Surface Chemistry of the Polyamide Series. I. Effect of Hydrogen Bonding on the Nature of Poly-α-amino Acid Monolayers

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The conformation of protein is maintained mainly by the hydrogen bonding between $\rangle C{=}O$ and $H{-}N\langle$ groups of the peptide linkages. In this connection, the monolayers of poly-α-amino acids, or polypeptides as model substances of protein, have been studied by many workers.[1-11] It has been found[8,9] that the monolayers of poly-α-amino acids with nonionic side chains are condensed and that their surface viscosities appear at larger areas than the crose-packed areas when they are spread at the air/water interface. This finding has been interpreted by assuming that the polypeptide chain is rigid as a result of the

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hydrogen bonding between the peptide linkages of backbones. They are spread in a β-configration. On the other hand, the monolayers of prolyl polypeptides are of the expanded type and the appreciable surface viscosities are manifested at much smaller areas than their close-packed areas.[8,9] Their chain configurations have been considered to be flexible as a result of the weak interaction due to the decrease in the number of hydrogen bonds between peptide groups. Thus, the hydrogen bonds between $\rangle C{=}O$ and $H{-}N\langle$ groups of peptide linkages are important for understanding the nature of the monolayers of polypeptides, or proteins. Further, such a hydrogen bonding is assumed to play a major role in the stability of the configuration of polyamides of the nylon type.[11] The monolayers of nylons have been studied by some workers,[1,2,4,12-15] but the effect of the number of CH_2 groups on polyamide monolayers has never been investigated.

In the studies of this series, the effect of hydrogen bonding on the nature of the monolayers of poly-α-amino acids and polyamides of the nylon type have been studied at the air/water and oil/water interfaces in order to elucidate the role of hydrogen bonding in the configurations of the polymers and proteins.

It appears that sarcosyl polypeptides are suitable for the present study, because the sarcosyl residue (that is, the *N*-methyl glycyl residue) cannot form a hydrogen bond with a carbonyl group of another residue, as in the case of the prolyl residue, because it lacks a hydrogen atom to be hydrogen-bonded. Accordingly, in the present work, the effect of hydrogen bonding on the polypeptide monolayers has been studied at the air/water and oil/water interfaces with polysarcosine, copoly-1 : 1-(glycine, sarcosine) and copoly-1 : 1-(DL-alanine, sarcosine). For the sake of comparison, the monolayers of poly-L-alanine and poly-DL-alanine have also been studied. Further, poly-α-aminoisobutyric acid has been studied in order to investigate the steric hindrance to the hydrogen bond formation.

Experimental

Samples.—The samples used in the present study were prepared by the polymerization or copolymerization of the *N*-carboxyanhydrides of the corresponding amino acids, using sodium methoxide as an initiator, according to the usual method.[16] The average degree of polymerization was determined by end-group analysis. Poly-DL-alanine (n=40), polysarcosine (n=27), copoly-1 : 1-(glycine, sarcosine) (n=33) and copoly-1 : 1-(DL-alanine, sarcosine) (n=40) were spread from solutions in a mixed solvent, water-isopropyl alcohol (1 : 1, v/v). The spreading solvents for poly-L-alanine and poly-α-aminoisobutyric acid were trifluoroacetic acid and a mixture of dichloroacetic acid and benzene (3 : 7, v/v) respectively.

Methods.—At the air/water interface, the surface pressure was measured using surface balances of both the float and hanging-plate types. The trough used was made of polymethyl methacrylate, the rim of which was coated with purified paraffin.

The surface potential was measured by the ionizing air-electrode method, using polonium as a radiation source. The potential was detected by a Cary Model-31 vibrating reed electrometer. The accuracy was ± 3 mV. The surface moment of the film, μ, was calculated from the observed surface potential, ΔV, utilizing the Helmholtz formula, $\mu = \Delta V A/4\pi$, where A is the area per residue.

The surface viscosity was measured by the rotatory oscillation of a suspended disk on the surface of the liquid; it was calculated by the following formula;[17]

$$\eta_s = \Delta\lambda_{10}\frac{2.303\,I}{2\pi P}\left(\frac{1}{a^2}-\frac{1}{b^2}\right)$$

where I is the moment of inertia of the disk; P, the period of oscillation; $\Delta\lambda_{10}$, the difference between logarithm decrements of oscillation in the presence of film and in its absence; a, the radius of the disk, and b, the radius of the film surrounding the oscillation disk. In the apparatus used in the present experiments, I was 30.25 g. cm.; P, 13.06 sec.; a, 1.00 cm., and b, 4.00 cm.

The compression of the film at the air/water interface was started 20 minutes after the polymer was spread; the film was compressed at the rate of 12 cm^2/min.

At the oil/water interface, the interfacial pressure was measured by the ring method. The interfacial concentration of the polymer was changed by the successive injection method, corrected by the Thomas theoretical correction formula.[10,18,19] The diameter of the ring was 3.014 cm. Petroleum ether (b. p. 85—115°C) was used as an oil phase. The interfacial tension at the interface of petroleum ether and distilled water was 49.6 dyn./cm. at 23°C. The measurement of the interfacial pressure was carried out five minutes after each injection. It was ascertained that the interfacial pressure-area curve of polyvinyl acetate agrees closely with that reported by Hotta[13] at the oil/distilled water interface.

All the measurements were performed at room temperature. The change of temperature, however, never exceeded one degree during the course of an experiment.

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Results

The surface pressure-area (Π-A), the surface moment-area (μ-A) and the surface viscosity-area (η_s-A) curves of poly-DL-alanine on distilled water are shown in Fig. 1. The Π-A curve of poly-L-alanine is also represented in the same figure. The surface viscosity of poly-DL-alanine was first detected at the area where the surface pressure is sufficiently low. The area per residue of poly-DL-alanine was much smaller than that of poly-L-alanine.

The effects of sulfuric acid on the surface pressure and surface moment of poly-DL-alanine are shown in Fig. 2. On aqueous sulfuric acid, the film was expanded and an increase in surface moment was observed.

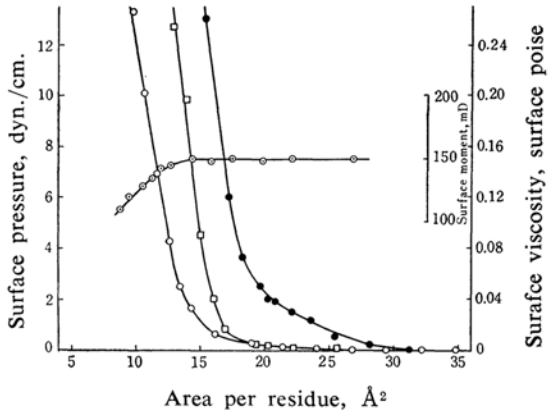

Fig. 1. Monolayers of poly-L-alanine and poly-DL-alanine on distilled water:
poly-L-alanine (15°C)—□, surface pressure
poly-DL-alanine (20°C)—○, surface pressure
⊙, surface moment; ●, surface viscosity.

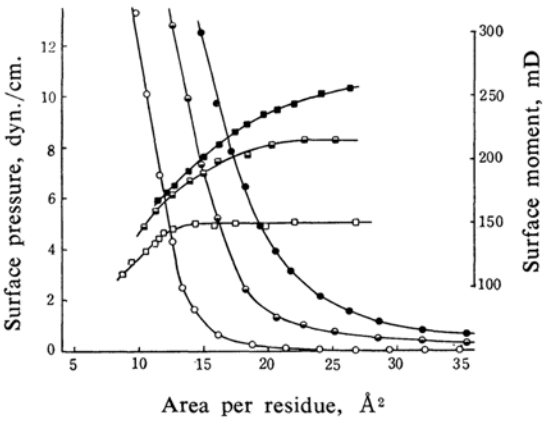

Fig. 2. Surface pressure-area and surface moment-area curves of poly-DL-alanine on distilled water (○, □) at 20°C and on the subsolutions of 3 N H_2SO_4 (◒, ◒) and 6 N H_2SO_4 (●, ■) at 20°C.

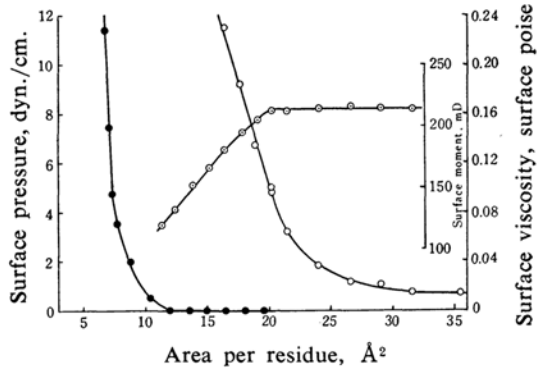

Fig. 3. Monolayer of poly-α-aminoisobutyric acid on distilled water at 17°C: ○, surface pressure; ⊙, surface moment; ●, surface viscosity.

The Π-A, μ-A and η_s-A curves of poly-α-aminoisobutyric acid on distilled water are shown in Fig. 3. The film of this polypeptide is much more expanded than that of polyalanine on distilled water, and a higher surface moment was obtained. In contrast with polyalanine, the surface viscosity was found first at a small area where the surface pressure is markedly high.

Figure 4 shows the Π-A and μ-A curves of copoly-1 : 1-(DL-alanine, sarcosine) on the 3 M potassium chloride solution. The film was of the expended type. The surface viscosity was not detected with our present apparatus because of its lower sensitivity. The surface moment was considerably lower than that of poly-DL-alanine. This might be caused by the partial dissolution of polymer chains in the aquous phase.

The film characteristics of the polymers described above are summarized in Table I, where A_δ is the area per residue at the minimum compressibility of the film; $A_{\Pi\to 0}$, the

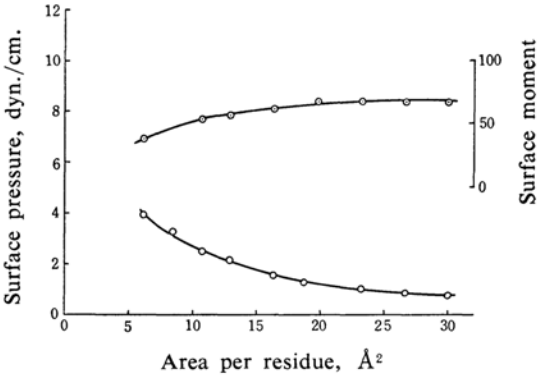

Fig. 4. Monolayer of copoly-1 : 1-(DL-alanine, sarcosine) on 3 M potassium chloride at 17°C: ○, surface pressure; ⊙ surface moment.

TABLE I. FILM CHARACTERISTICS OF POLYMERS AT THE AIR/WATER INTERFACE

Polymer	Subphase	A_δ Å²/res.	$A_{\Pi\to 0}$ Å²/res.	A_η Å²/res.	A_μ Å²/res.	μ mD
Poly-L-alanine	DW	15.0	15.9			
Poly-DL-alanine	DW	13.2	14.2	31	14.2	150 (c)
	3 N H_2SO_4	15.0	17.8		22.8	215 (c)
	6 N H_2SO_4	17.0	21.0			256 (at 26 Å²)
Poly-α-aminoisobutyric acid	DW	20.0	23.0	12	20.0	214 (c)
Copoly-1 : 1-(DL-alanine, sarcosine)	3 M KCl			No viscosity		66 (at 20 Å²)

DW: Distilled water

area per residue where the straight portion of the Π-A curve is extraporated to $\Pi=0$; A_μ, the area per residue where the surface moment, μ, begins to decrease; $\mu(c)$, the constant value of the surface moment, and A_η, the area per residue where the surface viscosity is first detected or begins to rise.

Although polysarcosine could not be spread as a monolayer at the air/water interface, even on the 3 M potassium chloride subsolution, because of its high solubility, a stable film was obtained at the oil/water interface. Figure 5 shows the interfacial pressure-area (Π_i-A) curves of polysarcosine, copoly-1 : 1-(glycine, sarcosine), and copoly-1 : 1-(DL-alanine, sarcosine), as well as that of poly-DL-alanine. The film of poly-DL-alanine was slightly expanded in a lower surface-pressure region, and above the pressure of 3 dyn./cm., the interfacial, pressure was nearly identical with the surface pressure in the same areas. The Π_i-A curve of copoly-1 : 1-(DL-alanine, sarcosine) agreed fairly well with that of polysarcosine. Both films can be considerably expanded and compressible. Copoly-1 : 1-(glycine, sarcosine) also gave an expanded film, although the area per residue was much smaller than that of polysarcosine.

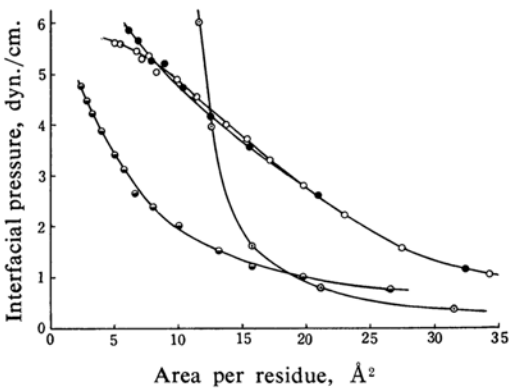

Fig. 5. Interfacial pressure-area curves of poly-DL-alanine (⊙), polysarcosine (○), copoly-1 : 1-(glycine, sarcosine) (◒) and copoly-1 : 1-(DL-alanine sarcosine) (●) at petroleum ether/distilled water interface (15°C).

Discussion

The Air/Water Interface. — *Polyalanine on Disrilled Water.* — The films of poly-L-alanine and poly-DL-alanine on distilled water are of the condensed type. The π-A curve of poly-DL-alanine is in good agreement with those reported by Isemura et al.[20] and by Glazer and Dogan,[21] and the μ-A curve, with Isemura and Ikeda's.[8] The surface viscosity of this polypeptide is first detected at a much larger area than its limiting area (A_δ or $A_{\Pi\to 0}$), as Fig. 1 and Table I show, and, accordingly, at the area where the surface pressure is sufficiently low. Similar results have been obtained by Ikeda and Isemura,[9] and by MacRitchie and Alexander.[22] Generally, a monolayer of the condensed type exibits such a suface viscosity-surface pressure relation.[9] In the monolayer of polyalanine, polypeptide chains are probably held together tightly, and the motion of residues or segments is strongly inhibited by the hydrogen bonding and by the partial double-bond nature of the peptide bonds.

It has been generally accepted[1-10] from the measurements of surface pressure, surface potential (or surface moment) and surface viscosity that on the aqueous sufaces poly-α-amino acids with nonionic side chains are spread in a β-configuration, with an alternation of side chains up and down to the surface. The data shown in Table I suggest that such a configuration is also plausible for the monolayers of poly-L-alanine and poly-DL-alanine.

It has been found[3] that the area per residue of the film of poly-γ-benzyl-L-glutamate is much smaller than that of its DL-isomers.

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Such a difference in area is to be ascribed to the much closer packing of L-polypeptide chains. On the contrary, poly-DL-alanine occupied a much smaller area in the film than poly-L-alanine. The A_δ value of the former is 13.2 Å^2 per residue, much smaller than the area calculated for the film in the β-form, (ca. 15 Å per residue), while that of the latter is 15.0 Å^2 per residue. As is well known, poly-DL-alanine is the only water-soluble polypeptide with nonpolar side chains, whereas poly-L-alanine is insoluble in almost all the solvents. Accordingly, the fact that the area of the film of poly-DL-alanine is smaller than that of its L-isomer may be ascribed to the greater solubility of the former film in the aqueous subphase. In the area, A_δ, a part of the alanyl residues of the DL-isomer might be already submerged into the aqueous subphase, even though in a β-form. The size of the side chains of polyalanine is smallest in poly-α-amino acids, and the van der Waals attractive force between side chains which contribute to the film stability will be quite weak, as will be discussed in a later section. Therefore, the smaller area of the DL-isomer, will be due to its greater solubility, caused by the partial presence of hydrogen bonds between the peptide group and the water molecule, because there may be some irregularity in the chain configuration.

The Effect of Sulfuric Acid on the Monolayer of Poly-DL-alanine.—The effect of concentrated sulfuric acid on the nylon monolayers has been studied by Crisp,[12] and by Hibberd and Alexander.[15] The expansion of films[12,15] and the increase in surface moments[15] have been observed by them on the concentrated sulfuric acid subsolutions. These findings were interpreted in terms of the weakening of hydrogen bonds between peptide groups. In the present study, as Fig. 2 and Table I show, a pronounced effect of sulfuric acid on the monolayer of poly-DL-alanine was observed on the 3 N and 6 N solutions. The expansion of the film of this polymer is caused by the increase in the flexibility of the polypeptide chain due to the breaking of hydrogen bonds, as in the case of nylon monolayers. The increase in surface moment seem to be caused by a similar reason. The breaking of hydrogen bonds will make the carbonyl groups more vertical as regards the interface, as was pointed out by Davies[5,7]; as a result, the surface moment increases.

Poly-α-aminoisobutyric Acid.—The monolayer of this polypeptide on distilled water is much more expanded than that of polyalanine. The shape of the Π-A curve is similar to those of poly-DL-alanine on the sulfuric acid subsolutions. As Table I shows, the discrepancy between the areas at A_δ and $A_{\Pi\to 0}$ is much greater than those of poly-L- and -DL-alanine on distilled water, as in the case of poly-DL-alanine on the acid subsolutions. In contrast with poly-DL-alanine on distilled water, the $\mu(c)$ value is quite high (214 mD), and the surface viscosity is first detected at a far smaller area than A_δ or $A_{\Pi\to 0}$ and, consequently, at the area where the surface pressure is sufficiently high.

In general, the monolayers of high polymers are of the condensed type under a strong interaction between constituent monomer units, while of the expanded type under a weak interaction.[8,9] The facts shown above indicate that the interaction between the monomer units or the polymer chains of poly-α-aminoisobutyric acid is very weak.

The steric hindrance to the hydrogen bond formation between peptide bonds has been studied with nylon monolayers by Hibberd and Alexander.[15] They found that nylon obtained from the condensation of sebacic acid with $\alpha:\alpha:\alpha':\alpha'$-tetramethyl tetramethylenediamine (Nylon TeMe 410) gives an expanded monolayer with no rigidity, while Nylons 210, 410 and 610 cause condensed monolayers. The anomaly of the film of Nylon TeMe 410 must arise from the considerable steric hindrance to the –CO·NH– groups afforded by the four α-substituted methyl groups. Poly-α-aminoisobutyric acid possesses two α-substituted methyl groups. Therefore, the steric hindrance to peptides bonds must be quite great, as in the case of Nylon TeMe 410. The expanded monolayer with a low surface viscosity can be obtained with this polypeptide because the formation of the hydrogen bonds between peptide groups is hindered. The higher value of the constant surface moment (214 mD) here than that of poly-DL-alanine on distilled water (150 mD) also suggests that there are few hydrogen bonds, as in the case of poly-DL-alanine film on the acid subsolutions.

Copoly-1 : 1-(DL-alanine, sarcosine).—This copolymer gives an expanded film on the 3 M potassium chloride solution, one with no viscosity. The sarcosyl residue has no hydrogen atom to be hydrogen-bonded like the prolyl residue in the polypeptide chain. The nature of the film observed is probably caused by the increase in the flexibility of the polymer chain due to the decreases in the number of hydrogen bonds between monomer units and in the double-bond nature of main chain. It has been found[8,9] that the films of prolyl polypeptides are of the expanded type and that their surface viscosities are observed at small areas per residue where the surface pressures are quite

high in contrast with those of nonionic polypeptides. This fact has been ascribed to the decrease in the number of hydrogen bonds between peptide linkages due to the presence of prolyl residues, and it is in good agreement with the results of our present investigation.

The Oil/Water Interface.—At the oil/water interface, the film of poly-DL-alanine was expanded slightly in the lower-pressure region, and under a higher pressure the Π_t-A curve was found to be nearly identical with the Π-A curve. The expansion of the film at this interface is much less than in the case of poly-DL-phenylalanine.[10] Poly-DL-alanine is a polypeptide with the smallest side chain. Therefore, the van der Waals attraction between side chains is rather weak. This is probably responsible for the slight expansion of the film of this polypeptide at the oil/water interface.

The behavior of the monolayer of polysarcosine is markedly different from that of poly-DL-alanine, even though these polymers have the same side chains. The film of polysarcosine is rather expanded and compressible. Methyl groups attach to nitrogen atoms of the polymer chain in polysarcosine, but to α-carbon atoms in poly-DL-alanine. Evidently, the difference between these polymer films is to be ascribed to the absence or presence of hydrogen bonds between monomer units. The absence of the hydrogen bonds makes the polymer chains more flexible. Consequently, the monolayer of polysarcosine is considerably expanded and conpresible. The lack of a double-bond nature in polymer chain might also be responsible for the expansion of the polysarcosine film. On the other hand, the film of poly-DL-alanine is rather condensed, even at the oil/water interface, because its polypeptide chains are held together rigidly by the hydrogen bonds and by the partial double-bond nature of the peptide bonds.

Copoly-1 : 1-(DL-alanine, sarcosine) gives a Π_t-A curve which is nearly identical with that of polysarcosine. The monolayer of copoly-1 : 1-(glycine, sarcosine) is also of the expanded type, although the area occupied per residue is much smaller. The effect of hydrogen bonding is small with these polymers because of the presence of sarcosyl residues.

Summary

The effect of hydrogen bonding on the nature of poly-α-amino acid monolayers has been studied at the air/water and oil/water interfaces with poly-L-alanine, poly-DL-alanine, polysarcosine, copoly-1 : 1-(glycine, sarcosine), copoly-1 : 1-(DL-alanine, sarcosine) and poly-α-aminoisobutyric acid.

Poly-L-alanine and poly-DL-alanine gave monolayers of the condensed type on distilled water, and the surface viscosity of poly-DL-alanine was first detected at a much larger area than its close-packed area where the surface pressure was sufficiently low. This fact suggests that there exists a strong interaction between peptide bonds of polyalanine. Both polymers would assume a β-configration. On the concentrated sulfuric acid subsolutions, the film of poly-DL-alanine was expanded and an increase in surface moment was observed because of the breaking of hydrogen bonds between peptide linkages.

A steric hindrance to the hydrogen bond formation was observed with the film of poly-α-amino isobutyric acid. This polypeptide gave an expanded monolayer on distilled water, and its surface viscosity could be first detected at a very small area where the surface pressure was remarkably high.

The film of copoly-1 : 1-(DL-alanine, sarcosine) was of the expanded type on the 3 M potassium chloride subsolution because of the decrease in the number of hydrogen bonds and that in the double-bond nature of the main chain.

At the oil/distilled water interface, a marked difference was found between the monolayers of poly-DL-alanine and polysarcosine, although these polymers have the same side chains. Poly-DL-alanine gave a considerably condensed monolayer, even at the oil/water interface. On the other hand, the film of polysarcosine was of the expanded type. The difference between the films of these polymers is mainly to be ascribed to the presence or the absence of hydrogen bonds. The 1 : 1-copolymer of sarcosine with glycine or DL-alanine also gave an expanded monolayer.

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