Surface Chemistry of the Polyamide Series. II. Effect of the Sarcosyl Residue on the Monolayer of Polyleucine

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The role of the prolyl residue in polypeptide monolayers has been studied by Isemura and Ikeda.[1,2] The surface viscosity of the poly-DL-alanine monolayer, which is of the condensed type, was found to rise at larger area than its close-packed area. This result was interpreted by assuming that the polypeptide chain is rigid because of the hydrogen bonds between peptide groups. On the other hand, the monolayers of prolyl peptides are of the expanded type, and the appreciable surface viscosities are manifested at much smaller areas than their close-packed areas. Their chain configuration are considered to be rather flexible as a result of the lack of hydrogen bonds between peptide groups.

In the preceding paper,[3] the view that the hydrogen bonds between peptide groups remarkably affect the nature of polypeptide monolayers has been supported by the results of studies of the monolayers of polysarcosine and of the copolymer of sarcosine with DL-alanine or glycine.

In the present investigation, the effect of hydrogen bonding on the polypeptide monolayers has been studied at the air/water and oil/water interfaces with copolypeptides of L-leucine with sarcosine of different compositions. The difference between monolayers of poly-L-leucine and its DL-isomer has also been studied.

Experimental

Samples. — Poly-L-leucine, poly-DL-leucine and polysarcosine were prepared by the polymerization of the N-caboxyanhydride of the respective amino acid, using sodium methoxide as an initiator.[4] Copolymers of L-leucine, with sarcosine of different compositions, were prepared from the mixtures of *N*-carboxyanhydrides of sarcosine and L-leucine by the same method. The spreading solution and the average degree of polymerization of each polymer are shown in Table I.

TABLE I. AVERAGE DEGREES OF POLYMERIZATION OF SAMPLES AND THEIR SPREADING SOLVENTS

Polymer	*n*	Solvent
Poly-L-leucine		TFA
Poly-DL-leucine		TFA
Copoly-3 : 1-(L-leucine, sarcosine)		DCA+TFA+IPA (5 : 2 : 3, v/v)
Copoly-1 : 1-(L-leucine, sarcosine)	20	DCA+TFA+IPA (4 : 3 : 3, v/v)
Copoly-1 : 3-(L-leucine, sarcosine)	28	DCA+TFA+IPA (5 : 2 : 3, v/v)
Polysarcosine	27	H_2O+IPA (1 : 1, v/v)

TFA Trifluoroacetic acid
DCA Dichloroacetic acid
IPA Isopropyl alcohol
n was determined by the end group analysis.

Methods. — The surface pressure, the potential and the viscosity were measured by a surface balance of the float type, an ionizing air-electrode and an oscillating rotatory disk respectively. The surface moment was calculated from the surface potential using the Helmholtz equation. The interfacial pressure was measured by the ring method. The details of these experimental methods have been described in the preceding paper.[3]

Results

The surface pressure-area (Π-A), surface moment-area (μ-A) and surface viscosity-area (η_s-A) curves of poly-L-leucine and the 3 : 1-, 1 : 1- and 1 : 3-copolymers of L-leucine with sarcosine on distilled water are shown in Figs. 1—4 respectively. Π-A curve of poly-DL-leucine is also illustrated in Fig. 1.

The film characteristics of these polymers are summarized in Table II, where A_δ is the area at the minimum compressibility of the film; $A_{\Pi\to 0}$, the area where the straight portion of the Π-A curve is extraporated to $\Pi=0$; A_μ, the area where the surface moment, μ, begins to decrease; $\mu(c)$, the constant value of the surface moment; A_η, the area where the surface viscosity begins to rise, and Π_η, the pressure at A_η.

Poly-L-leucine and poly-DL-leucine gave monolayers of the condensed type. The area per residue of the former at the limiting area, A_δ, was much smaller than that of the latter.

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1) T. Isemura and S. Ikeda, This Bulletin, 32, 178 (1959).
2) S. Ikeda and T. Isemura, ibid., 32, 659 (1959).
3) Part I of this series: T. Yamashita and T. Isemura, This Bulletin, 38, 420 (1965).
4) E. Katchalski and M. Sela, *Adv. Protein Chem.*, 13, 243 (1958).

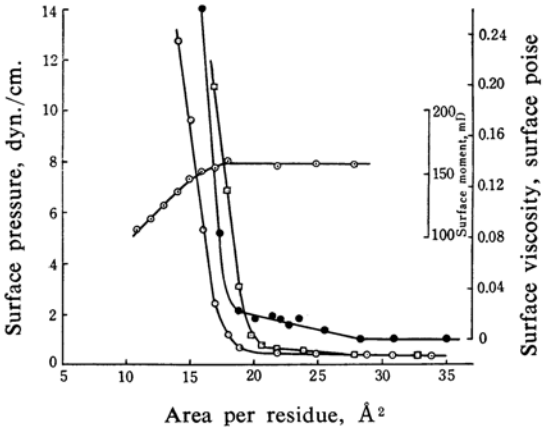

Fig. 1. Monolayers of poly-L-leucine and poly-DL-leucine on distilled water at 16°C: poly-L-leucine— ○, surface pressure; ⊙, surface moment; ●, surface viscosity: poly-DL-leucine— □, surface pressure.

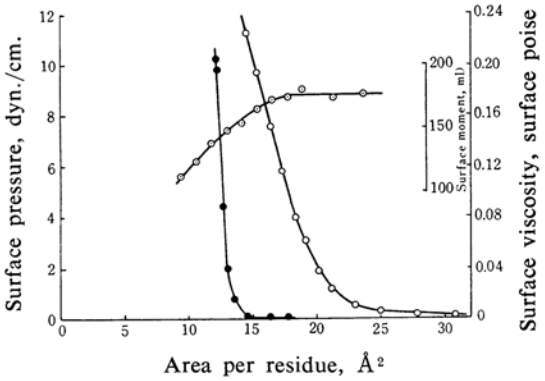

Fig. 2. Monolayer of copoly-3 : 1-(L-leucine, sarcosine) on distilled water at 16°C: ○, surface pressure; ⊙, surface moment; ●, surface viscosity.

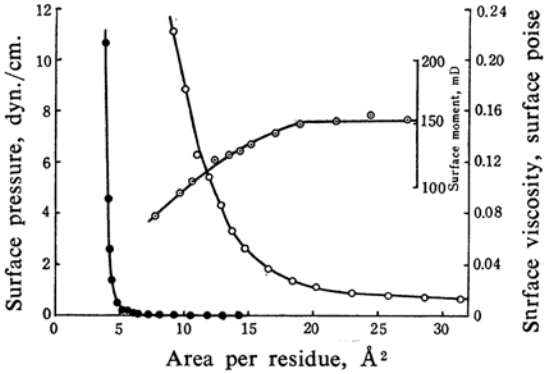

Fig. 3. Monolayer of copoly-1 : 1-(L-leucine, sarcosine) on distilled water at 16°C: ○, surface pressure; ⊙, surface moment; ●, surface viscosity.

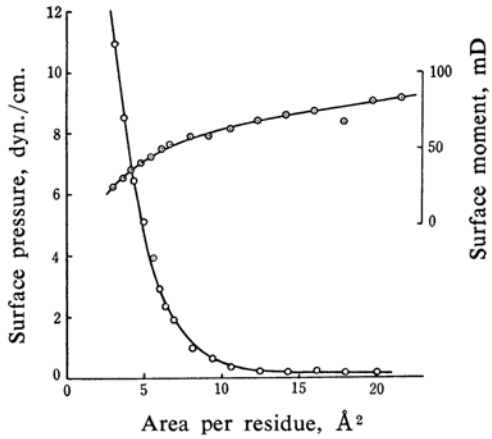

Fig. 4. Monolayer of copoly-1 : 3-(L-leucine, sarcosine) on distilled water at 16°C: ○, surface pressure; ⊙, surface moment.

The surface viscosity of poly-L-leucine was manifested at a larger area than A_δ and $A_{\Pi\to 0}$.

The film characteristics of poly-L-leucine were noticeably affected by the incorporation of the sarcosyl residue in the polypeptide chain. The monolayer of copoly-3 : 1-(L-leucine, sarcosine) was expanded much more than that of poly-L-leucine. In contrast, the surface viscosity could be detected at a much smaller area than A_δ and $A_{\Pi\to 0}$. The monolayer of copoly-1 ; 1-(L-leucine, sarcosine) was much more compressible than those of the two polymers cited above. The viscosity was first detected at a very small area (5.5 Å² per residue). The *Π-A* curve of copoly-1 : 3-(L-leucine, sarcosine) shifted to a smaller area than that of 1 : 1-copolymer, and the viscosity

Table II. Film characteristics of polyleucine and sarcosyl polypeptides at the air/water interface

Polymer	A_δ Å²/res.	$A_{\Pi\to 0}$ Å²/res.	A_μ Å²/res.	$\mu(c)$ mD	A_η Å²/res.	Π_η dyn./cm.
Poly-L-leucine	17.0	17.6	17.5	158	28.0	0.4
Poly-DL-leucine	19.0	19.6				
Copoly-3 : 1-(L-leucine, sarcosine)	18.0	20.3	18.0	176	14.5	10.5
Copoly-1 : 1-(L-leucine, sarcosine)	11.5	14.0	19.0	152	5.5	19.0
Copoly-1 : 3-(L-leucine, sarcosine)	4.4	6.5		80*	no viscosity	

* Surface moment at 20 Å² per residue.

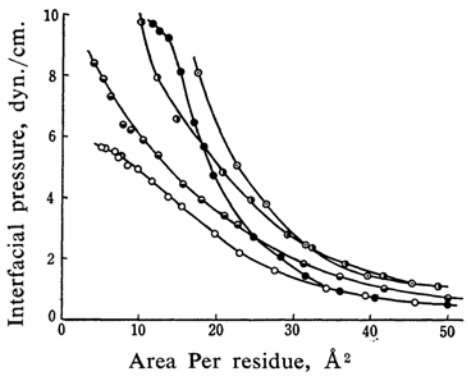

Fig. 5. Interfacial pressure-area curves of copolymers of L-leucine with sarcosine at the petroleum ether/distilled water interface at 17°C: ●, poly-L-leucine; ⊙, copoly-3 : 1-(L-leucine, sarcosine); ◑, copoly-1 : 1-(L-leucine, sarcosine); ◒, copoly-1 : 3-(L-leucine, sarcosine); ○, polysarcosine (15°C).

could not be detected. The low A_s and surface moment values suggest that the polymer chain is partially dissolved in the aqueous subphase.

The interfacial pressure-area (Π_t-A) curves of poly-L-leucine and the copolymers of L-leucine with sarcosine (3 : 1, 1 : 1, 1 : 3) at the oil/distilled water interface are shown in Fig. 5. All the films are expanded much more than at the air/water interface. The film of poly-L-leucine was found to be rather condensed in comparison with those of polysarcosine and copolymers containing the sarcosyl residue, although poly-L-leucine is also much more expanded at this interface than at the air/water interface. The compressibility of the copolymer film increased with the increasing content of the sarcosyl residue in the polymer chain; the polysarcosine film was highly compressible.

Discussion

Air/Water Interface.—*Polyleucine.*—The monolayers of poly-L-leucine and poly-DL-leucine are of the condensed type. As is shown in Table II, the A_s and $A_{\Pi\to 0}$ values for these films agree fairly well with each other, as in the case of poly-L-alanine and poly-DL-alanine,[3] whereas A_s differs to some extent from $A_{\Pi\to 0}$ with the films of sarcosyl polypeptides. The $\mu(c)$ value of poly-L-leucine agrees fairly well with that of poly-DL-alanine (150 mD[3]). The surface viscosity of poly-L-leucine is first detected at a much larger area than A_s and $A_{\Pi\to 0}$, as in the case of the poly-DL-alanine monolayer,[2,3] and the surface pressure is quite low at A_η. These facts suggest that the interaction between the monomer units or the polymer chains of polyleucine is very strong. The polypeptide chains of polyleucine are probably tightly held together, the motion of the residues or segment being strongly inhibited by the hydrogen bonding and by the partial double bond nature of peptide bonds. Judging from the data in Table II, poly-L-leucine is spread in a β-configuration, as in most polypeptide films,[1,2,3,5,6,9,10] orientating the side chains alternatively up and down in relation to the interface. Poly-DL-leucine is probably also in a β-form in the monolayer, as has been suggested by Cheesman and Davies,[9] although the area per residue is much larger than that of poly-L-leucine.

The A_s value of poly-L-leucine is 17.0 Å² per residue, while that of poly-DL-leucine is 19.0 Å² per residue. The larger area of the L-isomer may be ascribed to the closer packing of the L-polypeptide chains, as in the case of the isomers of poly-γ-benzylglutamate.[6]

Copolymers of L-Leucine with Sarcosine.—The film characteritics of poly-L-leucine are markedly changed by the incorporation of the sarcosyl residue in the polypeptide chains, as Table II shows. The films of the 3 : 1- and 1 : 1- copolymers of L-leucine with sarcosine are of the expanded type. In Fig. 6, the A_η and Π_η values of sarcosyl polyeptides are plotted against the sarcosyl residue content

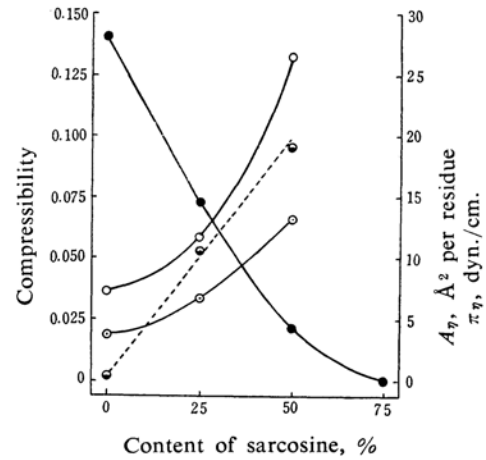

Fig. 6. Variations of compressibilites at 2 dyn./cm. (○) and 5 dyn./cm. (⊙), and of A_η (●) and Π_η (◒) with content of sarcosyl residue in polypeptide chain (at the air/distilled water interface).

5) C. W. N. Cumper and A. E. Alexander, *Trans. Faraday Soc.*, **46**, 235 (1950).
6) T. Isemura and K. Hamaguchi, This Bulletin, **27**, 125 (1954).
7) J. T. Davies, *Trans. Faraday Soc.*, **49**, 949 (1953).
8) J. T. Davies, *Biochim. Biophys. Acta*, **11**, 165 (1953).
9) D. F. Cheesman and J. T. Davies, *Adv. Protein Chem.*, **9**, 439 (1954).
10) T. Yamashita and T. Isemura, This Bulletin, **35**, 929 (1962).

in the polymer chain, together with the compressibilities of the film at 2 dyn./cm. and 5 dyn./cm. With the increase in the ratio of the sarcosyl residue in copolymers, the compressibility of the film and the Π_η value are increased, whereas the area at which the surface viscosity is first manifested is decreased.

In general, a monolayer of a polymer is of the condensed type and the surface viscosity is first observed in a region where the surface pressure is quite low as the result of strong interaction between polymer chains, while a film is of the expanded type and the surface viscosity is first detected in a high surface-pressure region. Ikeda and Isemura[2] found that the films of polypeptides are of the expanded type, and that the viscosities are detected in areas where the surface pressures are quite high. They attributed this finding to the decrease in the number of hydrogen bonds between polymer chains.

The results shown in Fig. 6 suggest that the interaction between polymer chains decreases with an increase in sarcosyl content. The number of hydrogen bonds between peptide groups is decreased when sarcosyl residues are copolymerized in the polypeptide chain because they lack the hydrogen atoms to be hydrogen-bonded in the residues. This is responsible for the weak interaction which has been observed with sarcosyl polypeptides. The flexibility of the segments or the polymer chain will be increased by the decrease in the number of hydrogen bonds and by the decrease in the double-bond nature of the main chain. The view[1-3] that the condensation and the expansion of the polypeptide films primarily depend on the hydrogen bonding between peptide groups has thus been confirmed by the present investigation.

The A_μ value of poly-L-leucine (17.5 Å^2 per residue) is nearly equal with the A_δ (17.0 Å^2 per residue). On the other hand, the film of copoly-1 : 1-(L-leucine, sarcosine) gives a much smaller A_δ value than A_μ. The A_δ and the A_μ of the 1 : 1-copolymer are 11.5 Å^2 per residue and 19.0 Å^2 per residue respectively. At the A_μ the electric dipole of the repeating unit begins to change its orientation. The sarcosyl residues of this copolymer begin to be submerged into the aqueous phase at A_μ, accompanying the decrease in surface moment. The A_δ of the 1 : 1-copolymer corresponds to the area occupied by the leucyl residues and by the sarcosyl residues remaining at the surface as a result of the effect of the hydrophobic leucyl residues. The sarcosyl residues of copoly-3 : 1-(L-leucine, sarcosine) will be more stable at the interface than those of the 1 : 1-copolymer because of the coexistence of a higher leucyl-residue content. This may be the cause of the lack of discrepancy between the A_δ and the A_μ values.

Copoly-1 : 3-(L-leucine, sarcosine) gives a somewhat condensed film. The A_δ value is quite small (4.4 Å^2 per residue). The surface moment is low compared with the other polymer films shown in Table II and decreases with compression. When the film is compressed, the sarcosyl residues of this polymer are submerged in the aqueous pahse; at A_δ, only leucyl residues remain at the surface. The A_δ value agrees closely with the area occupied by the remaining leucyl residues at the surface (A_δ of poly-L-leucine/4=4.3 Å^2 per residue).

The Oil/Water Interface. — A pronounced difference has been found[3] between the monolayers of poly-DL-alanine and polysarcosine at the oil/water interface, although the two polymers have the same side chains. The monolayer of poly-DL-alanine was rather condensed, even at the oil/water interface, while polysarcosine gave an expanded monolayer. The difference may be primarily ascribed to the presence or absence of hydrogen bonds between monomer units.

The monolayer of poly-L-leucine is of the expanded type at the oil/water interface. The film of this polypeptide is much more expanded than that of poly-DL-alanine at this interface. This expansion of the film is pbobably caused by the release of van der Waals force between side chains. The effect is much greater because the side chain is much larger than that of poly-DL-alanine.

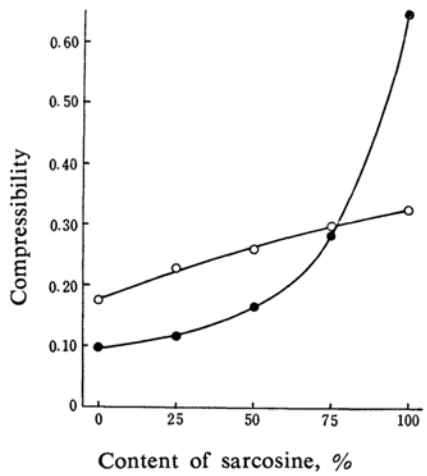

Fig. 7. Variations of compressibilities at 2 dyn./cm. (○), and 5 dyn./cm. (●) with content of sarcoyl residue in polypeptide chain (at the petroleum ether/distilled water interface).

The film of poly-L-leucine, however, is slightly more condensed than those of sarcosyl polypeptides.

Copoly-3 : 1-(L-leucine, sarcosine) gives a much more expanded film than poly-L-leucine, as well as at the air/water interface. A further increase in sarcosyl content increases the film compressibility, even at the oil/water interface, as Fig. 7 shows. This fact suggests that the polymer chain increases in flexibility as a result of the decrease in the number of hydrogen bonds between polymer chains and the decrease in the double-bond nature of the main chain.

Summary

The effect of hydrogen bonding on the nature of poly-α-amino acid monolayers has been studied at air/water and oil/water interfaces with poly-L-leucine, poly-DL-leucine, and the 3 : 1-, 1 : 1- and 1 : 3-copolymers of L-leucine with sarcosine.

Poly-L-leucine and poly-DL-leucine have been found to give condensed monolayers on distilled water. The surface viscosity of poly-L-leucine was first detected at a larger area than its close-packed area. The strong interaction between polymer chains of polyleucine was suggested by these findings.

On the other hand, at both air/water and oil/water interfaces, the compressibility of the film is increased much more by the incorporation of the sarocosyl residue in the polymer chain. At the air/water interface, the films of 3 : 1- and 1 : 1- copolymers of L-leucine with sarcosine are of the expanded type, and the surface viscosities have first been detected at much smaller areas than their close-packed areas. These findings suggest that the interaction between polymer chains diminishes because of the decrease in the number of intermolecular hydrogen bonds.

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