-benzyl-L-aspartate

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Recently great progress has been made in the studies on synthetic polypeptides, especially concerninig their solutions. Among them, the solutions of poly-γ-benzyl-L-glutamate in various solvents have been investigated extensively and it is found that this polymer has a right-handed α-helical configuration in some solvents[1]. More recently it has been reported, however, that poly-β-benzyl-L-aspartate, the side chain of which has a length by one methylene group less than that of poly-γ-benzyl-L-glutamate, is quite different from poly-γ-benzyl-L-glutamate in its behavior and that the α-helix of the former polymer may be left-handed rather than right-handed[2].

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[1] P. Doty, J. H. Bradbury and A. M. Holtzer, *J. Am. Chem. Soc.*, **78**, 947 (1956); J. T. Yang and P. Doty, ibid., **79**, 761 (1957); E. R. Blout, P. Doty and J. T. Yang, ibid., **79**, 749 (1957).

[2] E. R. Blout and R. H. Karlson, ibid., **80**, 1259, 6701 (1958); E. M. Bradbury, L. Brown, A. R. Downie, A. Elliott, W. E. Hanby and T. R. R. McDonald, *Nature*, **183**, 1736 (1959); R. H. Karlson, K. S. Norland, G. D. Fasman and E. R. Blout, *J. Am. Chem. Soc.*, **82**, 2268 (1960).

According to the surface chemical studies, synthetic polypeptides with non-polar or unionized side chains are known to give monolayers of condensed type on aqueous surfaces, the surface viscosity of which is manifest at a very low surface pressure region, but only those which contain prolyl residues in some high amounts give monolayers of expanded type, the surface viscosity of which is manifest first at a very high surface pressure region[3,4]. In general, therefore, the monolayers of ordinary unionized polypeptides are of condensed type and exhibit high surface viscosities, and the only difference among those which are composed of different amino acid residues are reflected explicitly in the behavior of the surface potential, according to the difference in polarities or electric moments of their side chains. Considering the striking difference in properties of poly-γ-benzyl-L-glutamate and poly-β-benzyl-L-aspartate in crystals or in solutions, as cited above, however, it is expected that the two polymers behave in a different way in their spread monolayers, since the difference of the two polymers in solutions and in crystals will be based on the different kinds of interactions of side chain with main chain or with other side chains and such an effect of side chain of polymers will be most distinctly revealed in monolayer states.

We have thus studied the monolayers of poly-γ-benzyl-L-glutamate (PBLG) and poly-β-benzyl-L-aspartate (PBLA) by the measurements of surface pressure, potential and viscosity. We will report any marked differences in their behavior and propose possible chain configurations of these polymers in monolayers and then discuss the differences on the basis of these configurations. Further we have examined the monolayer of a copolymer of γ-benzyl-L-glutamate and β-benzyl-L-aspartate, copoly-1:1-(γ-benzyl-L-glutamate, β-benzyl-L-aspartate) (CP(BLG, BLA)), and the equimolar mixture of PBLG and PBLA.

Experimental

PBLG (Code No. J11A) and PBLA (J826) were prepared by polymerizing *N*-carboxy-anhydrides of the corresponding L-amino acids in dioxane with triethylamine and CP(BLG, BLA) (A227B) by copolymerizing the equimolar mixture of *N*-carboxy-anhydrides of these amino acids in (1 : 1 v/v) chloroform-dioxane with triethylamine. Their intrinsic viscosities in dichloroacetic acid solution at 25°C were 0.13, 0.11 and 0.34, respectively. Their solutions in (1 : 4 v/v) dichloroacetic acid-benzene were spread on aqueous surfaces. The equimolar mixed monolayer of PBLG and PBLA was spread from their mixed solution in the same solvent.

The surface pressure was measured by a surface balance of the hanging plate type, the surface potential by a vibrating electrode and the surface viscosity by a rotatory oscillating disk[3,4]. The temperature was regulated by heating the inside of the shielding box with a voltage controlled lamp, if necessary.

Results

The surface pressure, Π, surface potential, ΔV, and surface viscosity, η, of PBLG, PBLA and CP(BLG, BLA) are plotted against the area, expressed in the unit of Å² per (average)

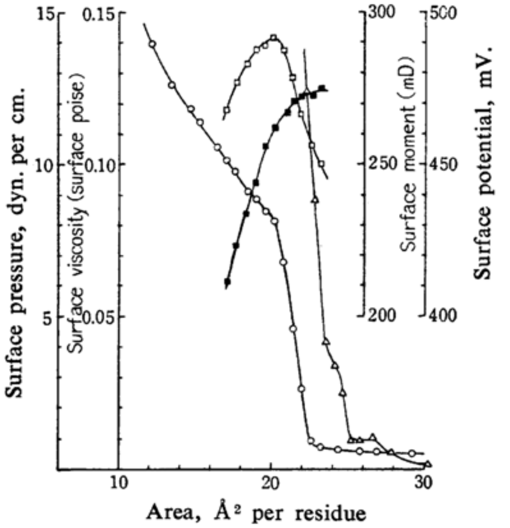

Fig. 1. Monolayer of PBLG at 9.0°C. ○, surface pressure; □, surface potential; ■, surface moment; △, surface viscosity.

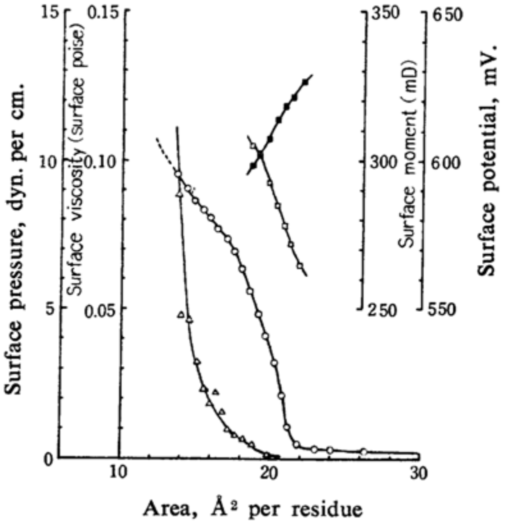

Fig. 2. Monolayer of PBLA at 10.8°C. ○, surface pressure; □, surface potential; ■, surface moment; △, surface viscosity.

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[3] T. Isemura and S. Ikeda, This Bulletin, 32, 178 (1959).

[4] S. Ikeda and T. Isemura, ibid., 32, 659 (1959).

TABLE I. MONOLAYER CHARACTERISTICS OF POLYPEPTIDES

	$A_{\Pi\to 0}$, Å²	$A_{\Delta V}$, Å²	$\mu_{21\text{Å}^2}$, mD	$A_{\eta=0.10}$, Å²
PBLG	22.8	23.2	270	22.7 (9.0°C)
PBLA	21.5	22.0	322	13.5 (10.8°C)
CP(BLG, BLA)	23.0	22.6	305	23.4 (12.3°C)
1 : 1 PBLG+PBLA	22.4	22.5	240	13.4 (19.3°C)

TABLE II. SURFACE COMPRESSIBILITIES

	δ_1, cm./dyn.	δ_2, cm./dyn.	δ_3, cm./dyn.
PBLG	0.0126 (22.8∼20.3Å²)	0.0855 (20.3∼ca. 11.4Å²)	—
PBLA	0.0222 (21.5∼20.3Å²)	0.0329 (20.3∼ca. 17Å²)	0.129 (ca. 17∼14.0Å²)
CP(BLG, BLA)	0.0195 (23.0∼18.3Å²)	1.21 (18.3∼ca. 12Å²)	—
1 : 1 PBLG+PBLA	0.0191 (22.4∼19.5Å²)	0.114 (19.5∼ca. 14Å²)	—

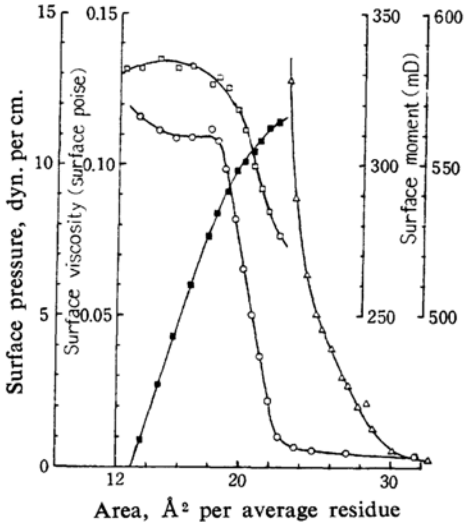

Fig. 3. Monolayer of CP(BLG, BLA) at 12.3°C. ○, surface pressure; □, surface potential; ■, surface moment; △, surface viscosity.

amino acid residue, as shown in Figs. 1, 2 and 3. All the monolayers are of condensed type. The surface potential was not definite but fluctuated from time to time at the region of large areas. However, reproducible values were observed as the surface pressure began to rise. Values of the area, $A_{\Pi\to 0}$, at which the surface pressure steeply increases on compression, and of the area, $A_{\Delta V}$, at which the surface potential becomes reproducible on compression, are tabulated in Table I. The surface pressure and potential were scarcely at all influenced by temperature. The $A_{\Pi\to 0}$ is nearly equal to $A_{\Delta V}$ for each polymer and they will represent the actual area occupied by an (average) amino acid residue.

The values of surface compressibility calculated by

$$\delta=-\frac{1}{A}\frac{\partial A}{\partial \Pi}$$

are given in Table II, which shows clearly the difference in surface pressure-area curves of the three polymers. In the region of low surface pressure the surface compressibility is in the order: PBLA>CP(BLG, BLA)>PBLG. The surface pressure-area curve of CP(BLG, BLA) is characterized by the region of very high surface compressibility such as that observed for poly-γ-benzyl-DL-glutamate[5].

The surface moments per amino acid residue calculated by the Helmholtz equation

$$\mu=\frac{A\cdot\Delta V}{4\pi}$$

are plotted against the area, as is also shown in Figs. 1, 2 and 3, and the values at 21 Å² are also listed in Table I. It is noticed that the surface moment of PBLA is about 50 mD higher than that of PBLG and CP(BLG, BLA) has an intermediate value of these two.

Although some differences are observed in surface pressure and potential between PBLG and PBLA, as described above, they are most distinctly reflected in surface viscosity behavior. The surface viscosity of PBLG is very high at very low surface pressure region, but that of PBLA is manifest first at high surface pressure region; the surface viscosity of the former is much lower than that of the latter. It is remarkable that, in spite of the condensed nature of monolayer reflected in surface pressure, PBLA has a very low surface viscosity. A monolayer of polymer with such composite properties, i. e., a monolayer of condensed type with a low surface viscosity, has never been reported. The surface viscosity-area curve of CP(BLG, BLA) is similar to that of PBLG rather than being an intermediate between PBLG and PBLA.

The surface viscosities of PBLG and PBLA were markedly influenced by temperature and they were higher as the temperature was high.

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[5] T. Isemura and K. Hamaguchi, ibid., 27, 125 (1954).

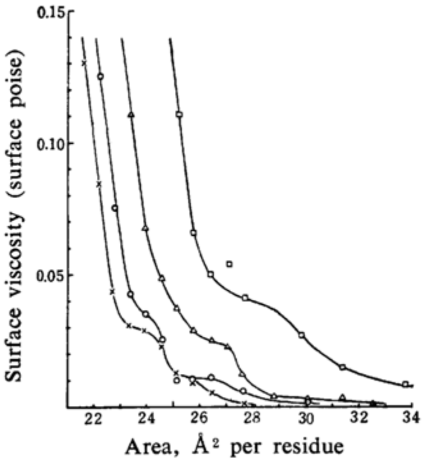

Fig. 4. Variation of surface viscosity of PBLG with temperature. □, 24.0°C; △, 16.2°C; ○, 9.0°C; ×, 5.6°C.

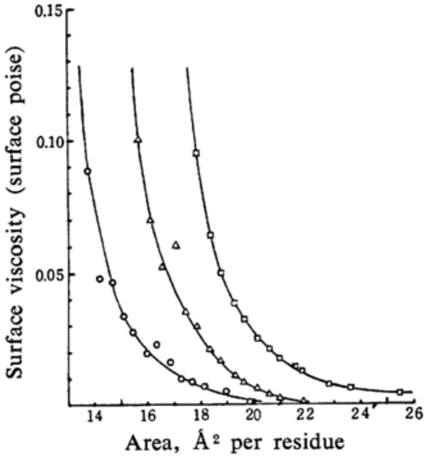

Fig. 5. Variation of surface viscosity of PBLA with temperature. □, 24.5°C; △, 17.9°C; ○, 10.8°C.

The variations of surface viscosity with temperature are shown in Figs. 4 and 5. Contrary to the common nature of the expanded monolayer[4)], PBLA has a positive temperature coefficient of surface viscosity rather than a negative one.

Values of the area, $A_{\eta=0.10}$, at which the surface viscosity reaches 0.10 surface poise, are also given in Table I.

Discussion

Chain Configurations of PBLG and PBLA in Monolayer.—The values of the limiting area, $A_{\Pi\to0}$, derived from the surface pressure-area curves imply that these three polymers assume a configuration such as the β-form in monolayers. Although the limiting area, 22 Å², of these polymers is somewhat larger than that of some other polypeptides such as poly-DL-alanine, 15 Å² [3,6)], this suggests that the area occupied by a residue is not defined by the main chain only but by the large side chain for these polymers. As the polymers are composed of L-amino acid residues exclusively, the side chains are oriented up and down alternatively when spread on an aqueous surface, assuming a β-configuration[6,7)] but the hydrophilic or hydrophobic nature and the orientation of side chain should be different from that of non-polar polypeptides owing to the presence of two oxygen atoms.

Since the three polymers have polar groups on a side chain as well as on the main chain and then the side chain has both hydrophilic and hydrophobic nature, the side chain can change its orientation with greater ease sometimes rising into the air, sometimes lying on the surface and sometimes submerging into the water. The fluctuation of surface potential at large areas might be ascribed to such a thermal motion of the side chain. On high compression, however, the orientation of the side chain is forced to be fixed into a definite position, as given below. Thus the area, $A_{\Delta V}$, at which the surface potential gives a definite value, is nearly equal to the limiting area, $A_{\Pi\to0}$.

As shown in Table I, the surface moments of these three polymers are much higher than those of polypeptides with non-polar side chains such as poly-DL-alanine, 160 mD[3)]. This can be attributed to the contributions of the polar groups on side chain, i. e., the electric moments of the carbonyl group and the $H_2C{-}O$ bond, to the surface moment besides that of the main chain. If the carbonyl group on the side chain is directed towards the aqueous surface with an angle 90° $-\theta$, then the 'surface moment' of the side chain of PBLG and PBLA is given by

$$\text{(A)} \qquad \mu_{C=O}\cos\theta-\mu_{C-O}\cos(\theta+30^\circ)$$

if all the bonds on the side chain are assumed to be in trans form, or by

$$\text{(B)} \qquad \mu_{C=O}\cos\theta+\mu_{C-O}\cos(\theta+30^\circ)$$

if the $\underset{|}{\mathrm{O}}{-}\mathrm{C}\langle_{\mathrm{O}}$ bond alone takes cis form. About half of the side chains are submerged into the aqueous subphase and surrounded by water molecules; this does not contribute to the surface moment[8)]. Thus the total surface moment observed experimentally consists of

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6) C. W. N. Cumper and A. E. Alexander, *Trans. Faraday Soc.*, **46**, 235 (1950).

7) J. T. Davies, *Biochim. Biophys. Acta*, **11**, 165 (1953).

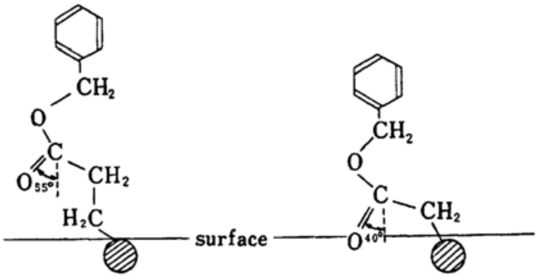
8) J. T. Davies, *Trans. Faraday Soc.*, **49**, 949 (1953).

TABLE III. CALCULATED VALUES OF SURFACE MOMENTS

$\mu=160\ \mathrm{mD}+1/2\,\mu(\text{side chain})$

θ (°)		0	30	35	40	45	50	55	60	90
μ(mD)	(A)	242	274	272	269	266	261	252	250	203
	(B)	439	359	344	327	309	291	271	250	117

the contributions of the main chain, 160 mD³⁾, and of half the 'surface moment' of the side chain. If it is assumed that $\mu_{C=O}=360$ mD and $\mu_{C-O}=170$ mD⁹⁾, the total surface moment can be calculated for various values of θ in either case, (A) or (B). The results are given in Table III. Comparing these calculated values with the observed values in Table I, it is found that the side chain of PBLA has a configuration with $\theta=40°$ in which all the bonds are trans except that the $\underset{|}{\mathrm{O}}-\mathrm{C}\Big\langle_{\mathrm{O}}$ bond is cis. On the other hand, the configuration of the side chain of PBLG has two possible configurations from the data on surface moment alone, but it is confined to the one with all the bonds being trans other than the $\underset{|}{\mathrm{O}}-\mathrm{C}\Big\langle_{\mathrm{O}}$ bond and with $\theta=55°$, if the limiting area derived from the surface pressure is taken into account. The configurations deduced thus are illustrated as follows:



PBLG PBLA

It can be seen that the orientations of side chains of PBLG and PBLA are quite different from each other, although the difference in their structure is only by one methylene group in side chain length. In other words, the carbonyl group on the side chain of PBLG seems to play the role of a hydrophobic part and does not serve for the hydrophility of the side chain very well, but that of PBLA is submerged into the aqueous subphase and is indistinguishable from the carbonyl group on the main chain, thus greatly increasing the hydrophilic nature of the side chain.

The value of surface moment of CP(BLG, BLA) intermediate between PBLG and PBLA supports the configuration similar to the β-form for these three polymers in monolayer and the value, 305 mD, slightly higher than the mean value, 296 mD, suggests that the side chains of γ-benzyl-L-glutamate residue are influenced by β-benzyl-L-aspartate residue in this copolymer.

The observed value of surface moment of γ-benzyl-L-glutamate residue in PBLG, $\mu_{21\mathrm{A}^2}=$ 270 mD, may be compared with that of γ-methyl-L-glutamate residue in copoly-1 : 1-(γ-methyl-L-glutamate, DL-phenylalanine). The latter copolymer has a surface moment per amino acid residue, $\mu_{19\mathrm{A}^2}=197$ mD⁸⁾. Since the surface moment of poly-DL-phenylalanine is 110 mD⁷⁾, the contribution of γ-methyl-L-glutamate residue to the surface moment of the copolymer should be 284 mD, which is in accord with the surface moment of γ-benzyl-L-glutamate residue in PBLG.

Relation of Surface Viscosity with Surface Pressure.—As was noted before, whereas PBLG gives a high surface viscosity at areas larger than the area at which the surface pressure begins to rise steeply or the limiting area, i. e., at a very low surface pressure region, PBLA shows a high surface viscosity only at areas far smaller than the limiting area, i. e., at a high surface pressure region. In other words, the surface viscosity is much higher for PBLG than for PBLA. The behavior as was observed for PBLG has the general characteristics of monolayers of condensed type. However, that for PBLA has not been noticed for monolayers of any polymers so far examined, but only for monolayers of long chain fatty acids¹⁰⁾ and their mixtures with each other or with long chain alcohols¹¹⁾. This striking difference between PBLG and PBLA may be interpreted on the basis of the configurations of side chains presented above.

The monolayer of PBLG would correspond to the glassy state polymers in three dimensional state in which the interaction between segments are very strong, and consequently the monolayer is condensed and the surface viscosity is very high⁴⁾. In this case the interaction between segments would mainly be the hydrogen bonding between peptide bonds in the main chain. The monolayer of PBLA would also correspond to the glassy state. In the case of PBLA, however, the carbonyl group on the side chain is submerged into water like

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9) A. E. Alexander and J. H. Schulman, *Proc. Roy. Soc.*, **A161**, 115 (1937).

10) G. E. Boyd and W. D. Harkins, *J. Am. Chem. Soc.*, **61**, 1188 (1939).

11) G. E. Boyd and F. Vaslow, *J. Colloid Sci.*, **13**, 275 (1958).

the one on the main chain, as referred to before, and, therefore, the interactions between segments are not necessary ascribed to the hydrogen bonding between peptide bonds but also possibly to the hydrogen bonding of the carbonyl group on the side chain with the imino group on the main chain. The monolayer configuration of PBLA would be less stable than that of other polypeptides and a part of the molecular chain can easily be submerged into the aqueous subphase under compression or under shear flow. Further, the hydrophilic nature of the side chain is greatly enhanced owing to the carbonyl group in contact with water and would be responsible for reducing the resistance against compression or shear. Thus the high surface compressibility and the low surface viscosity of PBLA will be exhibited after the surface pressure begins to rise or the monolayer is compressed to the limiting area.

The specific nature of the side chain of PBLA seems to be related to its reactivity when it is reduced to remove the benzyl group on the side chain. By this treatment, PBLA does not produce poly-α-L-aspartic acid but gives poly-anhydro-L-aspartic acid[12], while PBLG gives poly-α-L-glutamic acid. This also suggests that the carbonyl group on the side chain is scarcely distinguishable from that on the main chain.

The variations of surface viscosity with temperature for PBLG and PBLA are given in Figs. 4 and 5. If the logarithms of surface viscosity of PBLA at different temperatures are plotted against surface pressure, a part of each curve gives a straight line expressed by

$$\log \eta = \log \eta_0 + c\Pi$$

as shown in Fig. 6. The same type of equation has been found for some other monolayers[4,10,11,13]. The constants of this equation for PBLA are tabulated in Table IV. No further implication of these constants is clear.

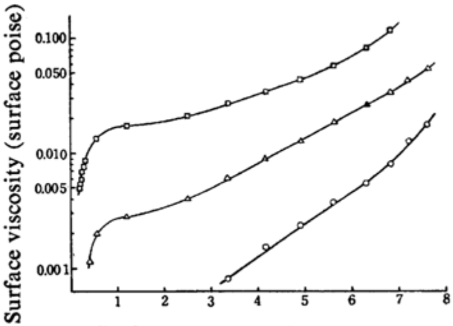

Fig. 6. The log η-Π plots for PBLA at different temperatures. □, 24.5°C; △, 17.9°C; ○, 10.8°C.

TABLE IV. CONSTANTS OF THE log η-Π RELATION FOR PBLA

Temperature, °C	log η_0	c	Π, dyn./cm.
24.5	−1.99	0.124	2.0∼5.0
17.9	−2.92	0.207	2.2∼6.5
10.8	−3.77	0.249	2.2∼7.0

The Equimolar Mixed Monolayes of PBLG and PBLA. — The monolayer characteristics of the equimolar mixture of PBLG and PBLA are shown in Fig. 7. The limiting areas derived from the surface pressure and from the surface potential are equal to each other and are about 22 Å², and the surface compressibility at the region of rise of surface pressure is intermediate between those for PBLG and PBLA and almost equal to that of CP(BLG, BLA). However, the surface moment and viscosity are much lower than those of CP(BLG, BLA). These characteristics are also given in Tables I and II. The reason for the low surface moment and the low surface viscosity of the mixed monolayer is not clear, since the dipole-dipole interaction would not be strong enough to influence monolayer properties very appreciably[14].

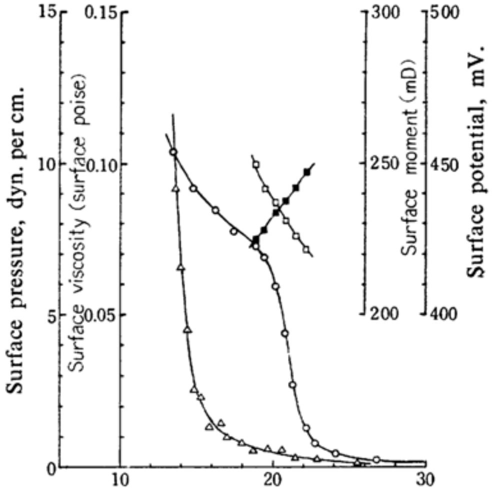

Fig. 7. Equimolar mixed monolayer of PBLG and PBLA at 19.3°C. ○, surface pressure; □, surface potential; ■, surface moment; △, surface viscosity.

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[12] J. Noguchi, T. Saito and M. Asai, *J. Chem. Soc. Japan, Pure Chem. Sec.* (*Nippon Kagaku Zasshi*), **81**, 620 (1960).

[13] T. Isemura and K. Fukuzuka, *Mem. Inst. Sci. Ind. Res., Osaka Univ.*, **13**, 137 (1956).

[14] J. Marsden and J. H. Schulman, *Trans. Faraday Soc.*, **34**, 748 (1938); W. D. Harkins and R. T. Florence, *J. Chem. Phys.*, **6**, 847 (1938).

Summary

In view of the marked differences of PBLG and PBLA in crystals and in solutions, we have investigated their differences in monolayer states and further examined the monolayers of CP(BLG, BLA) and of the equimolar mixture of PBLG and PBLA.

All these polymers were found to be of condensed type and their limiting areas to be about 22 Å^2. The surface compressibility at the region of rise of surface pressure was in the order: PBLA > CP(BLG, BLA) > PBLG. The surface moments of all these polymers were considerably higher than those of non-polar polypeptides. The surface moment of PBLA was 50 mD higher than that of PBLG, and that of CP(BLG, BLA) gave a value intermediate between them. PBLG and CP(BLG, BLA) exhibited high surface viscosities at a very low surface pressure region, but, in spite of the condensed nature of the monolayer, PBLA showed appreciable surface viscosity first at a high surface pressure region. The surface viscosities of PBLG and PBLA were higher at higher temperatures.

From the data on surface pressure and surface moment, all these polymers were considered to have a configuration similar to the β-form in the spread state and possible configurations of side chains of PBLG and PBLA were deduced. It was found that the carbonyl group on the side chain of PBLG is far from the aqueous surface but that of PBLA is in contact with the surface and scarcely distinguishable from the carbonyl group on the main chain. Accordingly, PBLG can take a stable configuration like the β-form in monolayer, but the correspounding configuration of PBLA will be less stable owing to possible hydrogen bonding of the carbonyl group on the side chain with the imino group on the main chain and the resulting enhanced hydrophility of the side chain. This might be ascribed to the differences in surface compressibility and surface viscosity of PBLG and PBLA.

The surface pressure behavior of the mixed monolayer of PBLG and PBLA was such as was expected, but its behavior in surface potential and viscosity was very anomalous.

We wish to acknowledge to Professor J. Noguchi of Kanazawa University our indebtedness for his kind guidance in preparing the polypeptides.

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