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By Takuya YAMASHITA

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Surface Chemistry of Polyamide Series.

By Takuya YAMASHITA

I. Effect of Hydrogen Bonding on the Nature of Poly-α-amino Acid Monolayers

The conformation of protein is maintained mainly by the hydrogen bonding between $>C=O$ and $H-N<$ 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 that the monolayers of poly-α-amino acids with nonionic side chains are condensed and their surface viscosities appear at larger areas than the close-packed areas when they are spread at air/water interface. This finding was interpreted by assuming that the polypeptide chain is rigid owing to the hydrogen bonds between peptide linkages of backbones. They

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1) C.W.N. Cumper and A.E. Alexander, Trans. Faraday Soc., 46, 235 (1950).
2) T. Isemura and K. Hamaguchi, Bull. Chem. Soc. Japan, 25, 40 (1952)
3) T. Isemura and K. Hamaguchi, ibid., 26, 424 (1953).
4) T. Isemura and K. Hamaguchi, ibid., 27, 125 (1954).
5) J.T. Davies, Trans. Faraday Soc., 49, 949 (1953).
6) J.T. Davies, Biochim. Biophys. Acta, 11, 165 (1953).
7) D.F. Cheesman and J.T. Davies, Adv. Protein Chem., 9, 439 (1954).
8) T. Isemura and S. Ikeda, Bull. Chem. Soc. Japan, 32, 178 (1959)
9) S. Ikeda and T. Isemura, ibid., 32, 659 (1959).
10) T. Yamashita and T. Isemura, ibid., 35, 929 (1962).
11) Y. Kinoshita, Macromol. Chem., 33, 1 (1959).

are spread in β-configuration. On the other hand, the monolayers of prolyl polypeptides were of expanded type and the appreciable surface viscosities were manifested at much less areas than their close-packed areas[8,9]. Their chain configurations were considered to be flexible owing to the weak interaction due to the decrease in number of hydrogen bonds between peptide groups. Thus, the hydrogen bonds between >C=O and H-N< groups of peptide linkages are important for understanding the nature of 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 nylon type[11]. The monolayers of nylons have been studied by some workers[1,2,4,12~15], but the effect of the numbers of CH_2 groups on polyamide monolayers has never been investigated.

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

It appears that sarcosyl polypeptides are suitable for the present study, because sarcosyl residue (namely, N-methyl glycyl) residue cannot form hydrogen bond with carbonyl group of other residue as in the case of prolyl residue owing to

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12) D.J. Crisp, J. Colloid Sci., 1, 161 (1946).

13) H. Hotta, ibid., 9, 504 (1954).

14) K. Inokuchi, Bull. Chem. Soc. Japan, 29, 490 (1956).

15) G.E. Hibberd and A.E. Alexander, Proc. 3rd Int. Cong. Surface Activity, 2, 144 (1960).

its lacking of hydrogen atom to be hydrogen bonded. Accordingly, in the present work, the effect of hydrogen bonding on the polypeptide monolayers has been studied at 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-amino-isobutyric acid was 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 corresponding amino acids using sodium methoxide as an initiator according to the usual method[16]. The average degree of polymerization was determined by the end group analysis. Polysarcosine (n=27), copoly-1:1-(glycine, sarcosine) (n=33) and copoly-1:1-(DL-alanine, sarcosine) (n=40) were spread from the solution in a mixed solvent, water-isopropyl alcohol (1:1, v/v). The spreading solvents for poly-L-alanine, and poly-α-amino-isobutyric acid were trifluoroacetic acid, and a mixture of dichloroacetic acid and benzene (3:7, v/v), respectively.

Methods. ----- At air/water interface, the surface pressure was measured using surface balances of both 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 radioactive air-electrode method using polonium as a radiation source.

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16) E. Katchalski and M. Sela, Adv. Protein Chem., 13, 243 (1958).

The potential was detected by Cary Model-31 vibrating read electrometer. The accuracy was ± 1 mV. The surface moment of the film, μ was calculated from the observed surface potential, ΔV utilizing the Helmholz 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 liquid, and 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 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 air/water interface was started 20 minutes after spreading the polymer and the film was compressed at the rate of 12 cm.^{2}/min.

At 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, being corrected by the Thomas theoretical correction formula[10,18,19].

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17) L.G. Fourt and W.D. Harkins, J. Phys. Chem., 42, 897 (1938).

18) A.G. Thomas, Nature, 179, 776 (1957).

19) E.G. Cockbain, K.J. Day and A.I. McMullen, Proc. 2nd Int. Cong. Surface Activity, 1, 56 (1957).

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. per 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].

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

Results

The surface pressure-area (Π-A), surface moment-area (μ-A) and 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 surface pressure is sufficiently low. The area per residue of poly-DL-alanine was somewhat less 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 the increase in surface moment was observed.

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 polyalanine on distilled water and the higher surface moment was obtained.

In contrast with polyalanine the surface viscosity was found first at small area where surface pressure was markedly high.

Fig. 4 shows the π-A and μ-A curves of copoly-1:1-(DL-alanine, sarcosine) on 3 M potassium chloride solution. The monolayer was considerably expanded. The surface viscosity was not detected with our present apparatus owing to the less sensitivity.

The film characteristics of polypeptides described above are summarized in Table I, where A_s is the area per residue at the minimum compressibility of film, $A_{\pi \to 0}$ the area where the straight portion of π-A curve is extraporated to $\pi = 0$, A_μ the area where the surface moment, μ begins to decrease, μ(c) the constant value of surface moment, and A_η the area where the surface viscosity is first detected or begins to rise.

Table I. Film characteristics of polypeptides at air/water interface

Polypeptide	Subphase	A_s 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	14.0	15.2	31	13.5	150(c)
	3 N H_2SO_4	16.0	17.8		22.8	215(c)
	6 N H_2SO_4	17.0	21.0		26.3	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.

Although polysarcosine could not be spread as a monolayer even on 3M potassium chloride solution because of its high solubility, a stable film was obtained at oil/water interface. Fig. 5 shows the interfacial pressure-area (π_i-A) curves of polysarcosine, copoly-1:1-(glycine, sarcosine), copoly-1:1-(DL-alanine, sarcosine) as well as poly-DL-alanine. Poly-DL-alanine was slightly expanded in lower pressure region, and under higher surface pressure, the interfacial pressure was identical with the surface pressure at air/water interface. The π_i -A curve of copoly-1:1-(DL-alanine, sarcosine) was nearly identical with that of polysarcosine. The films were considerably expanded. Copoly-1:1-(glycine, sarcosine) also gave an expanded monolayer, although the area was much less.

Discussion

Air/water interface. ------ Polyalanine on distilled water. ----- The π -A and μ-A (or ΔV-A) curves of poly-DL-alanine have already been reported by several workers[8,6,20]. Our results are in good agreement with Isemura and Ikeda's[8]. The surface viscosity of this polypeptide has been measured by Ikeda and Isemura[9] and MacRitchie and Alexander[21]. They found that the surface viscosity is first detected at the area where surface pressure is sufficiently low. The similar result was also obtained in the present study. It has been

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20) J. Glazer and M.Z. Dogan, Trans. Faraday Soc., 49, 448 (1953).

21) F. MacRitchie and A.E. Alexander, J. Colloid Sci., 16, 57 (1961).

pointed out[9] that the monolayer of poly-DL-alanine is much more condensed than some vinyl polymers with side chains of the same order of length, such as polyvinyl acetate and polyethyl acrylate. This is attributed to the fact that the polypeptide chains are held together rigidly by the interchain hydrogen bonds. The surface viscosity of poly-DL-alanine also suggests that there exists a strong interaction between polypeptide chains.

It has been generally accepted[1~10] from the surface pressure, surface potential and surface viscosity measurements that on aqueous surfaces poly-α-amino acids with nonionic side chains such as poly-DL-alanine and poly-DL-phenylalanine are spread in β-configuration with the alternation of side chains up and down to the surface. The data in Table I suggest that such a configuration is plausible for poly-DL-alanine. Poly-L-alanine appears also to be spread in β-form from the fact that the A_δ value is 15.0 A^2/residue. A_δ is considered to correspond to the close-packed area in protein monolayers[22].

It is found that the area per residue of poly-γ-benzyl-L-glutamate[3] is much less than its DL-isomer. This difference is ascribed to the fact that L-polypeptide chains pack more closely than DL-isomer. In contrast with this fact, poly-DL-alanine occupied somewhat less area than poly-L-alanine. As well known, poly-DL-alanine is the only water-soluble polypeptide with nonpolar side chain. On the other hand, poly-L-alanine is insoluble in almost all the solvents. Then, the less area of poly-DL-alanine than poly-L-alanine

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22) H.B. Bull, Adv. Protein Chem., 3, 95 (1947).

might be ascribed to the partial dissolution of polypeptide chain with compression. The hydrogen bonding between peptide bonds and van der Waals force between side chains are responsible for the stability of polypeptide monolayers. The hydrogen bonds between peptide bonds of poly-DL-alanine seem to be much weaker, because it has irregurality in its configuration and the hydrogen bonds with water molecules are present. Further, the van der Waals attraction between side chains of polyalanine is much less than those of other polypeptides owing to its small size of side chains. This is proved from the fact the π -A curve of this polypeptide is little affected at oil/water interface as shown in Fig. 5 in contrast with poly-DL-phenylalanine[1,10]. Thus, the less area of poly-DL-alanine than poly-L-alanine would be caused by the less stability of film due to the presence of hydrogen bonds between peptide bond and water molecule, and by its small size of side chains.

Effect of sulfuric acid on poly-DL-alanine monolayer. ----- The effect of concentrated sulfuric acid on nylon monolayers has been studied by Crisp[12], and by Hibberd and Alexander[14]. The expansion of films and increase in surface moments have been observed on concentrated sulfuric acid solutions owing to the breaking of hydrogen bonds between peptide groups. In the present study, as shown in Fig. 2 and Table I, the pronounced effect was observed with poly-DL-alanine on 3 N and 6 N sulfuric acid solutions. The expansion of the film would be caused by the increase in flexibility of the polypeptide chain due to the breaking of hydrogen bonds

between peptide bonds as in the case of nylon monolayers. The increase in surface moment would also be due to the breaking of hydrogen bonds, which would make the carbonyl group more vertical to the interface as pointed by Davies[5,7])

Poly-α-aminoisobutyric acid.----- The monolayer of this polypeptide is much more expanded than that of poly-alanine. The shape of the π-A curve is resemble those of poly-DL-alanine on sulfuric acid solutions. In contrast with poly-DL-alanine on distilled water, the $\mu(\alpha)$ value (214 mD) is much higher, and the surface viscosity is detected at far less area than A_δ or $A_{\pi\to 0}$ where the surface pressure is sufficiently high. The discrepancy between A_δ and $A_{\pi\to 0}$ is great as well as poly-DL-alanine on acid solutions compared with those of poly-L- and DL-alanine on distilled water.

In general, the monolayers of high polymers are of condensed type under the strong interaction between constituent monomer units, while of expanded type under the weak interaction. The fact shown with poly-α-aminoisobutyric acid indicates that the interaction between monomer units is very weak.

The effect of steric hindrance on the peptide bonds has been studied with nylon monolayers by Hibberd and Alexander. It was found that nylon obtained from condensation of sebatic acid with α:α:α':α'-, tetramethyl tetramethylenediamine (Nylon TeMe 410) gave an expanded monolayer possessing no rigidity, while Nylons 210, 410 and 610 caused the condensed monolayers. The anomaly 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. Thus, the steric hindrance to peptide bonds must be remarkable as in the case of Nylon TeMe 410. The formation of the hydrogen bonds between peptide groups would be hindered. Accordingly, the expanded monolayer with low surface viscosity would be obtained with this polypeptide. The higher value of constant surface moment (214 mD) than that of poly-DL-alanine on distilled water (150 mD) also suggests that the number of hydrogen bonds is little as in the case of poly-DL-alanine film on aicd.

Copoly-1:1-(DL-alanine, sarcosine). ----- This copolypeptide gives an expanded monolayer on 3 M potassium chloride solution possessing no viscosity. The spreading, however, is incomplete owing to its high solubility. Sarcosyl residue has no hydrogen atom to be hydrogen bonded as prolyl residue in polypeptide chain. The nature of film observed ~~this polymer~~ should be caused by the increase in flexibility due to the decreased number of hydrogen bonds. It has been found[8,9] that the films of prolyl polypeptides are of expanded type and their surface viscosities are observed at small areas per residue where the surface pressures are considerably high in contrast with nonionic polypeptides. This fact was attributed to the decrease in the number of hydrogen bonds between peptide linkages in these polypeptides, and is in good agreement with our present investigation.

Oil/water interface. ----- Poly-DL-alanine expanded slightly in lower surface pressure region at oil/water

interface, and ~~at~~ under higher surface pressure the π_i-A curve was found to be identical with π-A curve. The expansion of the film at oil/water interface is much less than in the case of poly-DL-phenylalanine[10]. Poly-DL-alanine is the polypeptide with the smallest side chain. The van der Waals attraction between side chains is rather weaker. This would be responsible for the condensed nature of this polypeptide at 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 expanded and compressible. Methyl groups attach to nitrogen atoms of polymer chain in polysarcosine, while to α-carbon atoms in poly-DL-alanine. Therefore, it is evident that the difference of these polymer films will be ascribed to the absence and presence of hydrogen bonds between peptide groups. The absence of hydrogen bonds makes the polymer chains more flexible. Consequently, the monolayer of polysarcosine becomes highly expanded and compressible. On the other hand, the film of poly-DL-alanine is condensed even at oil/water interface, because its polypeptide chains are held together rigidly by hydrogen bond and its side chains are small.

Copoly-1:1-(DL-alanine, sarcosine) gives the nearly identical π_i-A curve with polysarcosine. The monolayer of copoly-1:1-(glycine, sarcosine) is also of an expanded type, although the area occupied per residue is much less. The effect of hydrogen bonding is scace with these polymers owing

to the presence of sarcosyl residue.

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

The steric hindrance to hydrogen bond formation was found with poly-α-aminoisobutyric acid. This polypeptide gave an expanded monolayer on distilled water and its surface viscosity could be first detected at very small area where surface pressure was high.

The monolayer of copoly-1:1-(DL-alanine, sarcosine) was of expanded type on 3 M potassium chloride solution and its surface viscosity was not detected owing to the decrease in number of hydrogen bonds.

At oil/distilled water interface, the marked difference

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

The author wishes to express his gratitude to Prof. Toshizo Isemura of Osaka University for his kind guidance throughout the present work. He is also indebted to Prof. Junzo Noguchi of Hokkaido University for preparing the polypeptides, and to Dr. Sanshiro Kume of Osaka University for making the ionizing air-electrode.

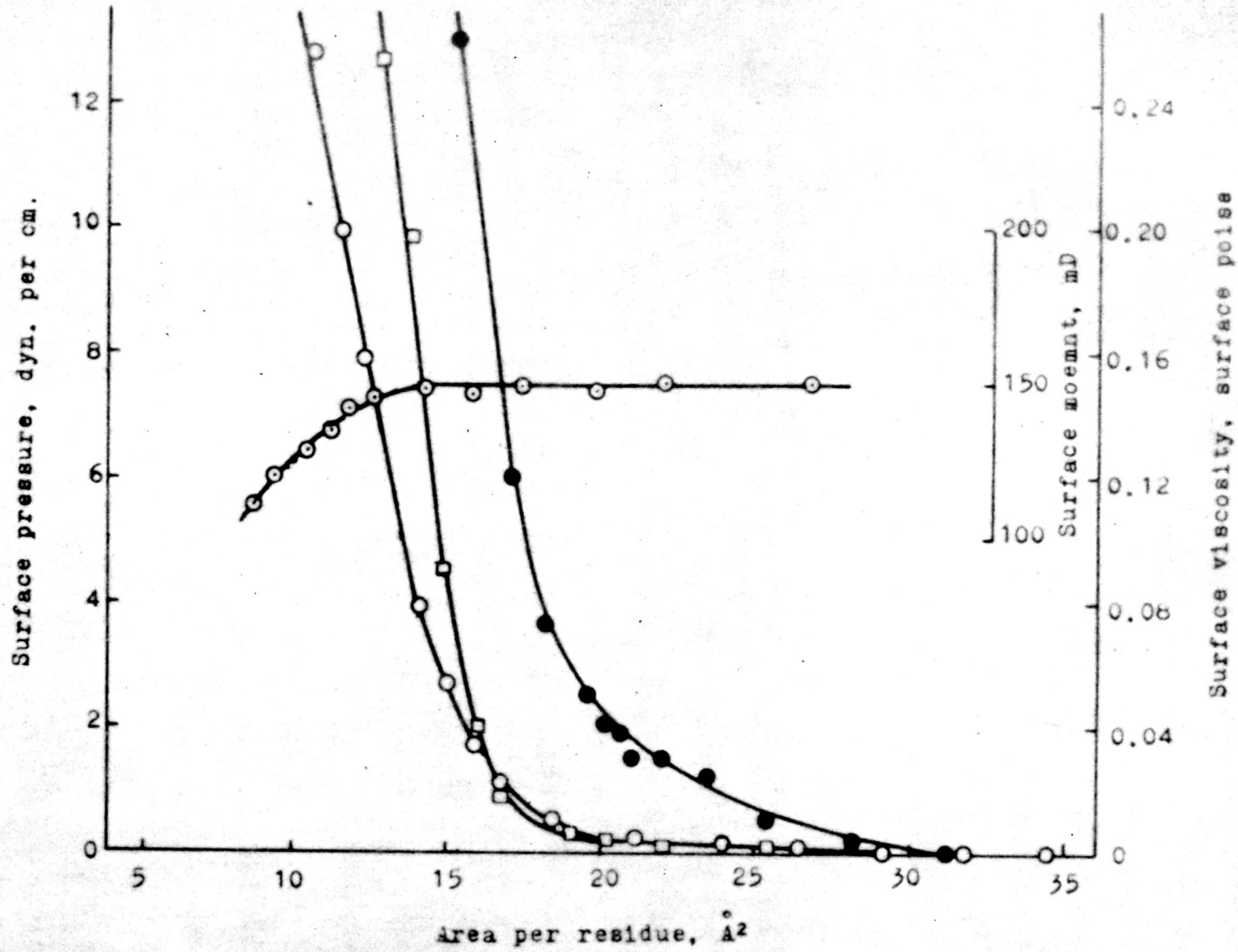

Fig. 1. Monolayers of poly-L-alanine and poly-DL-alanine on distilled water: poly-L-alanine (15°C) — □, surface pressure: poly-DL-alanine (19°C) — ○, surface pressure; ⊙, surface moment; ●, surface viscosity.

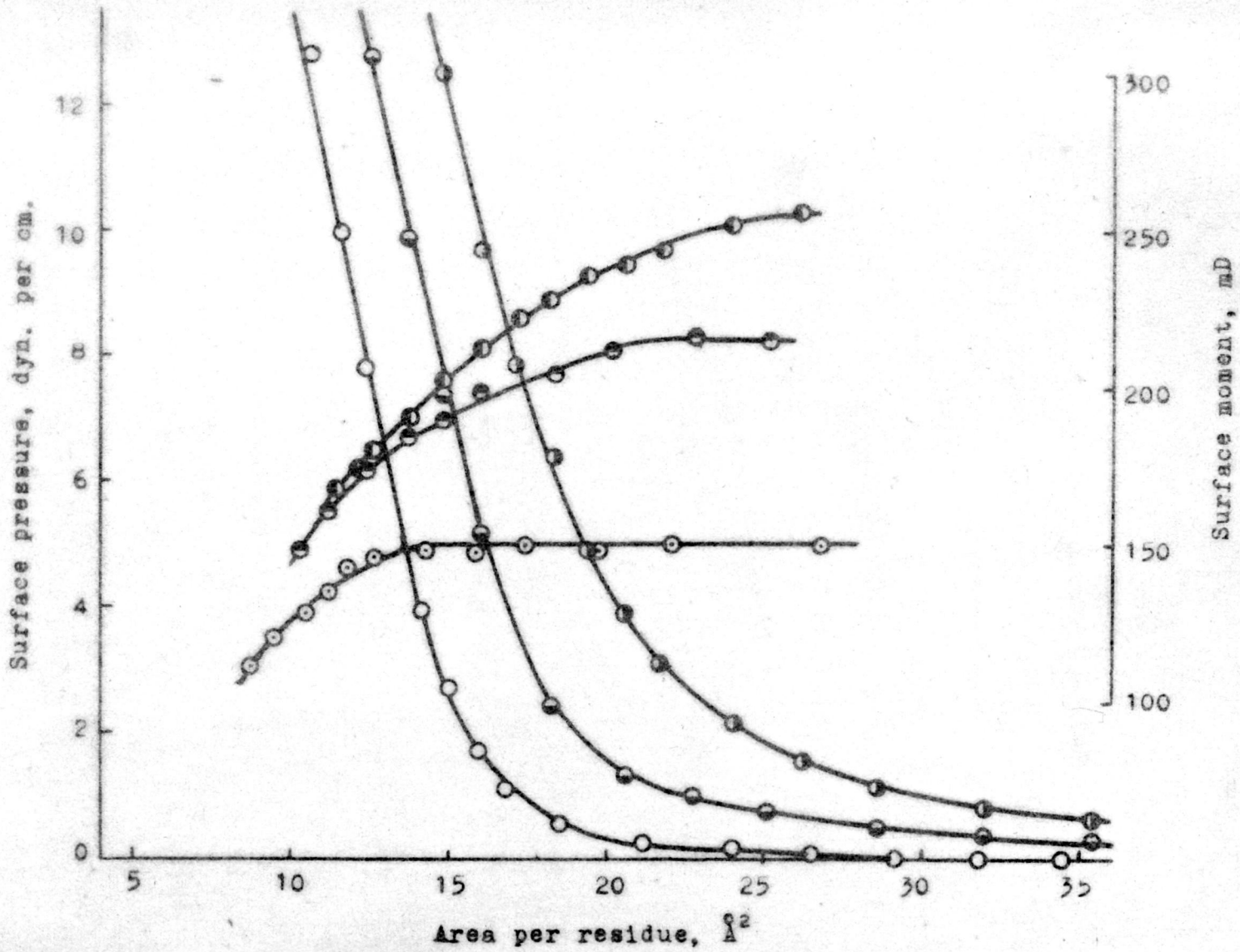

Fig. 2. Surface pressure-area and surface moment-area curves of poly-DL-alanine on distilled water (○ , ⊙) and on the subsolutions of 3 N H_2SO_4 (◒ , ●) and 6 N H_2SO_4 (◑ , ◐) at 20° C.

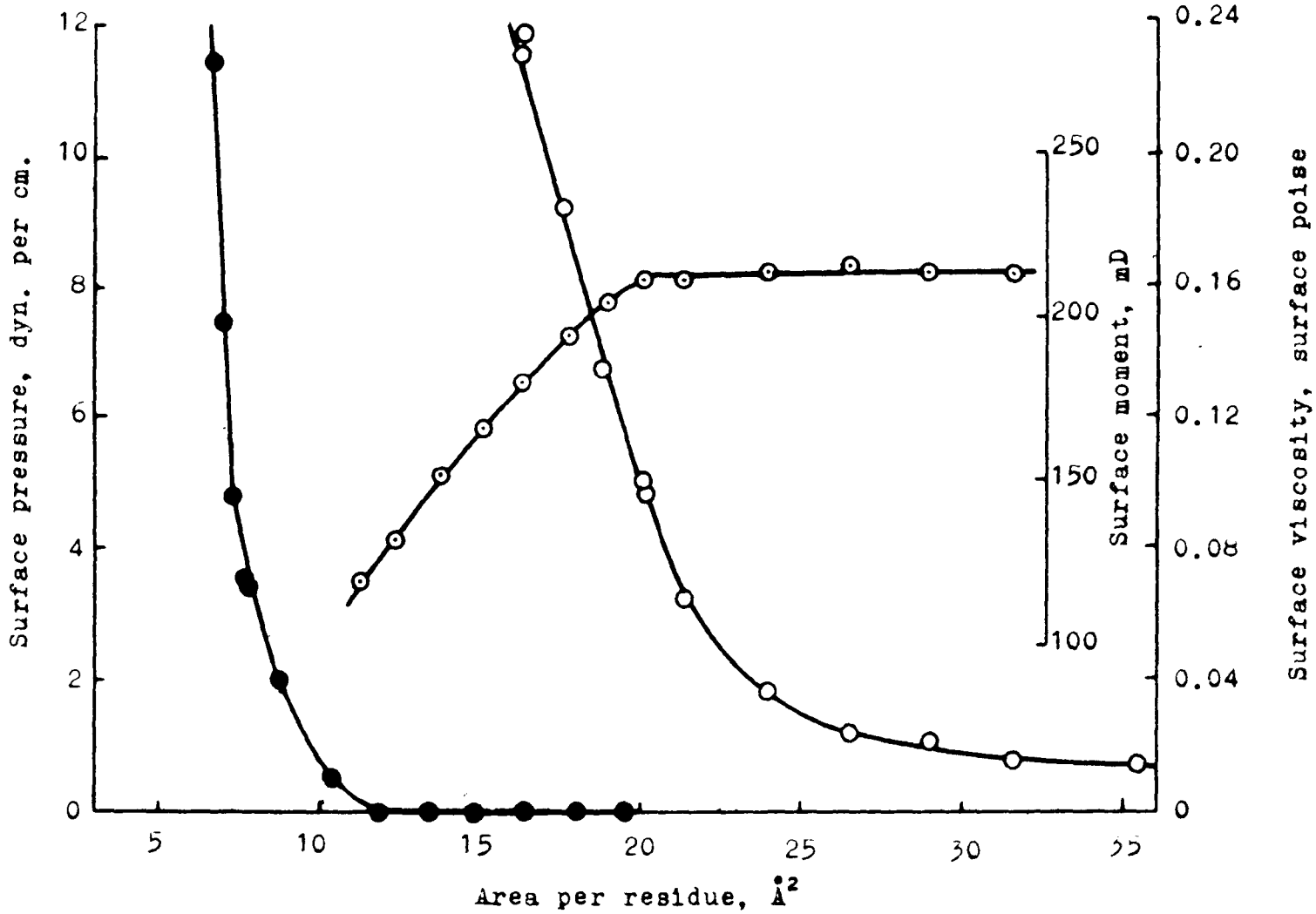

Fig. 3. Monolayer of poly-α-aminoisobutyric acid on distilled water at 17°C: ○ , surface pressure; ⊙ , surface moment; ● , surface viscosity.

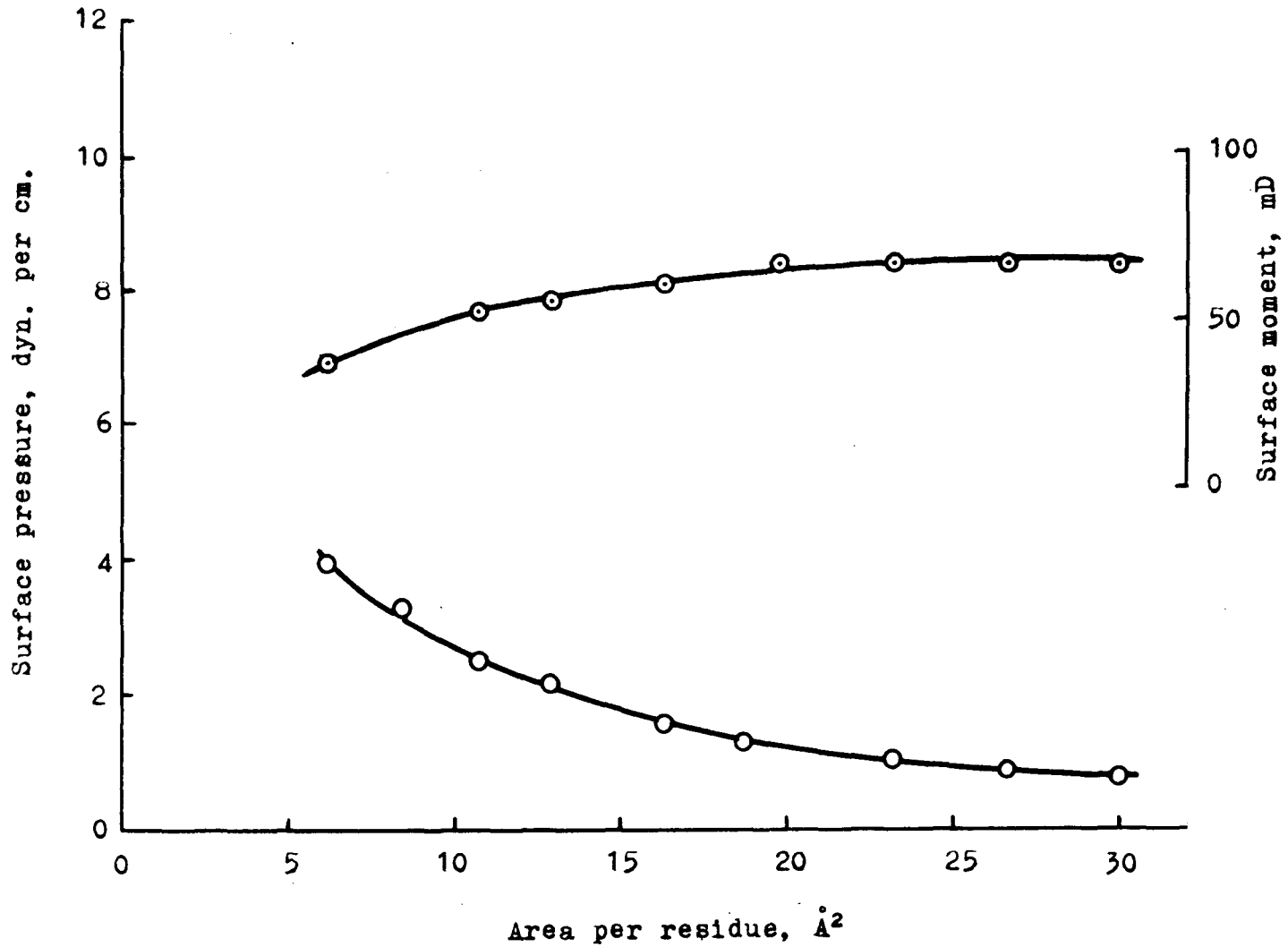

Fig. 4. Monolayer of copoly-1:1-(DL-alanine, sarcosine) on 3 M Kcl at 17°C: ○ , surface pressure; ⊙ , surface moment.

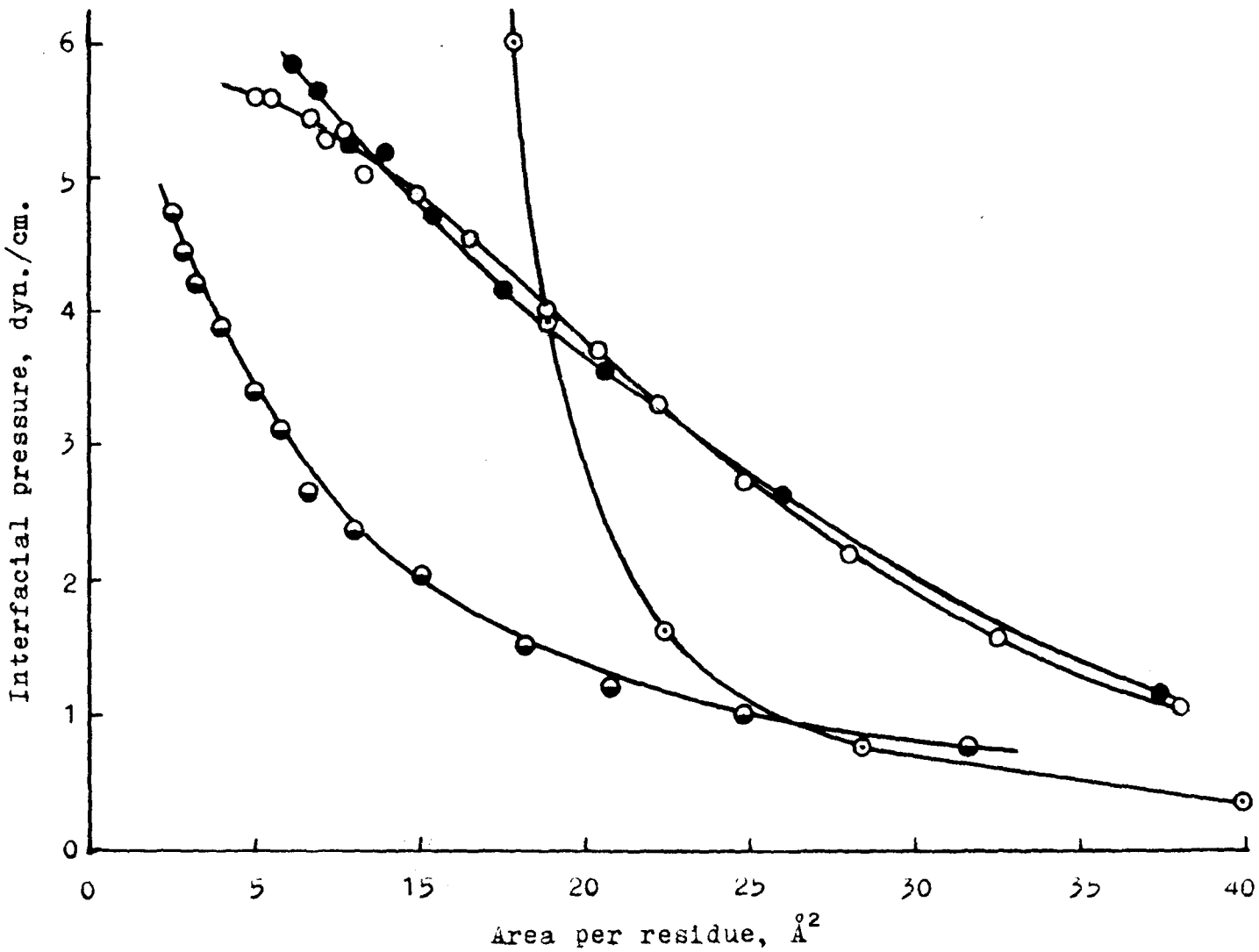

Fig. 5. Interfacial pressure-area curves of poly-*DL*-alanine (⊙), polysarcosine (○), copoly-1:1-(glycine, sarcosine) (◒) and copoly-1:1-(DL-alanine, sarcosine) (●) at petroleum ether/distilled water interface (15° C).

II. Effect of Sarcosyl Residue on the Monolayer of Polyleucine

The roles of prolyl residue in polypeptide monolayers have been studied by Isemura and Ikeda[1,2]. The surface viscosity of poly-DL-alanine monolayer which is of 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 owing to the hydrogen bonds between peptide groups. On the other hand, the monolayers of prolyl polypeptides are of expanded type and the appreciable surface viscosities are manifested at much less areas than their close-packed areas. Their chain configurations were considered to be rather flexible owing to the lack of hydrogen bonds between peptide groups.

The view that the hydrogen bonds between peptide groups affect remarkably the nature of polypeptide monolayers was supported with the studies on the monolayers of polysarcosine and of copolymer of sarcosine with DL-alanine or glycine as described in the previous paper[3].

In the present investigation, the effect of hydrogen bonding on the polypeptide monolayers has been studied at air/water and oil/water interfaces with copolypeptides of

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1) T. Isemura and S. Ikeda, Bull. Chem. Soc. Japan, 32, 178 (1959).

2) S. Ikeda and T. Isemura, ibid., 32, 659 (1959).

3) Part I of this series.

L-leucine with sarcosine of different compositions. The difference between monolayers of poly-L-leucine and its DL-isomer was also studied.

Experimental

Samples.----- Poly-L-leucine, poly-DL-leucine and polysarcosine were prepared by the polymerization of N-carboxyanhydride 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 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 end group analysis.

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4) E. Katchalski and M. Sela, Adv. Protein Chem., 13, 243 (1958).

Methods ----- Surface pressure, potential and viscosity were measured by a surface balance of float type, a radioactive air-electrode and an oscillating rotatory disk, respectively. Surface moment was calculated from surface potential using the Helmholtz equation. The interfacial pressure was measured by the ring method. The details of experimental methods have been described in the previous 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, 3:1-, 1:1- and 1:3-copolymers of L-leucine with sarcosine on distilled water are shown in Figs. 1-4, respectively. The 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 film, $A_{\pi\to 0}$ the area where straight portion of the π-A curve is extraporated to $\pi = 0$, A_μ the area where surface moment, μ begins to decrease, $\mu(c)$ the constant value of surface moment and A_η the area where viscosity begins to rise, and π_η the pressure at A_η.

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

Polymer	A_s 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	174	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	14.0	80*	no viscosity	

* Surface moment at 20 Å² per residue.

Poly-L-leucine and poly-DL-leucine gave monolayers of condensed type. The area per residue of the former at the limiting area, A_s was much less than that of the latter. The surface viscosity of poly-L-leucine was manifested at larger area than A_s and $A_{\pi 7}$.

The film characteristics of poly-L-leucine were remarkably affected by the incorporation of 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 much less area than A_s and $A_{\pi 7}$. The monolayer of copoly-1:1-(L-leucine, sarcosine) was much more compressible than those of both polymers cited above. The viscosity was first detected at very small area (5.5 $Å^2$ per residue). The film of copoly-1:3-(L-leucine, sarcosine) was somewhat condensed more than that of 1:1-copolymer, and the surface viscosity could not be detected. The low values of A_s and surface moment suggest that the polymer chain would be dissolved partially in the aqueous subphase.

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

copolymer film decreased with the increasing content of leucyl residue in polymer chain.

Discussion

Air/water interface. ---------- Polyleucine. ----- Monolayers of poly-L-leucine and poly-DL-leucine are of condensed type. As shown in Table II, the A_s and $A_{\pi\to 0}$ values for these films coincide 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 was manifested at much larger area than A_s and $A_{\pi\to 0}$ as in the case of poly-DL-alanine[2,3]. The surface pressure is very low at A_q. Then, the condensation of poly-L-leucine monolayer is ascribed to the hydrogen bonding between peptide linkages, which hold the polypeptide chains rigid. From the data in Table II, it seems that this polypeptide is spread in β-configuration on distilled water, orientating the side chains alternatively up and down to the interface as in the case of most polypeptide films if the film is closely packed[1,3,5~10].

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

6) T. Isemura and K. Hamaguchi, Bull. Chem. Soc. Japan, 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, Bull. Chem. Soc. Japan, 35, 929 (1962).

The A_δ value agrees with the close-packed area estimated using Stuart model. Poly-DL-leucine would also be spread in β-form in the monolayer.

Poly-L-leucine occupies much less area than poly-DL-leucine in the monolayer. Poly-γ-benzyl-L-glutamate has been found to occupy far less area per residue in the monolayer than its DL-isomer[7]. The difference in areas per residue between monolayers of L- and DL-isomers is ascribed to the closer packing of chains of L-polypeptide than its DL-isomer at the interface.

Copolypeptides of L-leucine with sarcosine. ----- The film characteristics of poly-L-leucine were markedly affected by the incorporation of sarcosyl residue in the polypeptide chain as shown in Table II. The film changed from condensed type to expanded type excepting copoly-1:3-(L-leucine, sarcosine), and the compressibility of the film was increased remarkably with the increase of the ratio of sarcosyl residue in copolypeptides. The area where the surface viscosity is first manifested is far less with copolypeptide film, and shifts to the small area with increasing content of sarcosine. The A_η and π_η values of sarcosyl polypeptides are plotted against the content of sarcosyl residue in the polypeptide chain in Fig. 6 together with the compressibilities at 2 dyn. per cm. and 5 dyn. per cm. The compressibility and π_η value are increased with sarcosyl content, whereas A_η value is decreased.

In general, monolayer of polymer is of condensed type and the surface viscosity is manifested in a region where

the surface pressure is very low under the strong interaction between monomer units, while the film is of expanded type and the surface viscosity is first detected in a high surface pressure region. Isemura and Ikeda[2] found that the films of prolyl polypeptides are of expanded type, and the viscosities are detected at the areas where the surface pressures are considerably high owing to the decrease in number of hydrogen bonds between peptide linkages.

The results shown in Fig. 6 suggest that the interaction between polymer chains decreases with increase in sarcosyl content. The number of hydrogen bonds between >C=O and H-N< groups is decreased when sarcosyl residues are copolymerized in polypeptide chain because they lack hydrogen atoms to be hydrogen bonded in the residues. This would be responsible for the weak interaction which has been observed with sarcosyl polypeptides. The view[1,2] that the condensation and expansion of the polypeptide films depend on the hydrogen bonding between peptide groups has been confirmed by the present investigation.

The A_{μ} value (17.6 $\mathring{A}^2$ per residue) is nearly equal with A_{δ} (17.0 $\mathring{A}^2$ per residue) in poly-L-leucine film. On the other hand, copoly-1:1-(L-leucine, sarcosine) has much less A_{δ} value than A_{μ}. The A_{μ} and A_{δ} of 1:1-copolymer are 19.0 $\mathring{A}^2$ per residue and 11.5 $\mathring{A}^2$ per residue, respectively. At A_{μ}, the electric dipole of repeating unit begins to change its orientation. The sarcosyl residues of this copolypeptide begins to get into aqueous phase accompanying the decrease in surface moment at A_{μ}. The A_{δ} of 1:1-copolymer would correspond to the area occupied by leucyl residue and by remaining sarcosyl

residue at the surface owing to the effect of the hydrophobic leucyl residue. The sarcosyl residue of copoly-3:1-(L-leucine, sarcosine) seems to be more stable at the interface than those of 1:1-copolymer owing to the coexistence of higher content of hydrophobic leucyl residue. This is the cause of no discrepancy between A_δ and A_μ values.

Copoly-1:3-(L-leucine, sarcosine) gives film with condensed nature. The A_δ value is very small (4.4 $Å^2$ per residue). The surface moment is low compared with other polymer films shown in Table II and decreased with compression. By compressing the film, sarcosyl residues of this polymer would be submerged in aqueous phase, and at A_δ, only leucyl residues remain at the surface. The A_δ value agrees closely with the area occupied by remaining leucyl residues at the surface (A_δ of poly-L-leucine/4 = 4.3 $Å^2$ per residue).

<u>Oil/water interface</u>.----- The pronounced difference has been found[3)] between the monolayers of poly-DL-alanine and polysarcosine at oil/water interface, although both polymers have the same side chains. The monolayer of poly-DL-alanine was rather condensed even at oil/water interface, while polysarcosine gave an expanded monolayer. The difference was ascribed to the presence and absence of hydrogen bonds between monomer units.

The monolayer of poly-L-leucine is of expanded type at oil/water interface. The film of this polypeptide is much more expanded than that of poly-DL-alanine at this interface. The expansion should be 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. The film of poly-L-leucine, however, is slightly condensed compared with those of sarcosyl polypeptides.

Copoly-3:1-(L-leucine, sarcosine) gives much more expanded film than poly-L-leucine as well as at air/water interface. Further increase in sarcosyl content increases the film compressibility more even at oil/water interface as shown in Fig. 7. This fact suggests that the polymer chain increases its flexibility owing to the decrease in number of hydrogen bonds between peptide linkages.

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 3:1-, 1:1- and 1:3-copolymers of L-leucine with sarcosine.

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

On the other hand, at both air/water and oil/water interfaces, the compressibility of the film was increased much more with incorporation of sarcosyl residues in the polymer chain. At air/water interface, the film changed from condensed type to expanded type, the surface viscosities being first detected at much less areas than their close-packed areas. The facts shown above suggest that the interaction between

polymer chains is diminished with decrease in number of intermolecular hydrogen bonds.

The author wishes to express his hearty thanks to Prof. Toshizo Isemura of Osaka University for his kind guidance throughout the present work. He is also indebted to Prof. Junzo Noguchi of Hokkaido University for his kind guidance in preparing the samples.

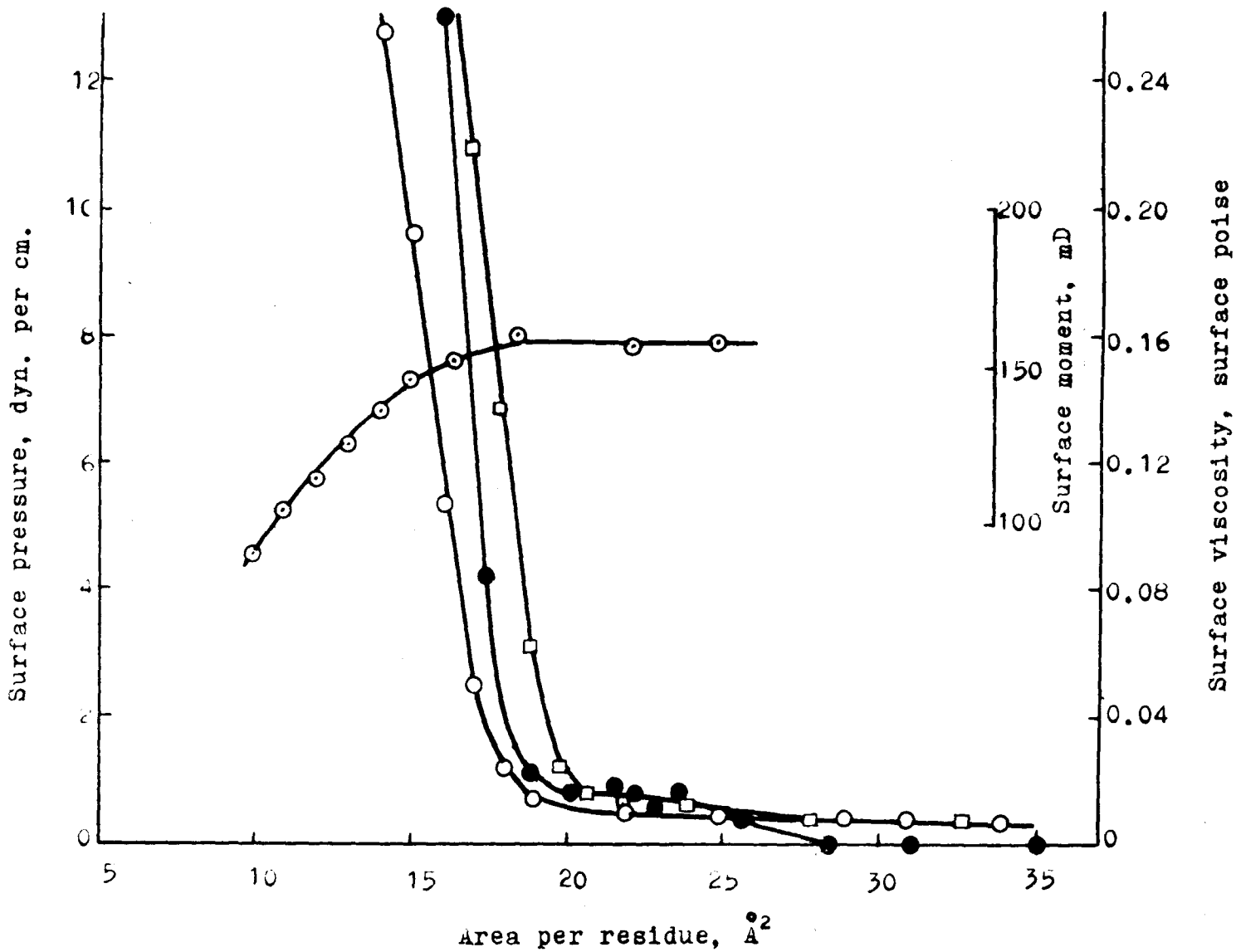

Fig. 1. Monolayers of poly-L-leucine and poly-DL-leucine on distilled water at 16°C: poly-L-leucine —○, surface pressure; ⊙, surface moment; ●, surface viscosity: poly-DL-leucine —□, surface pressure.

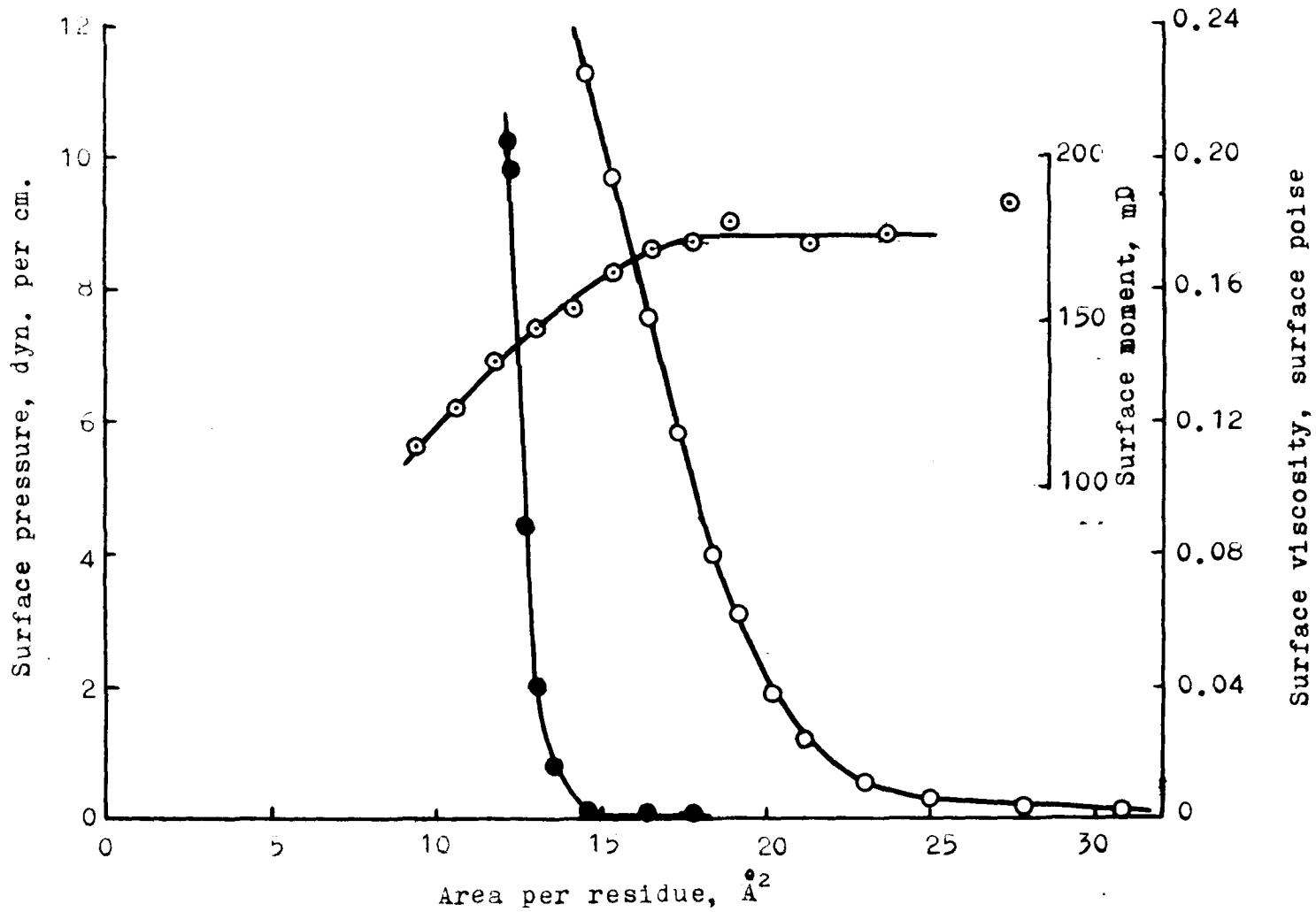

Fig. 2. Monolayer of copoly-3:1-(L-leucine, sarcosine) on distilled water at 16°C: ○ , surface pressure: ⊙ , surface moment; ● , surface viscosity.

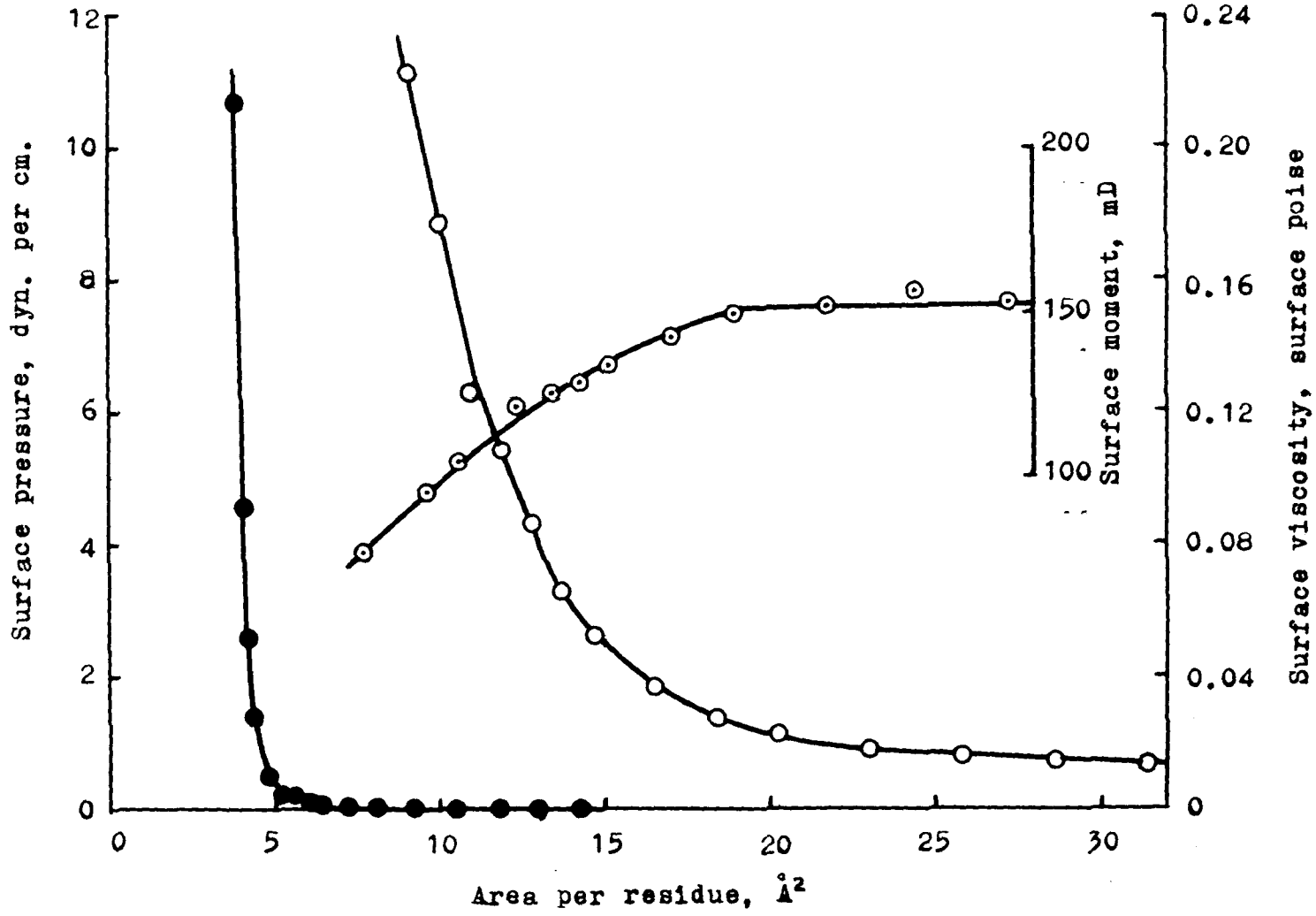

Fig. 3. Monolayer of copoly-1:1-(L-leucine, sarcosine) on distilled water at 16°C: ○ , surface pressure; ⊙ , surface moment; ● , surface viscosity.

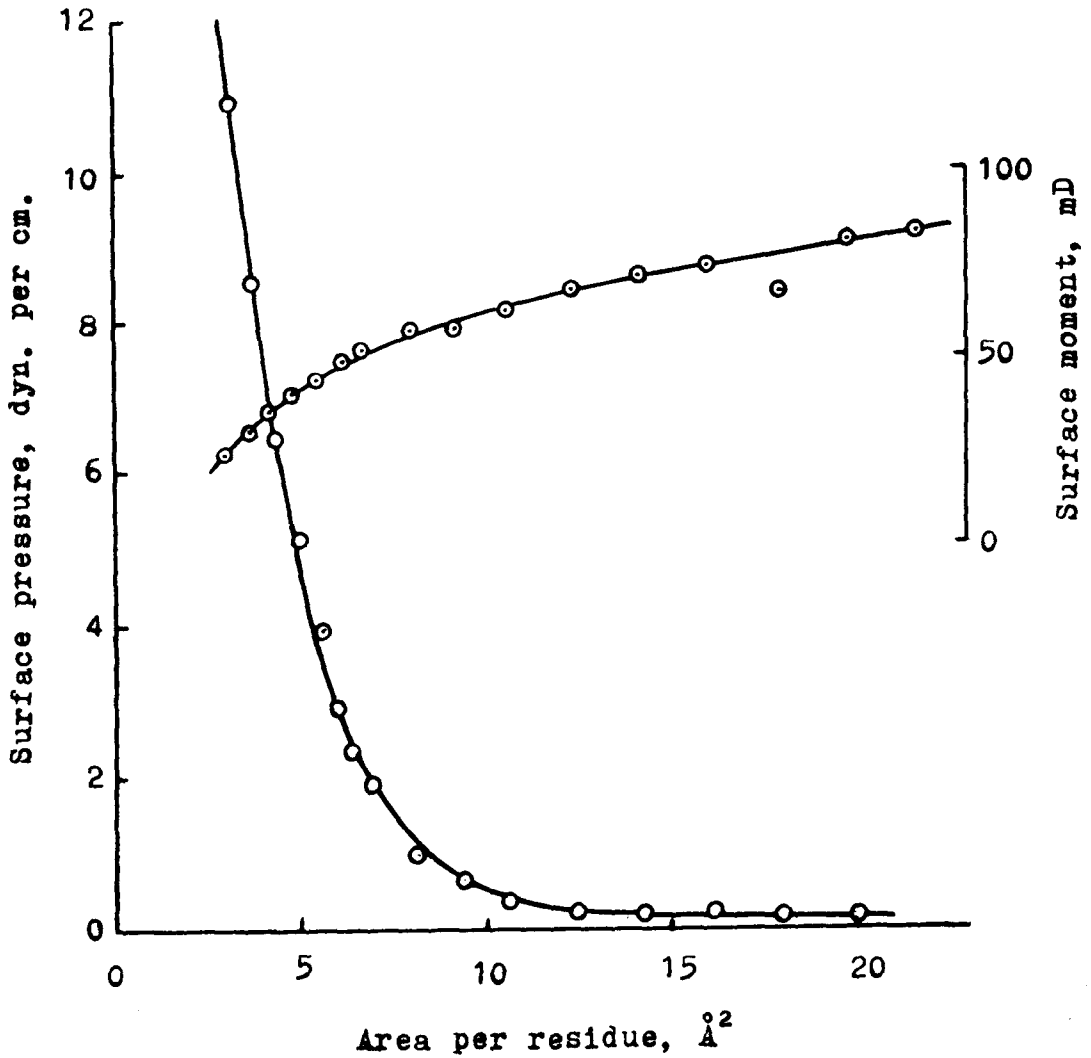

Fig. 4. Monolayer of copoly-1:3-(L-leucine, sarcosine) on distilled water at 16°C: ○, surface pressure; ⊙, surface moment.

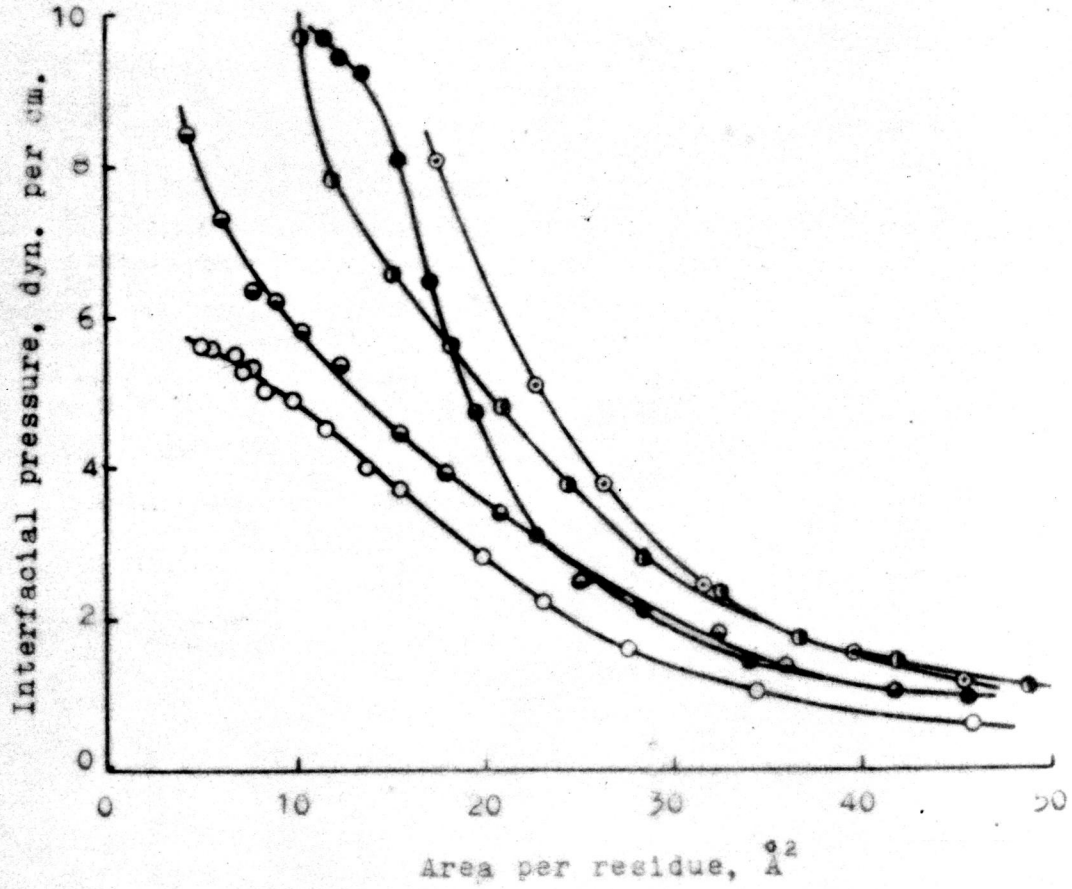

Fig. 5. Interfacial pressure-area curves of copolymers of L-leucine with sarcosine at petroleum ether/distilled water interface at 17°C: ●, poly-L-leucine; ⊙, copoly-3:1-(L-leucine, sarcosine); ◑, copoly-1:1-(L-leucine, sarcosine); ◒, copoly-1:3-(L-leucine, sarcosine); ○, polysarcosine (15°C).

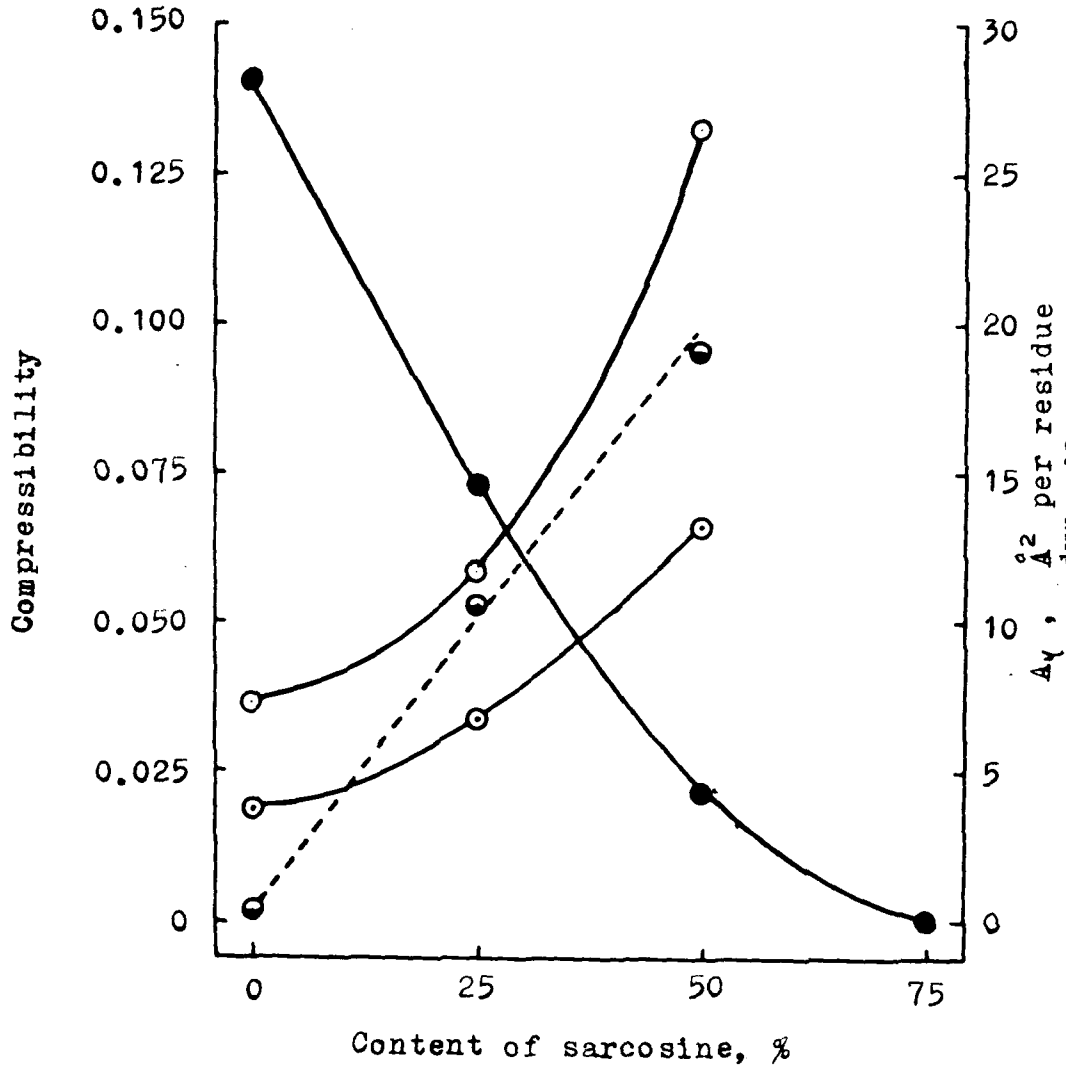

Fig. 6. Variations of compressibilities at 2 dyn. per cm. (○) and 5 dyn. per cm. (⊙), and of A_η (●) and π_η (◒) with content of sarcosyl residue in polypeptide chain (air/water interface).

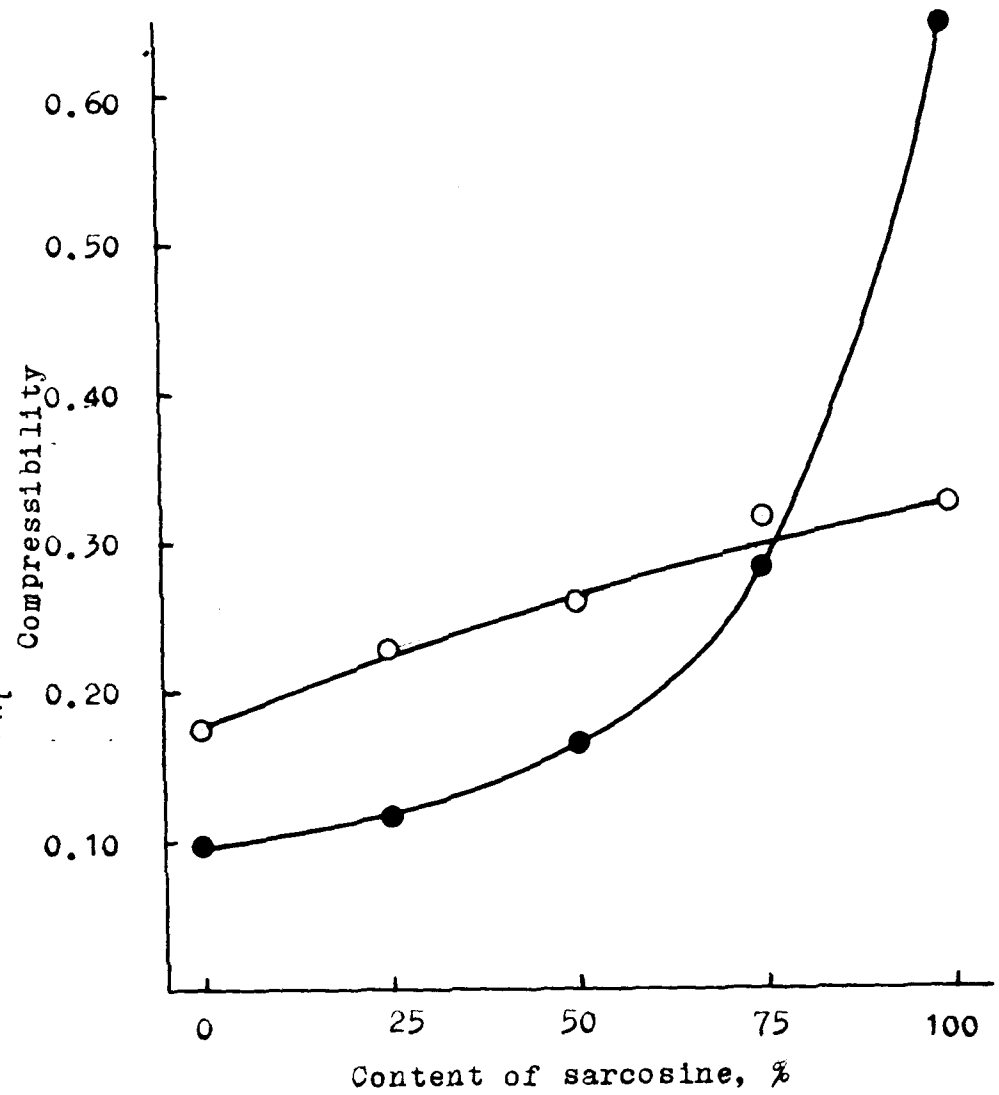

Fig. 7. Variations of compressibilities at 2 dyn. per cm. (○), and 5 dyn. per cm. (●) with content of sarcosyl residue in polypeptide chain (oil/water interface).

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III. *Surface Chemistry of Synthetic Protein Analogues. Polytyrosine and its Related Polypeptides*

(Received July 22, 1958)

We have previously studied[1-6] the monolayers of synthetic polypeptides with non-electrolytic side chains as well as those with electrolytic side chains at air/water interface. It is well known that tyrosine residue is very specific among other amino acid residues in protein because of the potency of its hydrogen bonding. In the present experiment, poly-L-tyrosine and poly-DL-tyrosine were studied at air/water and oil/water interfaces, in order to investigate the effect of hydrogen bond on the nature of polypeptide monolayer. The monolayer of poly-DL-phenylalanine which lacks only a phenolic OH group per residue was also studied in comparison with polytyrosine. The effect of the phenolic OH group was also investigated by its esterification with benzyl group.

Experimental

Among the samples used in the present investigation, poly-DL-phenylalanine, poly-DL-tyrosine and poly-*o*-benzyl-DL-tyrosine were synthesized by Dr. H. Tani and Dr. H. Yuki of our University, and poly-L-tyrosine by Dr. E. Katchalski and Dr. M. Sela of the Weizmann Institute of Science. The solvents used for the speading solution for each polypeptide are listed in Table I.

Surface pressure and surface potential were measured simultaneously. Surface pressure was measured by Wilhelmy's hanging plate method and surface potential by vibrating air electrode method. The pH of the substrate was adjusted by adding hydrochloric acid or potassium carbonate to any desired pH without buffering. The pH was measured with Beckman's glass electrode or with Toyo pH test paper. Interfacial pressure and interfacial potential were measured by the procedure which was reported previously by Hotta[7-9] of our laboratory, excepting the use of hanging plate instead of detaching ring. At oil/water interface, the interfacial concentration was changed by successive injection method using micrometer syringe. Measurement was carried out five minutes later after every injection. Petroleum ether of boiling point of 90~120°C was used as an oil phase. All the experiments were carried out at room temperature without any temperature regulation. The temperature change never exceeded more than one degree in the course of experiment.

Results and Discussion

Poly-DL-phenylalanine.—We studied at first the film of poly-DL-phenylalanine at air/water and oil/water interfaces as an example of the polypeptide film containing the side chain of benzyl group. In Fig. 1, surface (or interfacial) pressure and surface (or interfacial) moment were plotted per phenylalanyl residue. The surface (or interfacial) moment was calculated from

TABLE I
SOLVENTS USED FOR EACH POLYPEPTIDE

Polypeptide	Solvent	Interface
Poly-DL-phenylalanine	Benzene(9)+Dichloroacetic acid(1)	A/W
	Chloroform	A/W
	Benzene(25)+Dichloroacetic acid(1)	O/W
Poly-L-tyrosine (D. P. 45)	Pyridine(1)+Isopropyl alcohol(1)	A/W, O/W
	Benzene(3)+Dichloroacetic acid(7)	A/W
Poly-DL-tyrosine	Pyridine(1)+Isopropyl alcohol(1)	A/W, O/W
Poly-*o*-benzyl-DL-tyrosine	Benzene(3)+Dichloroacetic acid(2)	A/W, O/W

1) T. Isemura and K. Hamaguchi, This Bulletin, **25**, 40 (1952).
2) T. Isemura and K. Hamaguchi, ibid., **26**, 425 (1953).
3) T. Isemura and K. Hamaguchi, ibid., **27**, 125 (1954).
4) T. Isemura and K. Hamaguchi, ibid., **27**, 339 (1954).
5) K. Hamaguchi and T. Isemura, ibid., **28**, 9 (1955).
6) T. Isemura, K. Hamaguchi and H. Kawasato, ibid., **28**, 185 (1955).
7) T. Isemura, H. Hotta and T. Miwa, ibid., **26**, 331 (1953).
8) H. Hotta, ibid., **26**, 386 (1953).
9) H. Hotta, ibid., **27**, 412 (1954).

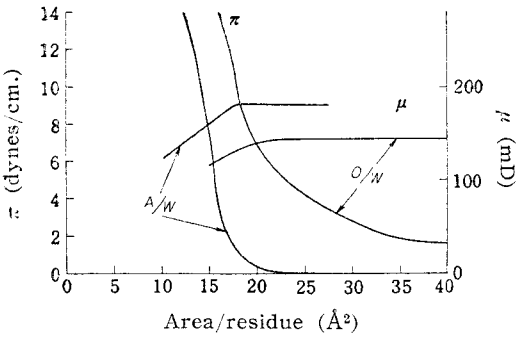

Fig. 1. Surface (or interfacial) pressure (π)-area and surface (or interfacial) moment (μ)-area curves of poly-DL-phenylalanine on distilled water. (A/W interface, 10°C and O/W interface, 13°C).

the observed surface (or interfacial) potential using Helmholtz's formula. $\Delta V = 4\pi n\mu$. The force-area curves of this polypeptide film spread from chloroform solution* and from benzene solution containing a small amount of dichloroacetic acid were exactly identical. A limiting area per residue was found from compressibility-area relation to be 15.8 Å². This area is somewhat larger than that of the typical polypeptide with non-electrolytic paraffine side chains, namely 14.7 Å². Mishuck and Eirich[10] reported that this polypeptide has a small limiting area and attributed this fact to the multilayer formation. On the other hand, Cumper and Alexander[11] found 14.4 Å as the limiting area per residue of this polypeptide from the surface viscosity-area relation. The area found per residue from the force-area relation by them is in rather good agreement with our present result. The surface moment at the area where the film begins to form multilayer is designated by μ_c. μ_c of this polypeptide is 182 mD and is in the same order of magnitude with that of most polypeptide films of non-electrolytic side chains which are spread in β-configuration, whereas Davies[12] reported the surface moment of this polypeptide to be about 100 mD at 30 Å²/residue. At oil/water interface the film is considerably expanded as shown in Fig. 1, because of release of van der Waals force between large side chains such as benzyl groups by oil phase. μ_c was found to be 144 mD and markedly higher than that found by Davies (about 80 mD at 30 Å²).

Poly-L-tyrosine and poly-DL-tyrosine.— The force-area relations of poly-L-tyrosine and poly-DL-tyrosine spread from solutions of respective polypeptide in a mixture of pyridine and isopropyl alcohol (1 : 1) was investigated as a function of pH of the substrate water. The surface pressure-area curves for poly-L-tyrosine and poly-DL-tyrosine are shown in Fig. 2 and Fig. 3, respectively. As shown in these figures on the substrate at pH's less than 9.4 the film considerably condensed. The small area of these polypeptides can not be attributed to partial dissolution of the film as shown in Fig. 4; the presence of salt such as potassium chloride in the substrate water at the concentration of 1.0 mol. per liter scarcely affects the expansion of film. On the other hand, the film is considerably expanded on a substrate at pH's more

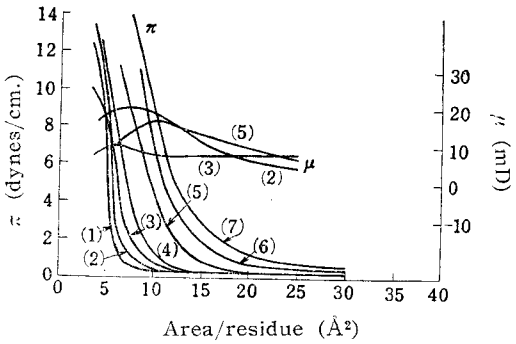

Fig. 2. Surface pressure (π)-area and surface moment (μ)-area curves of poly-L-tyrosine (spread from pyridine-isopropyl alcohol solution). (1) pH 2.0, (2) pH 5.6, (3) pH 7.2, (4) pH 9.4, (5) pH 11.0, (6) pH 11.3 and (7) pH 12.5. (26°C).

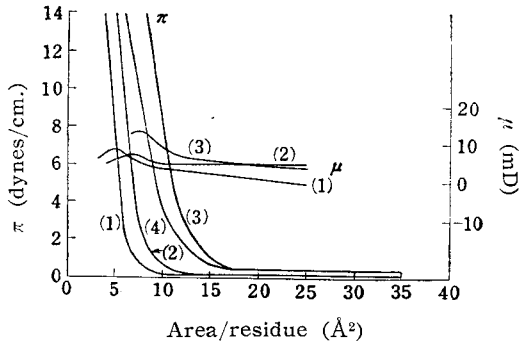

Fig. 3. Surface pressure (π)-area and surface moment (μ)-area curves of poly-DL-tyrosine. (1) pH 5.6, (2) pH 9.2, (3) pH 11.0 and (4) pH 12.5. (24°C).

* Unpublished experiment by Dr. K. Hamaguchi in our laboratory.

10) E. Mishuck and F. R. Eirich, *J. Polymer Sci.*, **16**, 397 (1955).

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

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

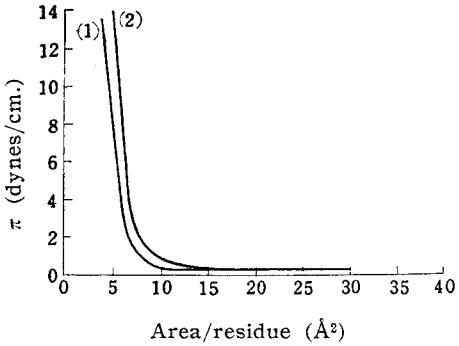

Fig. 4. Effect of the salt on poly-L-tyrosine (spread from pyridine-isopropyl alcohol solution) at pH 5.6. (1) no salt and (2) 1 mol. KCl. (26°C).

than 11.0. However, when the pH of the substrate becomes more than 13, it dissolves rather than expands. In Figs. 2 and 3, the surface moment-area relations of poly-L-tyrosine and poly-DL-tyrosine are also shown, respectively. Surface moment is very small in comparison with that of poly-DL-phenylalanine. The trend of the curve is very different from that of other polymer films. These facts may be caused by the cancellation of dipole by intramolecular hydrogen bond. Several years ago, Masuda and Eda[13] reported the small area per tyrosine residue for poly-DL-tyrosine film. They found a smaller area on acid substrate than on alkaline substrate. This may be caused by partial dissolution of polypeptide because of the smaller degree of polymerization of the polypeptide which they used.

Polytyrosine films spread at air/water interface occupy much less area than the films of other polypeptides such as polyphenylalanine and show much smaller surface moment as mentioned above. These anomalous properties of polytyrosine might come from the hydrogen bonding of phenolic OH group in tyrosine residue. Without this hydroxyl group, polytyrosine may assume exactly a structure of polyphenylalanine. Accordingly, the film would have a limiting area of about 15 Å² per residue. Nevertheless, the limiting area per residue for poly-L-tyrosine was found to be 5.7 Å² and that for poly-DL-tyrosine to be 5.8 Å² both at pH 5.6. These small areas per residue may be attributed to the spreading of these polypeptides in a coiled state, owing to the strong hydrogen bonding of phenolic OH groups. The extremely low value of surface moment supports also this inference. Even on alkaline substrate, the spreading of the film is not complete, although the film expands considerably at pH region between 8.0 and 11.5. As shown in Fig. 5, this finding corresponds to the partial breaking of hydrogen bonds and to the partial dissociation of phenolic OH groups, and to the result of spectrophotometric titration by Katchalski and Sela[14,15]. On the other hand, we obtain the film which seems to assume β-keratin configuration irrespective of the pH of the substrate if we spread it from a solution in dichloroacetic acid which is a strong hydrogen-bond breaking agent. The surface moment of the film thus obtained is about 120~130 mD, as shown in Fig. 6, suggesting the intramolecular cancellation of OH contributions to surface moment

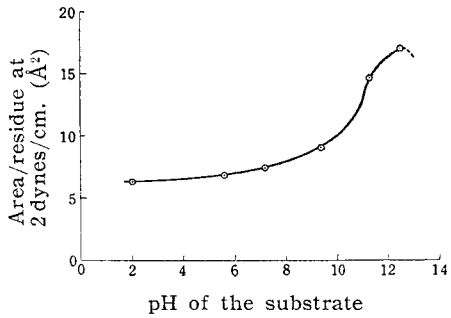

Fig. 5. Effect of pH of the substrate water on the area at 2 dynes/cm. of poly-L-tyrosine (spread from pyridine-isopropyl alcohol solution) at air/water interface.

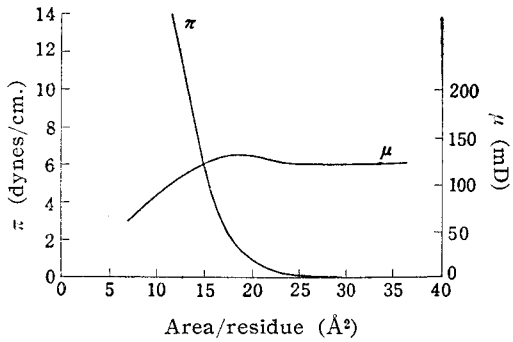

Fig. 6. Surface pressure (π)-area and surface moment (μ)-area curves of poly-L-tyrosine (spread from dichloroacetic acid and benzene solution) on distilled water. (16°C).

13) Y. Masuda and K. Eda, *Science [Japan] (Kagaku)*, **21**, 470 (1951).

14) E. Katchalski and M. Sela, *Bull. Research Council of Israel*, **2**, 315 (1952).

15) E. Katchalski and M. Sela, *J. Am. Chem. Soc.*, **75**, 5284 (1953).

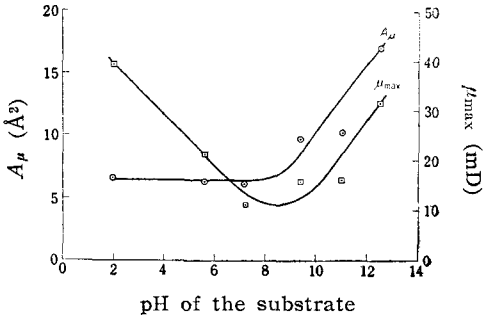

Fig. 7. Monolayers of poly-L-tyrosine (spread from pyridine-isopropyl alcohol solution). Area at maximum surface moment (A_μ)-pH and maximum surface moment (μ_{max})-pH curves.

and tending to the value of surface moment of ordinary polypeptide films. In Fig. 7, the surface moment of polytyrosine increases as the film is compressed and passes a maximum and then decreases, whereas most usual well-spread linear polymer shows a constant surface moment, μ_c until the polymer film is closely packed as the film is compressed and further compression causes the decrease of surface moment. The maximum surface moment, μ_{max} and the area where the maximum moment was found, A_μ, are plotted against pH. μ_{max} increases considerably above pH 8.5. This might be caused by the unfolding and reorientation of polypeptide owing to the breaking of some hydrogen bonds. The reason why μ_{max} increases at the extreme acid substrate is not clear but is presumably due to the formation of oxonium ions[16].

In Figs. 8 and 9, the results obtained at petroleum ether/water interface are shown. The film is considerably expanded.

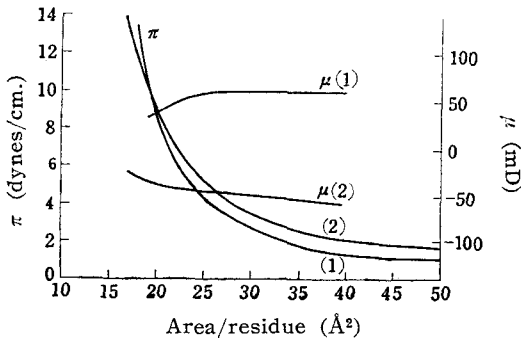

Fig. 8. Interfacial pressure (π)-area and interfacial moment (μ)-area curves of poly-L-tyrosine. (1) pH 2.0, 13°C and (2) 11.0, 15°C.

16) I. Sawai, *Trans. Faraday Soc.*, **31**, 765 (1938).

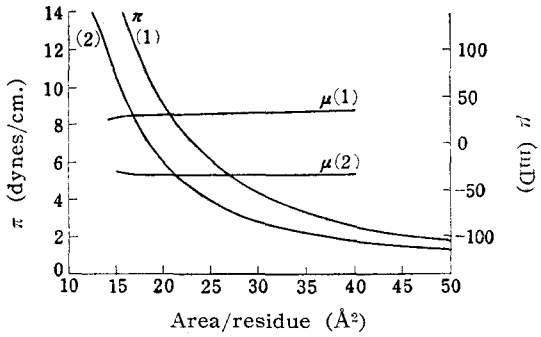

Fig. 9. Interfacial pressure (π)-area and interfacial moment (μ)-area curves of poly-DL-tyrosine. (1) pH 2.0 and (2) pH 11.0. (22°C).

Poly-L-tyrosine film is more expanded at pH 11.0 than at 2.0, whereas poly-DL-tyrosine is more expanded at pH 2.0 than at pH 11.0. The force-area curves are like those of the monolayers of polypeptide with nonpolar side chains such as poly-DL-phenylalanine. At pH 2 all the side chains of *p*-hydroxybenzyl group might be dissolved in the oil phase. Interfacial potential was found to be 60 mD (μ_c) for poly-L-tyrosine and 32 mD at 25 Å²/residue for poly-DL-tyrosine. These values are markedly less than that for poly-DL-phenylalanine (144 mD (μ_c)). This difference might be caused by the contribution of phenolic OH group of tyrosine. Poly-DL-tyrosine is more expanded than poly-L-tyrosine corresponding to the general tendency of expansion of poly-DL-amino acid more than poly-L-amino acid as previously reported[3]. On the other hand, the fact that at air/water interface the film of poly-DL-tyrosine occupies less area than poly-L-tyrosine does at pH 12.5 is due to the general tendency of higher solubility of poly-DL-amino acid than poly-L-amino acid. If the aqueous phase is alkaline, the phenolic OH dissociates and dissolves in the aqueous phase at both air/water and oil/water interfaces. In this case the contribution of dipole is inverse. The negative surface moment was found at oil/water interface, namely −44 mD at 25 Å²/residue for poly-L-tyrosine and −34 mD (μ_c) for poly-DL-tyrosine.

From these results we can conclude that both poly-L-tyrosine and poly-DL-tyrosine spread incompletely at air/water interface and the film is in a partially coiled state because of the hydrogen bonding of phenolic OH group if it is not ionized. However, at oil/water interface by the release of van der Waals force between

side chains due to the intervening of oil, hydrogen bonds are readily broken, and the monolayer which seems to be in β-configuration is obtained. Even at air/water interface, similar monolayer is obtained when we spread the film from a solution in hydrogen bond breaking agent. The obtained force-area curve is very similar to that of poly-DL-phenylalanine. With this monolayer, surface moment of 132 mD (μ_{max}) is found.

Poly-*o*-benzyl-DL-tyrosine. — Force-area and surface (interfacial) moment-area

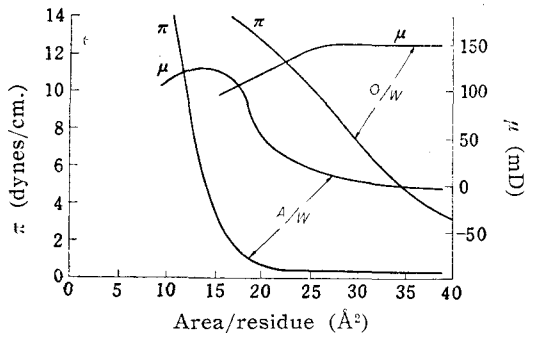

Fig. 10. Surface (or interfacial) pressure (π)-area and surface (or interfacial) moment (μ)-area curves of poly-*o*-benzyl-DL-tyrosine on distilled water. (24°C).

curves of poly-*o*-benzyl-DL-tyrosine at air/water and oil/water interfaces are shown in Fig. 10. Because of lacking of hydrogen bonding agency by esterification of phenolic OH groups, the film is well spread at air/water interface. The area per residue at minimum compressibility is somewhat less than that of poly-DL-phenylalanine and polytyrosine which was spread from the solution containing dichloroacetic acid. The large van der Waals force between side chains seems to cause the condensation of the film. The maximum value of surface moment reached 123 mD. Surface moment increased as the film was compressed, especially when the force-area curve begins to stand steeply at about 20 Å² residue. This change is based on the reorientation of large polar side chains. At oil/water interface, poly-*o*-benzyl-DL-tyrosine expanded considerably more than any other polypeptides mentioned above. All large side chains may be in the oil phase, and there is no interaction between the side chain because of the release of van der Waals force between them by intervening of oil. The size and difficulty of packing such large chains may be the cause of the expansion of the film. Interfacial moment was found to be 150 mD

TABLE II

AREAS AND SURFACE AND INTERFACIAL MOMENTS FOR EACH POLYPEPTIDE

	Interface	Aqueous phase	pH	Temp. (°C)	A_π (Å²/residue)	$A_{\pi=5}$ (Å²/residue)	μ (mD)	A_μ (Å²/residue)
Poly-DL-phenylalanine	A/W	d.w	5.4	10	15.8	15.6	182(C)	17.8
	O/W	d.w	5.4	13		22.8	144(C)	23.0
Poly-L-tyrosine (Pyridine-Isopropyl alcohol)	A/W	d.w	5.6	26	5.7	5.7	21(M)	6.2
		0.01 N K_2CO_3	11.0	26	10.4	10.0	10(M)	16.0
	O/W	0.01 N HCl	2.0	13		23.7	60(C)	25.0
		0.01 N K_2CO_3	11.0	15		25.0	−44 (at 25 Å²)	
Poly-L-tyrosine (Dichloroacetic acid-Benzene)	A/W	d.w	3.0	16	15.4	15.6	132(M)	18.8
Poly-DL-tyrosine	A/W	d.w	5.6	24	5.8	5.6	9(M)	4.8
		0.01 N K_2CO_3	11.0	24	11.0	11.0	8(M)	14.0
	O/W	0.01 N HCl	2.0	22		28.0	32 (at 25 Å²)	15.2
		0.01 N K_2CO_3	11.0	22		22.5	−34(C)	16.0
Poly-*o*-benzyl-DL-tyrosine	A/W	d.w	4.0	24	13.8	13.9	123(M)	14.2
	O/W	d.w	4.2	24		33.7	150(C)	29.2

A_π: Area at minimum compressibility (limiting area), $A_{\pi=5}$: Area at 5 dynes/cm., μ: Surface (or interfacial) moment, (C): Constant value, (M): Maximum value, A_μ: Area at which μ begins suddenly decrease, or area at the maximum value of μ, d.w: Distilled water.

(μ_c) which corresponds namely to the value of poly-DL-phenylalanine.

All the results are summarized in Table II.

Summary

The monolayer properties of synthetic polypeptides which have phenolic OH groups in the side chains such as poly-L-tyrosine and poly-DL-tyrosine were studied to investigate the effect of hydrogen bonding by tyrosyl OH groups. Poly-*o*-benzyl-DL-tyrosine and poly-DL-phenylalanine were also studied for comparison.

At air/water interface, poly-L-tyrosine and poly-DL-tyrosine occupy much smaller area per residue than any other polypeptide films, and have very small surface moment, if they were spread from the solution in pyridine and isopropyl alcohol mixture. These polypeptides might be spread in a strict sense not in an extended monolayer but in a coiled state, suggesting the hydrogen bond between phenolic OH and —CO— or —NH— groups in the main chain. On the alkaline substrate the film expands considerably because of the breaking of hydrogen bonds by the ionization of phenolic OH. At petroleum ether/water interface, the film is also expanded by releasing of van der Waals force. Interfacial moment changes its sign due to whether the aqueous phase is acid or alkaline. It suggests that *p*-hydroxybenzyl groups are in the oil phase at oil/acid solution, whereas they are in the aqueous phase at oil/alkaline solution. Poly-L-tyrosine assumes the configuration very similar to that of poly-DL-phenylalanine, if poly-L-tyrosine is spread from a solution in dichloroacetic acid which is a strong hydrogen bond breaking agent.

Poly-*o*-benzyl-DL-tyrosine gives a monolayer which is somewhat more condensed than poly-DL-phenylalanine at air/water interface, whereas it occupies a larger area per residue than poly-L-tyrosine, poly-DL-tyrosine and poly-DL-phenylalanine at oil/water interface. All these findings are caused by the existence of very large side chains in poly-*o*-benzyl-DL-tyrosine.

The authors express their hearty thanks to Dr. H. Tani and Dr. H. Yuki of the Faculty of Science of Osaka University and Professor E. Katchalski and Dr. M. Sela of the Weizmann Institute of Science in Israel who gave them the valuable samples of synthetic polypeptides. The expense for the experiment was partly defrayed from the grant given by the Ministry of Education, to which the authors' thanks are due.

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IV. *Effects of Tyrosyl Residue on the Polypeptide Monolayers*

(Received November 21, 1961)

Protein has a definite conformation which is responsible for its biological activity. This conformation is believed to be chiefly maintained by the intramolecular hydrogen bonding. Tyrosyl residue is very specific among other amino acid residues in protein because of the potency of its hydrogen bonding. In our previous investigations[1,2], we have studied the monolayers of poly-L-tyrosine, poly-DL-tyrosine, poly-*o*-benzyl-DL-tyrosine and poly-DL-phenylalanine in terms of the hydrogen bonding of the phenolic hydroxyl group of tyrosyl residue at the air/water and oil/water interfaces.

Poly-*o*-benzyl-DL-tyrosine was found, like most polypeptide films, to be spread in a β-configuration. Poly-L-tyrosine and poly-DL-tyrosine, however, showed solvent effects at the air/water interface owing to their phenolic hydroxyl group. If they were spread from the solution in a mixture of pyridine and isopropyl alcohol, they occupied much less area per residue than polypeptide films such as poly-DL-phenylalanine in a β-configuration. This fact suggests that poly-L-tyrosine might not be spread in an extended monolayer in a strict sense, but in a coiled state probably owing to the hydrogen bonding between the phenolic hydroxyl group and the carbonyl or imino group in a main chain. On the other hand, if poly-L-tyrosine is spread from the solution in a mixture of dichloroacetic acid and benzene, it probably assumes a β-form.

In the present study, we have investigated the effects of the phenolic hydroxyl group of tyrosyl residue on the polypeptide monolyers. For this purpose, the monolayers of poly-L-tyrosine, copoly-1 : 1-(L-tyrosine, L-phenylalanine), copoly-1 : 1 : 2-(L-tyrosine, *o*-benzyl-L-tyrosine, L-phenylalanine) and copoly-1 : 1-(L-tyrosine, glycine) have been investigated. For comparison, the monolayer of poly-DL-phenylalanine was also studied.

Experimental

Materials.—The same samples of poly-L-tyrosine and poly-DL-phenylalanine were used as in the preceding study. Copolypeptides containing tyrosyl residue were kindly supplied by Professor J. Noguchi of Hokkaido University.

As spreading solvents, mixtures of dichloroacetic acid and benzene were used. The ratio of dichloroacetic acid (DCA) to benzene is various for different polypeptides, as can be seen in Table I.

TABLE I. THE RATIOS OF DCA TO BENZENE IN SPREADING SOLUTIONS

Sample	DCA : Benzene (v/v)
Poly-DL-phenylalanine	3 : 7
Poly-L-tyrosine	7 : 3
Copoly-1 : 1-(L-tyrosine, L-phenylalanine)	3 : 7
Copoly-1 : 1 : 2-(L-tyrosine, *o*-benzyl-L-tyrosine, L-phenylalanine)	3 : 7
Copoly-1 : 1-(L-tyrosine, glycine)	1 : 1

Method.—The films were spread at the air/water and oil/water interfaces. The surface pressure and surface potential were measured simultaneously by the Wilhelmy hanging plate method and by the vibrating electrode method respectively. Interfacial pressure was measured by the ring method. At the oil/water interface, the interfacial concentration was changed by the successive injection method, using a micrometer syringe, and was corrected by the Thomas theoretical correction formula[3]. Measurements were carried out five minutes after every injection. The pH value of the subphase was adjusted to any desired value by adding hydrochloric acid or potassium carbonate and was measured with a Horiba M-3 glass electrode pH meter. Petroleum ether with a boiling point of 85~115°C was used as an oil phase. All the experiments were carried out at room temperature without any temperature regulation. However, the change never exceeded more than one degree in the course of the experiment.

The limiting area per residue of the film was obtained from the minimum of its compressibility, $\delta = -dA/AdII$, and the surface moment, μ, was calculated from the surface potential, ΔV, using the Helmholtz formula, $\mu = \Delta VA/4\pi$.

The average residual weight of copolypeptide was calculated on the basis of its polymerization ratio.

Results

The surface pressure-area (Π-A) and surface moment-area (μ-A) curves of poly-DL-phenylalanine at the air/water interface are shown

1) T. Isemura, S. Ikeda and T. Yamashita, *Mem. Inst. Sci. and Ind. Research, Osaka Univ.*, 15, 167 (1958).
2) T. Isemura and T. Yamashita, This Bulletin, 32, 1 (1959).
3) A. G. Thomas, *Nature*, 179, 776 (1957).

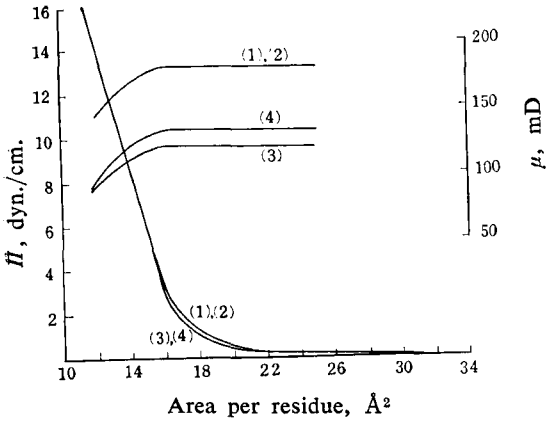

Fig. 1. Surface pressure-area and surface moment-area curves of poly-DL-phenylalanine (24°C): (1), on 0.01 N HCl; (2), on distilled water; (3), on 0.02 N K_2CO_3; (4), on 0.1 N K_2CO_3.

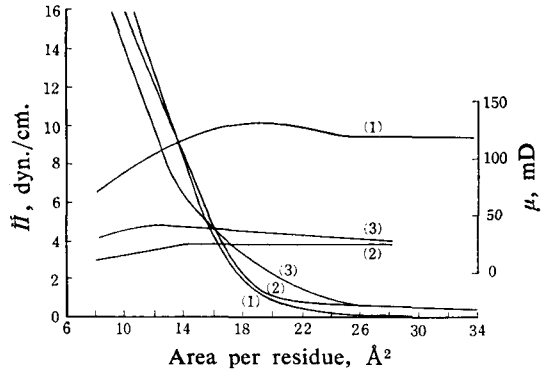

Fig. 2. Surface pressure-area and surface moment-area curves of poly-L-tyrosine (16°C): (1), on distilled water (Ref. 2); (2) on 0.02 N K_2CO_3; (3), on 0.1 N K_2CO_3.

in Fig. 1. The *Π-A* curves were nearly independent of the pH value of the subphases, although the *μ-A* curves were affected. The limiting area per residue was 15.6 Å² irrespective of the pH value of the subphases. On the alkaline subphase, the surface moment is lower by 50~60 mD than on the acid subphase.

The *Π-A* and *μ-A* curves of poly-L-tyrosine spread from a solution in a dichloroacetic acid-benzene mixture at the air/water interface are given in Fig. 2. In a low pressure region the film expands slightly more on 0.02 N potassium carbonate than on distilled water. Among the three subphases the film expands most on 0.1 N potassium carbonate. On the alkaline subphase, the surface moment is lower by 90~100 mD than on distilled water.

The *Π-A* and *μ-A* curves of copoly-1 : 1-(L-tyrosine, L-phenylalanine) and copoly-1 : 1 : 2-

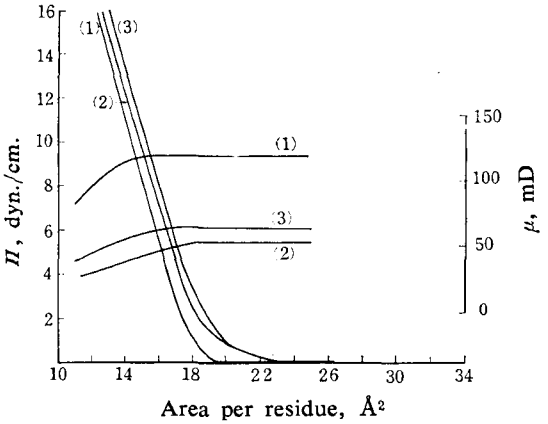

Fig. 3. Surface pressure-area and surface moment-area curves of copoly-1 : 1-(L-tyrosine, L-phenylalanine (19°C): (1), on distilled water; (2), on 0.02 N K_2CO_3; (3), on 0.1 N K_2CO_3.

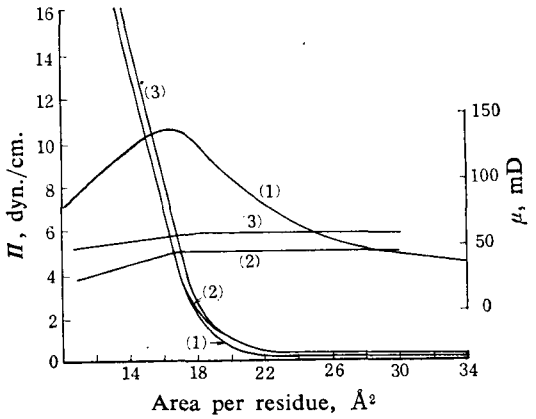

Fig. 4. Surface pressure-area and surface moment-area curves of copoly-1 : 1 : 2-(L-tyrosine, *o*-benzyl-L-tyrosine, L-phenylalanine) (10°C): (1), on distilled water; (2), on 0.02 N K_2CO_3; (3), on 0.1 N K_2CO_3.

(L-tyrosine, *o*-benzyl-L-tyrosine, L-phenylalanine) at the air/water interface are given in Figs. 3 and 4 respectively. The effects of the change in the pH values of the subphases on the *Π-A* relations of these copolypeptides are smaller than in the case of poly-L-tyrosine. The difference between surface moments of copoly-1 : 1-(L-tyrosine, L-phenylalanine) on acid and alkaline subphases is comparable to that between those of poly-DL-phenylalanine. The surface moment of copoly-1 : 1 : 2-(L-tyrosine, *o*-benzyl-L-tyrosine, L-phenylalanine) increased steeply on distilled water, as the film was compressed, as has been found with the film of poly-*o*-benzyl-DL-tyrosine[2]; it reached 137 mD at its maximum.

The Π-A and μ-A curves of copoly-1 : 1-(L-tyrosine, glycine) at the air/water interface are shown in Fig. 5. This copolypeptide occupies much less limiting area per residue in the surface film than those of any other

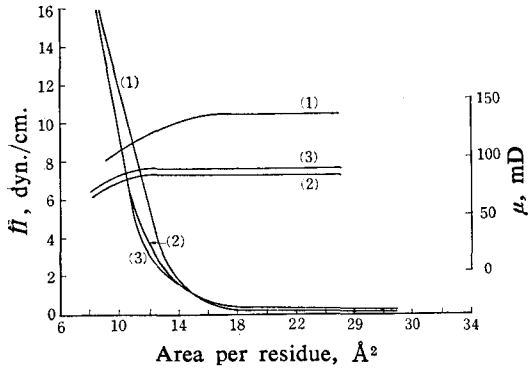

Fig. 5. Surface pressure-area and surface moment-area curves of copoly-1 : 1-(L-tyrosine, glycine) (10°C): (1), on distilled water; (2), on 0.01 N K_2CO_3; (3), on 0.1 N K_2CO_3.

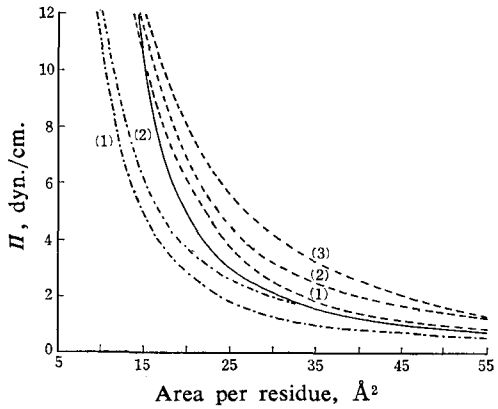

Fig. 6. Interfacial pressure-area curves of various polypeptides (16.5°C).
—— Poly-DL-phenylalanine, on distilled water.
---- Copoly-1 : 1-(L-tyrosine, L-phenylalanine) : (1), on 0.01 N HCl; (2), on 0.02 N K_2CO_3; (3), on 0.1 N K_2CO_3.
-·-· Copoly-1 : 1-(L-tyrosine, glycine) : (1), on 0.01 N HCl and distilled water; (2), on 0.01 N K_2CO_3 and 0.1 N K_2CO_3.

TABLE II. FILM CHARACTERISTICS OBTAINED FROM Π-A AND μ-A RELATIONS AT AIR/WATER INTERFACE

Polypeptide	Subphase	Temp. °C	$A\Pi$ Å²/residue	$A\mu$ Å²/residue	μ_c mD	$\Delta\mu$ mD
Poly-DL-phenylalanine	0.01 N HCl	24	15.6	16.0	182	
	dw	24	15.6	16.0	182	
	0.02 N K_2CO_3	24	15.6	16.0	124	58
	0.1 N K_2CO_3	24	15.6	16.0	134	48
Poly-L-tyrosine	dw**	16	15.6	18.8	132*	
	0.02 N K_2CO_3	16	15.7	14.0	26	106
	0.1 N K_2CO_3	16	—	12.4	42*	90
Copoly-1 : 1-(L-tyrosine, L-phenylalanine)	dw	19	17.0	15.2	120	
	0.02 N K_2CO_3	19	17.2	18.0	54	66
	0.1 N K_2CO_3	19	17.2	17.0	66	54
Copoly-1 : 1 : 2-(L-tyrosine, o-benzyl-L-tyrosine, L-phenylalanine)	dw	10	17.0	17.0	137*	
	0.02 N K_2CO_3	10	17.0	17.2	46	91
	0.1 N K_2CO_3	10	17.2	18.2	62	75
Copoly-1 : 1-(L-tyrosine, glycine)	dw	10	11.5	16.0	138	
	0.02 N K_2CO_3	10	10.9	12.0	85	53
	0.1 N K_2CO_3	10	10.9	11.5	90	48

$A\Pi$: Limiting area (area at minimum compressibility)
$A\mu$: Area at which μ begins to decrease
μ_c : Value of constant surface moment
$\Delta\mu$: Difference between constant (or maximum) values of surface moments on distilled water (or acid subphase) and on alkaline subphase
dw : Distilled water
* : Maximum value
** : Ref. 2

polypeptide mentioned above.

The film characteristics obtained from the *Π-A* and *μ-A* relations are listed in Table II.

The *Π-A* curves of poly-DL-phenylalanine, copoly-1 : 1-(L-tyrosine, L-phenylalanine) and copoly-1 : 1-(L-tyrosine, glycine) at the oil/water interface are shown in Fig. 6. The *Π-A* curve of poly-DL-phenylalanine measured by the ring method was slightly shifted to the left (namely, to the smaller area) in comparison with the curve obtained by the hanging plate method employed in the previous study[2]. This discrepancy is probably caused by the neglect in the previous work of the change in the contact angle of the glass handging plate. At the oil/water interface, the films expanded more than at the air/water interface. The film of copoly-1 : 1-(L-tyrosine, glycine) occupied much less area per residue than any other polypeptides at the oil/water interface as well as at the air/water interface.

Discussion

Effect of Subphase pH on the Surface Potential or Moment.—*Poly-DL-phenylalanine.*—The surface potential or moment of poly-DL-phenylalanine changes considerably in spite of its lack of ionizable side chains. The difference between constant values of the surface moments on the acid and alkaline subphases, $\Delta\mu$, is 50~60 mD. Because the change cannot be attributed to the ionization of the side chain, it must be caused by a change in the main chain. A similar change in surface potential or moment has been found for several synthetic polypeptides with a non-polar side chain, e. g., poly-DL-alanine[4], poly-DL-leucine[5], and poly-α-aminolauric acid[6].

Glazer and Dogan[4] found that the maximum value of the surface potential of poly-DL-alanine decreased with the increase in subphase pH. They attributed the decrease in surface potential with pH to the varying degree of ionization of the carboxyl and amino end groups. However, in the polypeptide of a high degree of polymerization, the ionization effect of end groups on the surface potential or moment might not be so effective.

Davies[5] found that values of the surface moment of poly-DL-leucine at 20 Å^2 per residue were 188 mD on 0.01 N hydrochloric acid, 137 mD on 0.02 N phosphate (pH 6.8), and 113 mD on 0.1 N sodium hydroxide. From this finding, he suggested that on the neutral and alkaline subphases the hydrogen bond between keto-imide groups in neighboring chains was formed and that on the acid subphase the hydrogen bond would be broken. However, his view that the hydrogen bond is maintained on such a strong alkaline subphase as 0.1 N sodium hydroxide is very doubtful, because the protein is unfolded in an alkaine solution, owing to the breaking of the hydrogen bonding as commonly accepted, while the breaking of the hydrogen bonding on the acid subphase is probable, although the effect of the acid seems to appear at a very high acidity.

The probable explanation of the change in surface potential or moment of non-polar synthetic polypeptide with pH seems to be the keto-enol transformation of the peptide bond. According to Pankhurst[7], the keto-imide group of polypeptide chain in gelatin is capable of resonance between the keto and the enol forms as amides, while the addition of a hydrogen ion would prevent the resonance. Schauenstein and Perko observed this effect for 3, 6-dioxo-1, 2, 4-triazine[8] with $HO\cdot C_6H_4\cdot CH_2-$, $C_6H_5\cdot CH_2-$ or CH_3- in the 5 position, and poly-L-tyrosine[9] from the differences in ultraviolet absorption spectra between their acid and alkaline solutions; they called this effect "peptenol effect". Owing to the peptenol effect, the surface moment or potential of the polypeptide monolayer can decrease more on alkaline than on the acid subphase, because most of the keto-imide groups in the main chain are in an enolate form, and the film would be more condensed on the subphase of a high pH, as has been found for the poly-DL-leucine monolayer[10], since the increase in the double bond nature of the peptide bond enhances the rigidity of the film.

Accordingly, the change in the surface moment or the potential of non-polar synthetic polypeptides with the pH might be caused primarily by the keto-enol transformation of peptide bond, i. e., the peptenol effect, although the ionization of the end groups and the breaking of the hydrogen bond on a strong acid solution may be effective to some extent on the change in the surface potential or the moment with pH. The *Π-A* relation of poly-DL-phenylalanine seems not to be affected by the change in the pH value of the subphase because of the effect of its large side chains.

Polypeptides other than Poly-DL-phenylalanine.—The difference between the constant or maximum values of the surface moments on

4) J. Glazer and M. Z. Dogan, *Trans. Faraday Soc.*, **49**, 448 (1953).
5) J. T. Davies, ibid., **49**, 949 (1953).
6) K. Eda and Y. Masuda, This Bulletin, **24**, 140 (1951).
7) K. G. A. Pankhurst, "Surface Chemistry", Butterworth Scientific Publication, London (1949), p. 109.
8) E. Schauenstein and G. M. Perko, *Z. Elektrochem.*, **57**, 927 (1953).
9) E. Schauenstein and G. M. Perko, ibid., **58**, 45 (1954).
10) D. F. Cheesman and J. T. Davies, "Advance in Protein Chemistry", Vol. 9, Academic Press, New York (1954), p. 439.

acid and alkaline subphases, $\Delta\mu$, is about 100 mD for poly-L-tyrosine and higher by 40~50 mD than that for poly-DL-phenylalanine. The abnormally large difference in the surface moment is probably due both to the peptenol effect of the main chain and to the ionization of the phenolic hydroxyl groups of the side chains.

The $\Delta\mu$ value of copoly-1 : 1-(L-tyrosine, L-phenylalanine) is about 60 mD and is comparable to that of poly-DL-phenylalanine. From the fact that the film expands slightly more on the alkaline than on the acid subphase, the decrease in surface moment seems to be due not only to the peptenol effect of the main chain, but also to the partial ionization of the side chains.

The surface moment of copoly-1 : 1 : 2-(L-tyrosine, *o*-benzyl-L-tyrosine, L-phenylalanine) on distilled water steeply increases with the compression of the film, as was found with the film of poly-*o*-benzyl-L-tyrosine[2]. This change is probably caused by reorientation of the large polar side chain of the *o*-benzyl-L-tyrosyl residue. On the other hand, the surface moment is nearly constant on the alkaline subphase. This fact suggets that the remarkable reorientation of the side chain is not accompanied by a compression of the film on the alkaline subphase. The $\Delta\mu$ values of this copolypeptide are 91 mD on 0.02 N potassium carbonate and 75 mD on 0.1 N potassium carbonate. These values are greater than those for copoly-1 : 1-(L-tyrosine, L-phenylalanine) film, although this copolypeptide has much less ionizable groups.

The $\Delta\mu$ value of copoly-1 : 1-(L-tyrosine, glycine) is about 50 mD and is comparable to that of poly-DL-phenylalanine. This finding suggests that the effect of the ionization of the phenolic hydroxyl group is slight in this case.

Effect of Subphase pH on the *Π-A* Relation. —*Air/Water Interface.*— The film of poly-L-tyrosine spread from a solution in pyridine-isopropyl alcohol mixture is affected remarkably by the pH of the subphase, as has been previously reported[1,2]. On the other hand, the effect of subphase pH on the *Π-A* relation is not so remarkable if it is spread from a solution containing dichloroacetic acid as a component of the solvent, and the limiting area per residue is close to 15 Å^2. Therefore, in this case, there is no hydrogen bonding between the phenolic hydroxyl group and the carbonyl or imino group in the main chain, and poly-Ltyrosine is probably spread in the β-form.

Schauenstein and Perko[9] found the pK value of the phenolic hydroxyl group of poly-L-tyrosine to be 11.0, and Katchalski and Sela[11] found that the spectrophotometric titration curve fitted the theoretical curve computed by assuming the pK_0 to be 9.5. Therefore, on 0.02 N potassium carbonate (pH 10.3), about a half of the side chains may be dissociated. However, the *Π-A* relation here is little affected compared with that on distilled water, although the monolayers of poly-L-glutamic acid[12] and copoly-1 : 2 : 1-(L-lysine, L-leucine, L-glutamic acid)[13] are affected remarkably by the pH of the subphase. This is probably due to the peptenol effect of the main chain and to the van der Waals attraction between the large side chains, both of which prevent the expansion of the film. On 0.1 N potassium carbonate (pH 10.7), however, the film rather expands since the side chains dissociate more, and the repulsive force between the ionized side chains can overcome the van der Waals force and the peptenol effect.

The effect of the subphase pH on copoly-1 : 1-(L-tyrosine, L-phenylalanine) is between that on poly-DL-phenylalanine and that on poly-L-tyrosine. Since this copolypeptide consists of equal numbers of ionizable and unionizable side chains, the effect of the pH appears between poly-DL-phenylalanine and poly-L-tyrosine.

The *Π-A* relation of copoly-1 : 1 : 2-(L-tyrosine, *o*-benzyl-L-tyrosine, L-phenylalanine) is little affected by the change in the subphase pH because it consists of fewer ionizable side chains (25%) than the tyrosine-polypeptides mentioned above.

The film of copoly-1 : 1-(L-tyrosine, glycine) condenses more on the alkaline subphase than on distilled water, probably because of the peptenol effect. This copolypeptide can condense by peptenol effect because only half of all its residues have side chains and the contraction of the main chain is rather easy owing to the steric effect.

All the polypeptide monolayers mentioned above except copoly-1 : 1-(tyrosine, glycine) seem to be in the β-form because their limiting areas per residue are very close to 15 Å^2.

Oil/Water Interface.—At the oil/water interface, the films of poly-DL-phenylalanine, copoly-1 : 1-(L-tyrosine, L-phenylalanine) and copoly-1 : 1-(L-tyrosine, glycine) expand more than at the air/water interface and change from a condensed type at the air/water interface into an expanded type. These changes are caused by the release of van der Waals force between the side chains due to oil.

11) E. Katchalski and M. Sela, *J. Am. Chem. Soc.*, **75**, 5284 (1953).
12) T. Isemura and K. Hamaguchi, This Bulletin, **27**, 339 (1954).
13) T. Isemura, K. Hamaguchi and S. Ikeda, *J. Polymer Sci.*, **23**, 651 (1957).

The change in the subphase pH appears to be more effective than at the air/water interface. The films of copoly-1 : 1-(L-tyrosine, L-phenylalanine) and copoly-1 : 1-(L-tyrosine, glycine) are more expanded on the alkaline than on the acid subphase, while at the air/water interface, the films of these copolypeptides are slightly affected by the change in pH. At the oil/water interface, the release of the van der Waals force between the side chains causes the expansion of the film by the repulsion among ionized groups.

Effect of Glycyl Residue on Polypeptide Monolayers.—At both the air/water and oil/water interfaces, the film of copoly-1 : 1-(L-tyrosine, glycine) occupies much less area per residue than in any other polypeptide mentioned above.

This copolypeptide contains the same number of tyrosyl and glycyl residues, so both the residues should affects its film properties. However, the tyrosyl residue would not be responsible for a small area per residue of copoly-1 : 1-(L-tyrosine, glycine) because poly-L-tyrosine and tyrosine-copolypeptides without glycyl residue, such as copoly-1 : 1-(L-tyrosine, L-phenylalanine) and copoly-1 : 1 : 2-(L-tyrosine, *o*-benzyl-L-tyrosine, L-phenylalanine), were spread in the β-form. The presence of glycyl residue in polypeptide chain might result in a small limiting area per residue.

Polyglycine (in trifluoroacetic acid) and copoly-1 : 2-(L-tyrosine, glycine) (in a DCA-benzene mixture) could not be spread as a monolayer at the air/water interface[14]. Davies[5] also found polyglycine not to be spread as a monomolecular film. Isemura and Hamaguchi[15] found that at the air/water interface the film of copoly-1 : 1-(glycine, DL-alanine) occupied a very small area per residue. They attributed this fact to the folding of the polypeptide chain due to the presence of the glycyl residue.

From these facts, the reason why copoly-1 : 1-(L-tyrosine, glycine) occupies much less area at both the air/water and oil/water interfaces than any other polypeptides studied here may be attributed to the folded structure caused by strong intrachain hydrogen bonding due to the presence of a glycyl residue which lacks a side chain.

14) T. Yamashita, unpublished results.
15) T. Isemura and K. Hamaguchi, This Bulletin, 27, 339 (1954).

Summary

The monolayers of poly-DL-phenylalanine (I), poly-L-tyrosine (II), copoly-1 : 1-(L-tyrosine, L-phenylalanine) (III), copoly-1 : 1 : 2-(L-tyrosine, *o*-benzyl-L-tyrosine, L-phenylalanine) (IV), and copoly-1 : 1-(L-tyrosine, glycine) (V) have been investigated at the air/water and oil/water interfaces.

The surface moment of I decreased by 50~60 mD more on alkaline than on the acid subphase, probably owing to the keto-enol transformation of the peptide bond. The decrease in the surface moment of II is about 100 mD; this decrease seems to be caused by the ionization of the side chains and by the enolation of the peptide bond.

At the air/water interface, the effects of the dissociation of the tyrosyl residue on the *Π-A* relations of II, III, IV and V were not very remarkable, probably because of the peptenol effect and the van der Waals force between side chains. It was found that the monolayers of I, II, III and IV assume a β-configuration. On the other hand, the film of V occupies much less area per residue than those in the β-form, which might be attributed to the folded structure caused by the strong intrachain hydrogen bond due to the glycyl residue.

At the oil/water interface, the films of I, III and V expand more and the change in subphase pH seems to be more effective than at the air/water interface. These facts might be caused by the release of van der Waals force between the side chains.

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V. Monolayers of Nylons prepared from ω-Aminocarboxylic Acids at Air/Water Interface

Polyamides of nylon type are prepared from diamines and dicarboxylic acids or from ω-aminocarboxylic acids, and they are commonly designated by the numbers of carbon atoms per monomer unit. For example, polyhexamethylene sebacamide, $(-HN(CH_2)_6NHCO(CH_2)_8CO-)_n$, and polyundecanamide, $(-HN(CH_2)_{10}CO-)_n$ are Nylon 610, and Nylon 11, respectively. It has been found that the melting points of nylons with odd numbers of CH_2 groups per monomer unit are lower than those with even numbers[1~4]. This fact was ascribed to the deficient hydrogen bond formation of the former nylons.[1,2] Kinoshita[5,6] has shown that the odd number of CH_2 groups in polyamides does not lead to the deficient hydrogen bond formation, but in general gives rise

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1) G. Champetier and R. Aélion, Bull. Soc. Chim. France, 1948, 683.

2) R. Hill and E.E. Walker, J. Polymer Sci., 3, 609 (1948).

3) D.D. Coffman, G.J. Berchet, W.R. Peterson, and E.W. Spanagel, ibid., 2, 306 (1947).

4) D.D. Coffman, N.L. Cox, E.L. Martin, W.E. Mochel and F.J. Van Natta, ibid., 3, 85 (1948).

5) Y. Kinoshita, Makromol. Chem., 33, 1 (1959).

6) Y. Kinoshita, ibid., 33, 21 (1959).

to somewhat similar configuration to the pleated sheets structure proposed by Pauling and Corey[7] for the structure of polypeptide. This configuration was called γ-form. On the other hand, polyamides with even numbers of CH_2 groups usually contain two different crystalline forms, α and β, which have the same chain repeat distance and differ only in the side-by-side arrangement of molecules.

In this connection, it is interesting to study the effect of the numbers of CH_2 groups on the nature of monolayers of a series of nylons. Although the monolayers of some nylons have been studied by several authors[8~15], no studies have been carried out in this line. Accordingly, in the present work, a series of nylons with 2 to 11 CH_2 groups per monomer unit (Nylons 3 to 12) prepared from ω-aminocarboxylic acids

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7) L. Pauling and R.B. Corey, Proc. Nat. Acad. Sci. U.S.A., 39, 253 (1953).

8) D.J. Crisp, J. Colloid Sci., 1, 161 (1946).

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

10) T. Isemura and K. Hamaguchi, Bull. Chem. Soc. Japan, 25, 40 (1952).

11) T. Isemura and K. Hamaguchi, ibid., 27, 125 (1954).

12) H. Hotta, J. Colloid Sci., 9, 504 (1954).

13) K. Inokuchi, Bull. Chem. Soc. Japan, 28, 453 (1955).

14) K. Inokuchi, ibid., 29, 490 (1956).

15) G.E. Hibberd and A.E. Alexander, Proc. 3rd Int. Cong. Surface Activity, 2, 144 (1960).

has been studied at air/water interface in order to investigate the correlation of the film properties with the numbers of CH_2 groups per monomer unit. The film of Nylon 9 at oil/water interface and that of polyurea, polynonamethylene urea $(-(CH_2)_9HNCONH-)_n$ at air/water interface have also been studied to prove the role of van der Waals attractive force between hydrocarbon groups in polyamide monolayers.

Experimental

Materials. ----- Nylon 3 (poly-β-alanine) which was prepared from acrylamide was supplied by Prof. S. Murahashi of Osaka University. The insoluble fraction in boiling water was used in the present experiment. Its intrinsic viscosity was 0.432 (in 90 % formic acid at 25°C). Nylon 4 ($n = 87$) and Nylon 5 ($n < 25$) were prepared from pyrrolidone by Dr. Y. Joh of Mitsubishi Rayon Co., and from piperidone by Mr. T. Konomi of Toyo Spinning Co., respectively. Nylons 7, 8 and 10 were supplied by Dr. Y. Kinoshita and Nylon 12 by Dr. M. Ito of Toyo Rayon Co. The intrinsic viscosities of these nylons were more than 0.9 (in cresol at 25°C). Nylons 7, 8 and 10 are the same samples as those used in Kinoshita's investigation[8]. Nylon 11 ($m = 16,000$) is a gift from Dr. V. Ambekar of École Supérieure de Physique et de Chimie de Paris. Nylon 6 (Amilan), Nylon 9 and polynonamethylene urea $(-(CH_2)_9HNCONH-)_n$ are commercial products.

Nylons 3, 4 and 5 were used without further purification. Nylon 6 was purified by precipitation from a solution in formic acid by adding water. The chips or fibers of Nylons

7 to 12 and polyurea were immersed in benzene for 24 hours and then in ethyl alcohol for the same hours. All the samples were dried at 80°C in vacuo for 3-4 hours.

Nylons 3 to 9 were spread from solutions in 90 % formic acid, Nylons 10, 11 and 12 from solutions in 1:1-mixture (v/v) of dichloroacetic acid and trifluoroacetic acid, and polyurea from trifluoroacetic acid. Nylon 9 was also spread from a solution in trifluoroacetic acid. The concentrations of these solutions were 0.2-0.3 mg. per ml.

Subsolution containing 40 % ammonium sulfate, or concentrated sulfuric acid was treated with active charcoal before use to remove surface active contaminants.

Methods ----- The surface pressure was measured by the Wilhelmy method, the surface potential by the radioactive air-electrode method, the surface viscosity by the rotatory oscillation method, and the interfacial pressure by the ring method. The surface moment was calculated from the surface potential using the Helmholtz equation. The details of the experimental methods have already been described in Part I of this series.

Results

Nylons 3, 4 and 5 were spread on 40 % ammonium sulfate subsolution because stable films were not obtained on distilled water. For the sake of comparison, Nylon 6 was also spread on the same subsolution. The surface pressure-area (Π-A) curves of these nylons are shown in Fig. 1. The film of Nylon 3 was of condensed type, while those of Nylons 4, 5

and 6 were of expanded type.

The Π-A, surface moment-area (μ-A) and surface viscosity-area (η_s-A) curves of Nylons 6 to 12 on distilled water are shown in Figs. 2 to 8, respectively. The interfacial pressure-area (Π_i-A) curve of Nylon 9 is also shown in Fig. 5. The Π-A and μ-A curves of Nylons 6 to 9 on concentrated sulfuric acid subsolutions (3 N and 6 N) are shown in the corresponding figures.

The film of Nylon 6 on distilled water was of expanded type. The area per residue was somewhat less than that on 40 % ammonium sulfate. The film of Nylon 7 on distilled water was condensed rather more than that of Nylon 6, although the film was of expanded type.

The films of Nylons 8 to 12 were of condensed type on distilled water and their areas per residue were considerably small. Trifluoroacetic acid is one of the strongest hydrogen bond breaking agents. The film of Nylon 9 spread from a solution in this solvent gave a completely identical Π-A curve on distilled water (not shown in Fig. 5) with that spread from a solution in formic aicd on the same subsolution (shown in Fig. 5). Further, the Π-A curve of this nylon spread from a solution in formic aicd on 40 % ammonium sulfate (not shown in the figure) was nearly identical with that on distilled water. From these facts, the poor spreading of Nylons 8 to 12 seems to be ascribed to neither to the presence of insoluble fraction in the spreading solution nor to the dissolution of polymers in aqueous phase.

The constant values of surface moments were not obtained

with the films of Nylons 8 to 12 on distilled water. The surface moments were increased with compression of the films, and reached at the maxima. The surface moment of Nylon 12 was not reproducible. The maximum value, however, was lower than that of Nylon 11.

On 3 N and 6 N sulfuric acid subsolutions, the remarkable expansion of films and the increase in surface moments were found with Nylons 6 to 9. The stronger the acidity was, the greater the effect was. The π-A, μ-A and η_s-A curves of polynonamethylene urea on distilled water are shown in Fig. 9. This polymer also gave a condensed film.

The film characteristics of Nylons 6 to 12 and polyurea are summarized in Table I, where A_δ is the area per residue at the minimum compressibility of film, $A_{\pi\to 0}$ the area per residue where the straight portion of π-A curve is extraporated to $\pi = 0$, A_μ the area per residue where the surface moment begins to decrease, $\mu(m)$ and $\mu(c)$ the maximum and constant values of surface moments, respectively, and A_η the area per residue where the surface viscosity begins to rise.

For the sake of comparison with the observed limiting areas per residue, namely, A_δ's and $A_{\pi\to 0}$'s, the close-packed areas of Nylons 6 to 12 and polynonamethylene urea were calculated from the unit cell dimensions of their crystals estimated from X-ray diffraction data. The results are shown in Table I, where the distance between chain atoms of Nylons 6 to 12, and polyurea, with the exception of Nylons 6, 7, and 11 for which the X-ray diffraction data are

Table I. Film characteristics and calculated close-packed areas of Nylons 6 to 12 and polynonamethylene urea

Polymer		A_s Å²/residue	$A_{\pi\to 0}$ Å²/residue	A_μ Å²/residue	μ mD	A_η Å²/residue	Calculated close-packed area Å²/residue
Nylon 6	DW	14.4	19.2	37	180 (c)	80	$\frac{17.24}{2} \times \frac{9.56}{2} = 41.2$
	3 N H_2SO_4				400 (at 51 Å²/res.)		
	6 N H_2SO_4				445 (at 46 Å²/res.)		
Nylon 7	DW	25.0	26.5	40	355 (c)	40	9.85 x 4.9 x sin 77° = 47.0
	3 N H_2SO_4			53	505 (m)		
	6 N H_2SO_4			56	700 (m)		
Nylon 8	DW	17.6	19.0	25	200 (m)	87	1.23 x 9 x 4.77 = 52.8
	3 N H_2SO_4			54	420 (m)		
	6 N H_2SO_4				600 (at 48 Å²/res.)		
Nylon 9	DW	20.0	23.0	26	252 (m)	100	1.23 x 10 x 4.77 = 58.7
	3 N H_2SO_4			56	490 (c)		
	6 N H_2SO_4			57	620 (c)		
Nylon 10	DW	18.0	20.0	25	205 (m)	72	1.23 x 11 x 4.77 = 64.5
Nylon 11	DW	21.5	23.0	26	240 (m)	85	14.9 x 4.9 x sin 77° = 71.1
Nylon 12	DW	18.0	20.0			60	1.23 x 13 x 4.77 = 76.3
Polynonamethylene urea	DW	28.0	30.0	32	225 (c)	84	1.23 x 12 x 4.77 = 70.4

DW: Distilled water.

available[16,17], is assumed to be 1.23 Å for fully extended chains from the distance between chain atoms of Nylon 6 ($\frac{17.24}{2 \times 7}$[16] = 1.231 Å), Nylon 7 ($\frac{9.35}{4}$[17] = 1.231 Å), Nylon 11 ($\frac{14.9}{12}$[17] = 1.24 Å), and Nylon 66 ($\frac{17.2}{8}$[16] = 1.23 Å). The distance between neighboring chains is assumed to be 4.9 X sin 77° = 4.77 Å from those of Nylons 7[17], 11[17] and 66[18]. These values are also used for the calculation of close-packed areas of Nylon 3 and Nylon 4.

Discussion

Nylon 3.------ Nylon 3 gives a film of condensed type on 40 % ammonium sulfate. Its A_s and $A_{\pi \to 0}$ values are 8.2 Å² per residue and 9.0 Å² per residue, respectively. These values are much less than the calculated close-packed area for fully extended chain (23.5 Å² per residue). It has been found[19,20] that polyglycine cannot be spread under any conditions because glycyl residues are hydrogen bonded particularly strong owing to the absence of side chains. As Nylon 3 also lacks side chains, and the chain repeat distance is rather short, the hydrogen bonds would be very strong as in the case of polyglycine. This might be the cause of the poor spreading and condensation of Nylon 3 film.

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16) D.R. Holmes, C.W. Bunn and D.J. Smith, J. Polymer Sci., 17, 159 (1955).

17) W.P. Slichter, ibid., 36, 259 (1959).

18) C.W. Bunn and E.V. Garner, Proc. Roy. Soc., A189, 39 (1947).

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

20) T. Yamashita and T. Isemura, Bull. Chem. Soc. Japan. 35, 929 (1962).

Nylons 4, 5 and 6, ----- In contrast with the condensed film of Nylon 3, the films of Nylons 4, 5 and 6 are of expanded type on 40 % ammonium sulfate subsolution. Nylon 6 also gives an expanded film on distilled water. Kink points are found in the π-A curves of Nylons 4 and 6.

The kink point of π-A curve of Nylon 6 appears at 37.5 Å^2 per residue on distilled water and 40 Å^2 per residue on 40 % ammonium sulfate. The π-A curve of this nylon on distilled water has already been reported by some workers[10, 12, 14]. The present result is in general agreement with theirs. The kink point is also found by them at nearly the same area as in the present study. The surface moment of Nylon 6 on distilled water begins to decrease at 37 Å^2 per residue, which is in good agreement with the area at kink point and the A_μ values reported by Hotta[12] and Inokuchi[14]. The surface viscosity of this nylon begins to rise at 80 Å^2 per residue. This value is much less than that found by Isemura and Hamaguchi[11]. The larger area found by them seems to be caused by the interaction between polymer and solvent because they used a mixture of cresol and benzene as a spreading solvent. They, however, found a characteristic area at 80 Å^2 per residue, which agrees with the A_η value in the present study.

It has been found[21] that poly-α-aminoisobutyric acid gave a monolayer of expanded type. The striking difference, however, exists in the surface viscosities between the films of this polymer and Nylon 6. The surface viscosity of the former first

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21) Part I of this series.

appears at considerably small area (12 $\mathring{A}^2$ per residue) than its limiting area (A_δ = 20.0 $\mathring{A}^2$ per residue) where the surface pressure is remarkably high, while that of the latter is detected at larger area (80 $\mathring{A}^2$ per residue) than the calculated close-packed area for fully extended chains (41.2 $\mathring{A}^2$ per residue), where the surface pressure is sufficiently low. In the film of the former, the interaction between polymer chains is scarce because the hydrogen bonds between keto and imino groups are hardly formed owing to the steric hindrance of α-disubstituted methyl groups. On the other hand, the much larger A_γ value of Nylon 6 suggests that the interaction between polymer chains is considerably great. Near A_γ, the polymer molecules at the surface would begin to contact with each other to construct a loose network structure owing to the hydrogen bonding between amide groups.

The areas at kink points of π-A curves of Nylon 4 (28 $\mathring{A}^2$ per residue on 40 % ammonium sulfate) and Nylon 6 (37.5 $\mathring{A}^2$ per residue on distilled water, 40 $\mathring{A}^2$ per residue on 40 % ammonium sulfate) agree fairly well with the calculated close-packed areas (29.3 $\mathring{A}^2$ per residue for Nylon 4, 41.2 $\mathring{A}^2$ per residue for Nylon 6). All the chain atoms of these polymers would lie on the water surface above the areas at kink point. A kink point is not found in the π-A curve of Nylon 5. This polymer, however, might be spread in a similar state to those of Nylons 4 and 6, because the film is remarkably expanded. The hydrogen bonds between keto and imino groups might not be so strong in the monolayers of Nylons 4, 5 and 6 as in the case of Nylon 3 because the ratio of these

groups in polymer chains is decreased more with the increase in number of CH_2 groups, and the polymer chains would be rather flexible. This would be the cause of expansion of films of Nylons 4, 5 and 6.

There is some difference among the films of Nylons 4, 5 and 6 on 40 % ammonium sulfate subsolution, although they are of expanded type. Below the surface pressure of ca. 4 dyn. per cm., the film of Nylon 5 is most expanded as shown in Fig. 5. The surface pressure of Nylon 6 is much lower than that of Nylon 5 at the same area. The pressure reduction in film of Nylon 6 would be caused by the increased van der Waals attractive force between hydrocarbon groups. It has been found in the present work that van der Waals attractive force plays an important role in the monolayers of Nylons 7 to 12 as will be discussed in the later sections.

Nylon 7.----- The film of Nylon 7 on distilled water is of expanded type. The film, however, is somewhat condensed than that of Nylon 6 on the same subsolution. This fact would be due to the increased van der Waals attractive force between hydrocarbon groups. The film of Nylon 7 might be in a intermediate state between expanded films obtained with Nylons 4, 5 and 6, and condensed films obtained with Nylons 8 to 12.

The surface moment of this nylon is remarkably high, which is the highest among those of nylons investigated. The A_μ value is 40 $Å^2$ per residue, which agrees with the A_γ value. The surface moments of polyamides of nylon type would be mainly contributed by the C=O dipoles as in the case of poly-α-amino acid with only hydrocarbon side chains[22].

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

The more vertical the C=O dipoles become to the interface, the closer the surface moment will approach the maximum value of 360 mD. The observed surface moment of Nylon 7 ($\mu_{(c)}$= 355 mD) is in good agreement with this value. Then, the C=O groups of Nylon 7 might orientate rather vertically to the interface. In such an orientation of C=O groups, the hydrogen bonds would hardly be formed between amide linkages above the area at A_μ. It has already been found for the monolayers of poly-α-aminoisobutyric acid[21] on distilled water, poly-DL-alanine[20] and poly-DL-leucine[19] on acid subsolutions that the surface moment increases if the number of hydrogen bonds is reduced.

The surface viscosities of polymers primarily depend on the number of hydrogen bonds between different groups as has been found with prolyl polypeptides[23], poly-α-aminoisobutyric acid[21] and sarcosyl polypeptides[21,24]. The agreement of A_μ with A_η supports the view that the hydrogen bonds would hardly be formed between amide groups above the area at A_μ because of the unfavorable orientation of C=O groups for the hydrogen bond formation.

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23) S. Ikeda and T. Isemura, Bull. Chem. Soc. Japan, 32, 655 (1959).

24) Part II of this series.

Differences in film properties between odd and even numbered nylons.----- It has been found[1~5] that the melting points of nylons with odd and even numbers of CH_2 groups are considerably different from each other. Some film characteristics of Nylons 6 to 12 on distilled water are shown in Fig. 10 against the number of carbon atoms per monomer unit. There exist striking differences between odd and even numbered nylons as in melting points. It should be noted here that the amino acid nylons with even numbers of CH_2 groups are described by odd numbers, and vice versa.

It is striking that the Π-A curves of Nylons 8, 10 and 12 are nearly identical. Accordingly, the limiting areas, namely, A_{δ}'s or $A_{\Pi \to 0}$'s of these nylons are nearly the same per residue with each other as shown in Table I. Nylon 9 and Nylon 11 also have nearly the same limiting areas per residue with each other, although the areas are somewhat larger than even numbered nylons. The surface moments of odd numbered nylons are somewhat high compared with those of even numbered nylons in the maximum values. The surface moment of Nylon 12 is not shown in Table I and Fig. 10 because of its poor reproducibility. The maximum value, however, lower than that of Nylon 11. Surface viscosities of odd numbered nylons with an exception of Nylon 7 begin to rise at larger areas than those of even numbered nylons. These differences in film properties between odd and even numbered nylons will be discussed in the succeeding sections.

Condensation of films of Nylons 8 to 12.----- In contrast with the condensed films of Nylon 9 at air/water interface, the film of this polymer at oil/water interface is highly expanded as shown in Fig. 5.

It has been found that the films of poly-α-amino acids[9,20] and some vinyl polymers[12] are expanded much more at oil/water interface than at air/water interface owing to the release of van der Waals force between hydrocarbon groups. Accordingly, it seems that van der Waals attractive force between hydrocarbon groups plays an important role in the remarkable condensation of these nylon films.

The hydrogen bonds between keto and imino groups of amide linkages would also be responsible for the condensation of these films, because the film properties of nylons are affected remarkably on concentrated sulfuric acid subsolution which causes the breaking of hydrogen bonds, and the surface viscosities of Nylons 8 to 12 are manifested at considerably large areas as will be discussed in the succeeding sections.

Thus, it seems that both van der Waals force between hydrocarbon chains and hydrogen bonds between keto and imino groups of amide linkages are responsible for the remarkable condensation of the films of Nylons 8 to 12 on distilled water.

Holt and Went[25] reported that with compression of the films the hydrophilic amide groups of polyamide films remain at surface, but the polymethylene groups are, presumably, pushed out of the surface. The consistency of the observed small limiting areas per residue among even or odd numbered nylons suggests that the hydrocarbon chains leave the surface with compression of the films and are folded owing to the van der Waals attractive force between them. The hydrogen bonding between amide groups might also play a significant role in the stability of such folded structures.

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25) P.F. Holt and C.W. Went, Proc. 3rd Int. Cong. Surface Activity, 2, 49 (1960).

Similar folded structures have been found by Isemura and Hotta[26] for adsorbed films of long chain dibasic acids. If the hydrocarbon groups leave the surface and are folded, the contributions of monomer units of even or odd numbered nylons to the limiting areas should be identical with each other. The somewhat larger areas of odd numbered nylons than those of even numbered ones might be due to the difference in packings of folded chains. The difference in maximum surface moments between both series of nylons would be caused by the same reason.

The maximum values of surface moments of Nylons 8 to 11 are between 200 and 250 mD. These values are somewhat higher than the constant values of surface moments of poly-α-amino acids, which are in the range from 150 mD to 180 mD on neutral subsolutions[20~22,24]. It has been found that[21] for poly-α-aminoisobutyric acid on distilled water and poly-DL-alanine on acid subsolution (3 N H_2SO_4) that the constant values of surface moments are increased to ca. 210 mD because the hydrogen bonds between keto and imino groups are scarce in these films. The higher $\mu(m)$ values of Nylons 8 to 11 on distilled water than $\mu(c)$ values of poly-α-amino acids on neutral subsolutions would not be caused by a small number of hydrogen bonds, but by a somewhat different orientation of $>C=O$ and $H-N<$ groups from that in poly-α-amino acids accompanied by a remarkable change in orientation of these groups during the folding process, because the presence of hydrogen bonds is proved as has been described in this and succeeding sections.

<u>Surface viscosities of Nylons 8 to 12</u>.----- The surface viscosities of Nylons 8 to 12 begin to rise at much larger areas than their limiting areas as shown in Table I. Near the area at A_γ, the polymer chains of these nylons would

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26) T. Isemura and H. Hotta, Bull. Chem. Soc. Japan, 23, 193 (1950).

begin to contact with each other to form a network structure owing to the interaction between keto and imino groups of different amide groups.

The A_γ values of odd numbered nylons are much larger than those of even numbered nylons. This fact suggests that hydrogen bond formation between amide groups takes place more easily in the former nylons than in the latter nylons. It seems that above the areas at A_γ almost all the chain atoms of these nylons lie on the water surface because the A_γ values are larger than the calculated close-packed areas excepting Nylon 12, and with high compression of films the hydrocarbon groups would leave the surface and be folded.

It has been reported by several authors[1,2,17] for the crystals of nylons that in the fully extended chains of nylons with even numbers of CH_2 groups all the amide groups on one chain lie directly next to amide groups on neighboring chains, so that the maximum degree of coupling is attainable, while in the fully extended chains of nylons with odd numbers of CH_2 groups the parallel array leads to close coupling of only half of the polar groups although the antiparallel array allows the coupling of all the amide groups. The difference in A_γ values in the present study between odd and even numbered nylons might be related to the difference in probabilities of hydrogen bond formation between both series of nylons. Although the deficient hydrogen bond formation in crystals of nylons with odd numbers of CH_2 groups was denied by Kinoshita,[5,6] it might have any effect on the areas where surface viscosities appear because these areas are closely related to the initial stage of hydrogen bond formation.

Polynonamethylene urea ----- The monolayer of this polymer has been investigated in order to prove the role of van der Waals attractive force between hydrocarbon groups for the stabilization of a folded structure of polyamides of nylon type. Polyurea was found to give a condensed film on distilled water as well as Nylons 8 to 12, although the limiting area is somewhat larger than those for nylons. The constant value of surface moment was obtained in a very narrow range, and was 225 mD., which is the same order of magnitude as the μ(m) values of Nylons 8 to 12. From these facts, this polyurea is considered to assume a folded structure near the limiting area like even numbered nylons, because this polymer contains 9 CH_2 groups per residue. Somewhat larger values of A_s (28 $\mathring{A}^2$ per residue), $A_{\pi\rho}$ (30 $\mathring{A}^2$ per residue) and A_μ (32 $\mathring{A}^2$ per residue) would be due to the contribution from another N-H group in the residue. At 84 $\mathring{A}^2$ per residue, the surface viscosity was first detected. Near this area, the polymer molecules begin to contact ~~each other~~ to interact with each other. With further compression, the hydrocarbon groups would be folded. The folded structure might be maintained both by van der Waals attractive force and by hydrogen bonds.

Effect of sulfuric acid on the monolayers of nylons ----- As has been shown in Figs. 2-5 and in Table I, on concentrated sulfuric acid, the remarkable expansion of films and the increase in surface moments are found with Nylons 6 to 9. The stronger the acidity is, the greater the effect is. It has been reported in Part I of this series that on concentrated sulfuric acid, poly-DL-alanine film is expanded much more

than on distilled water, and its surface moment increases from 150 mD ($\mu(c)$) to 215 mD ($\mu(c)$) and 256 mD (at 26 $Å^2$ per residue) on 3 N and 6 N solutions, respectively. These changes would be due to the breaking of hydrogen bonds between keto-imino groups. Poly-α-amino-isobutyric acid monolayer[22], in which hydrogen bonds are hardly formed because of the steric hindrance of α-disubstituted methyl groups, gives also high surface moment ($\mu(c)$, 214 mD) compared with poly-DL-alanine on distilled water. The surface moments of nylons on acid subsolutions are much higher than those of poly-DL-alanine on acid and poly-α-aminoisobutyric acid on distilled water[22], and the maximum contribution from C=O group (360 mD)[23]. Accordingly, a different cause must be sought for the remarkably high surface moments besides the breaking of hydrogen bonds. Such a high surface moment has been found by Hibberd and Alexander[15] for Nylon 610 (1038 mD per two amide groups at maximum on 3 N sulfuric acid). However, the reason why this nylon has a high surface moment is not given by the authors.

The adsorption of hydrochloric acid by nylon fibers has been studied as a function of acid concentration[27]. In concentrated hydrochloric acid solutions, nylon absorbs more acid than would be expected from the number of amino groups present. This excess absorption was attributed to the interaction of the acid with amide linkages. The amide groups of

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27) F.T. Wall and A. Beresniewicz, J. Phys. Chem., 60, 692 (1956).

nylon behave as base in acid medium. The interaction of hydrochloric acid with CONH groups of nylon has been proved by Larose[28, 29] from the measurements of infrared and near-infrared spectra of nylon treated with hydrochloric acid.

The addition of acid to the CONH group of nylon will be represented by

$$-CONH- + H^+ \text{-----} -CONH_2^+- .$$

Thus, nylon film would be charged positively. As a result of protonation to amide linkage, the surface moments would be increased remarkably.

The folded structures of higher nylons on distilled water are unfolded on the subsolutions of sulfuric acid because the hydrogen bonds which would stabilize the folded structure are broken. The repulsion between charged groups causes a remarkable expansion of the film. The polymer chains would be in a rather flexible state.

Summary

The surface films of a series of nylons with 2 to 11 CH_2 groups per monomer unit (Nylons 3 to 12) prepared from ω-amino-carboxylic acids have been studied at air/water interface in order to investigate the correlation of the film properties with numbers of CH_2 groups. The films of Nylon 9 at oil/water interface and of polynonamethylene urea at air/water interface have also been studied.

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28) P. Larose, Can. J. Chem., 35, 1239 (1957).

29) P. Larose, ibid., 39, 2394 (1961).

The film of Nylon 3 was of condensed type and the area per residue was very small probably owing to the especially strong hydrogen bonds between keto and imino groups as in the case of polyglycine. The films of Nylons 4, 5 and 6 on 40 % ammonium sulfate and of Nylon 6 on distilled water were of expanded type. These polymers would be spread in rather flexible states because the hydrogen bonds between keto and imino groups are not so strong as in Nylon 3 owing to the effect of increased CH_2 groups. The film of Nylon 7 was somewhat condensed on distilled water than that of Nylon 6, although the film was of expanded type. This fact might be caused by the increased van der Waals attractive force between hydrocarbon groups.

Nylons 8 to 12 gave condensed films on distilled water and the limiting areas per residue were remarkably small compared with the calculated areas for fully extended chains. The remarkable expansion of film was observed with Nylon 9 at oil/water interface. This fact suggests that van der Waals attractive force between hydrocarbon groups is responsible for the condensation of films of these nylons. The very small limiting areas per residue of these nylons were interpreted by assuming that the hydrocarbon chains are folded owing to van der Waals attractive force between CH_2 groups. Hydrogen bonds between keto and imino groups seem also to stabilize such folded structures because the subsolutions of concentrated sulfuric acid affect remarkably the film properties.

The striking differences have been found between odd numbered and even numbered nylons in the surface pressure-area,

surface moment-area, and surface viscosity-area relations of Nylons 8 to 12. The limiting areas per residue of odd numbered nylons were somewhat larger than even numbered ones, and the maximum surface moments of the formers were rather higher than those of the latters. These facts would be due to the difference in the packing of folded chains between odd and even numbered nylons. The areas of Nylons 8 to 12 where surface viscosities were first detected were much larger than the observed limiting areas per residue. The hydrogen bonds between keto and imino groups would be formed at such large areas. The areas of even numbered nylons were much less than those of odd numbered ones. This fact would be due to the difference in the modes of hydrogen bond formation between amide groups.

The film of polynonamethylene urea was also condensed type on distilled water and its limiting area per residue was much less than the calculated area. This polymer might be spread in a folded state as in the case of Nylons 8 to 12.

The remarkable effect of concentrated sulfuric acid on the film properties of Nylons 6 to 9 was found, being interpreted by the protonation to the amide linkages.

The author wishes to express his gratitude to Prof. Toshizo Isemura of Osaka University for his kind guidance throughout the present work and to Dr. Shoichi Ikeda of the same University for his helpful discussion. He is also indebted to Dr. Yukio Kinoshita and Dr. Masatoshi Ito of Toyo Rayon Co., Prof. Shunsuke Murahashi of Osaka University,

Dr. Yasushi Joh of Mitsubishi Rayon Co., Mr. Tsuyoshi Konomi of Toyo Spinning Co., and Dr. Vassant Ambekar of Ecole Supérieure de Physique et de Chimie de Paris for the supply of samples.

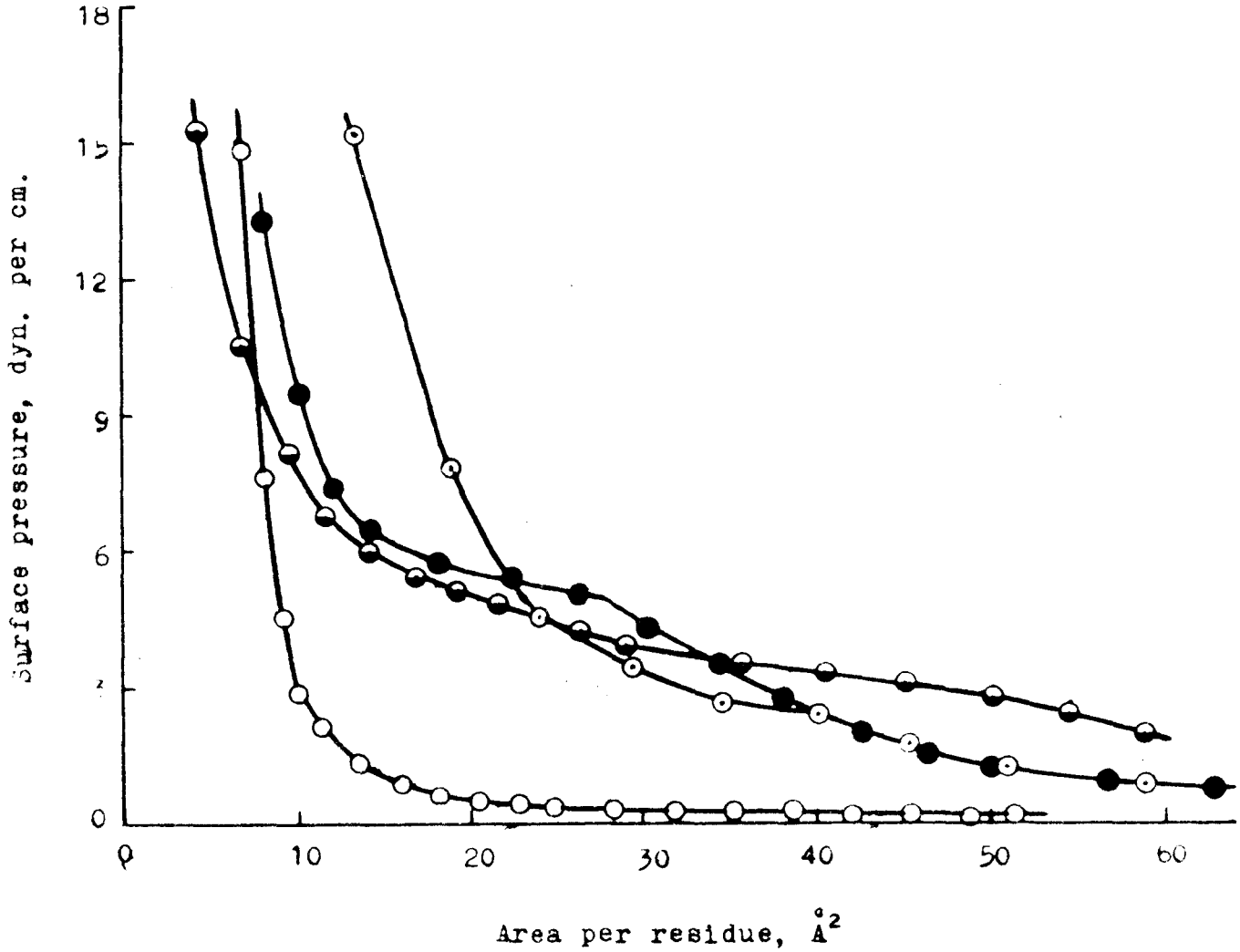

Fig. 1. Surface pressure-area curves of Nylon 3 (○), Nylon 4 (●), Nylon 5 (◒) and Nylon 6 (⊙) on 40 % ammonium sulfate at 23°C.

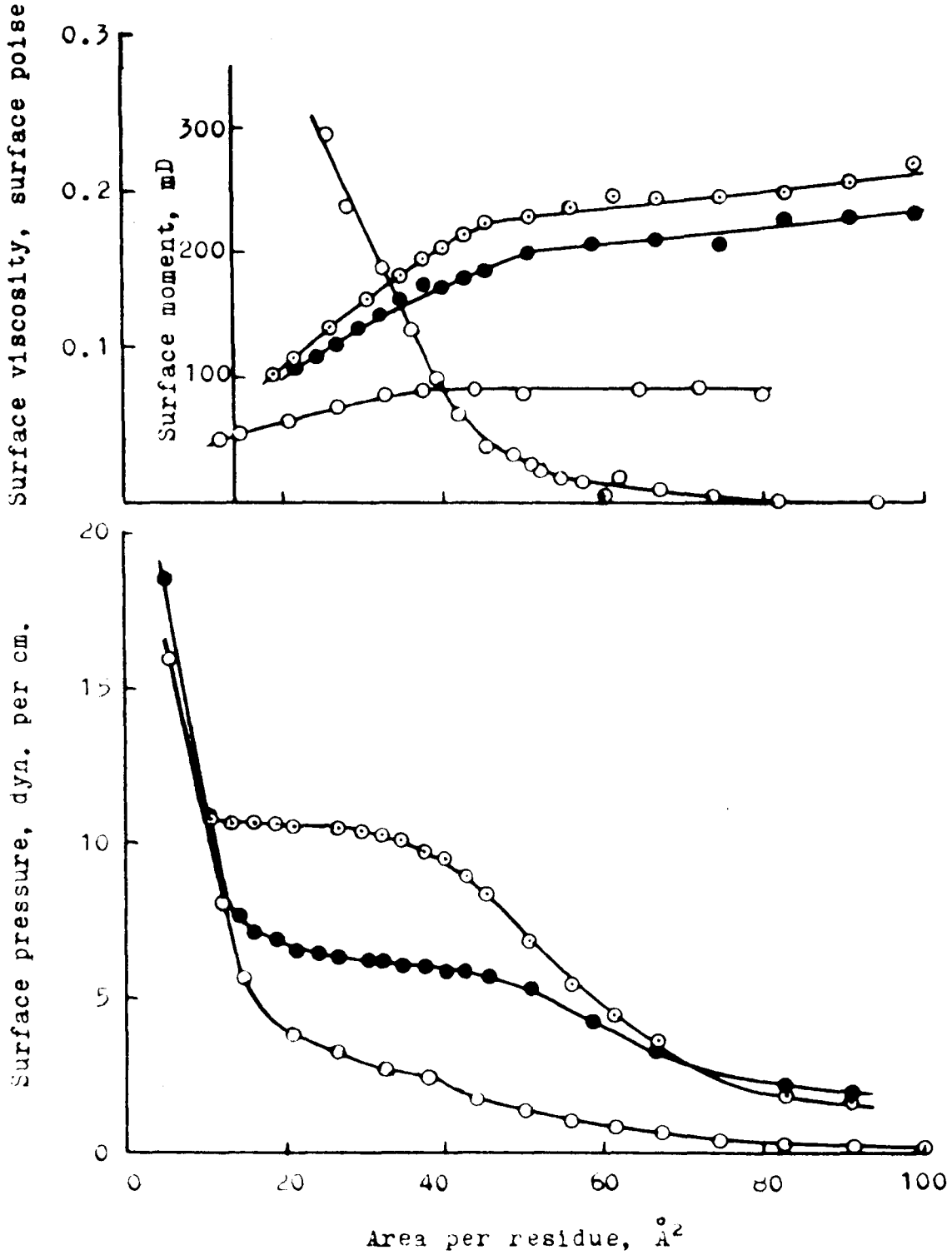

Fig. 2. Surface pressure-area and surface moment-area curves of Nylon 6 on distilled water (○) and on sulfuric acid (3N, ●; 6N, ⊙), and its surface viscosity-area curve on distilled water (◒) at 22°C.

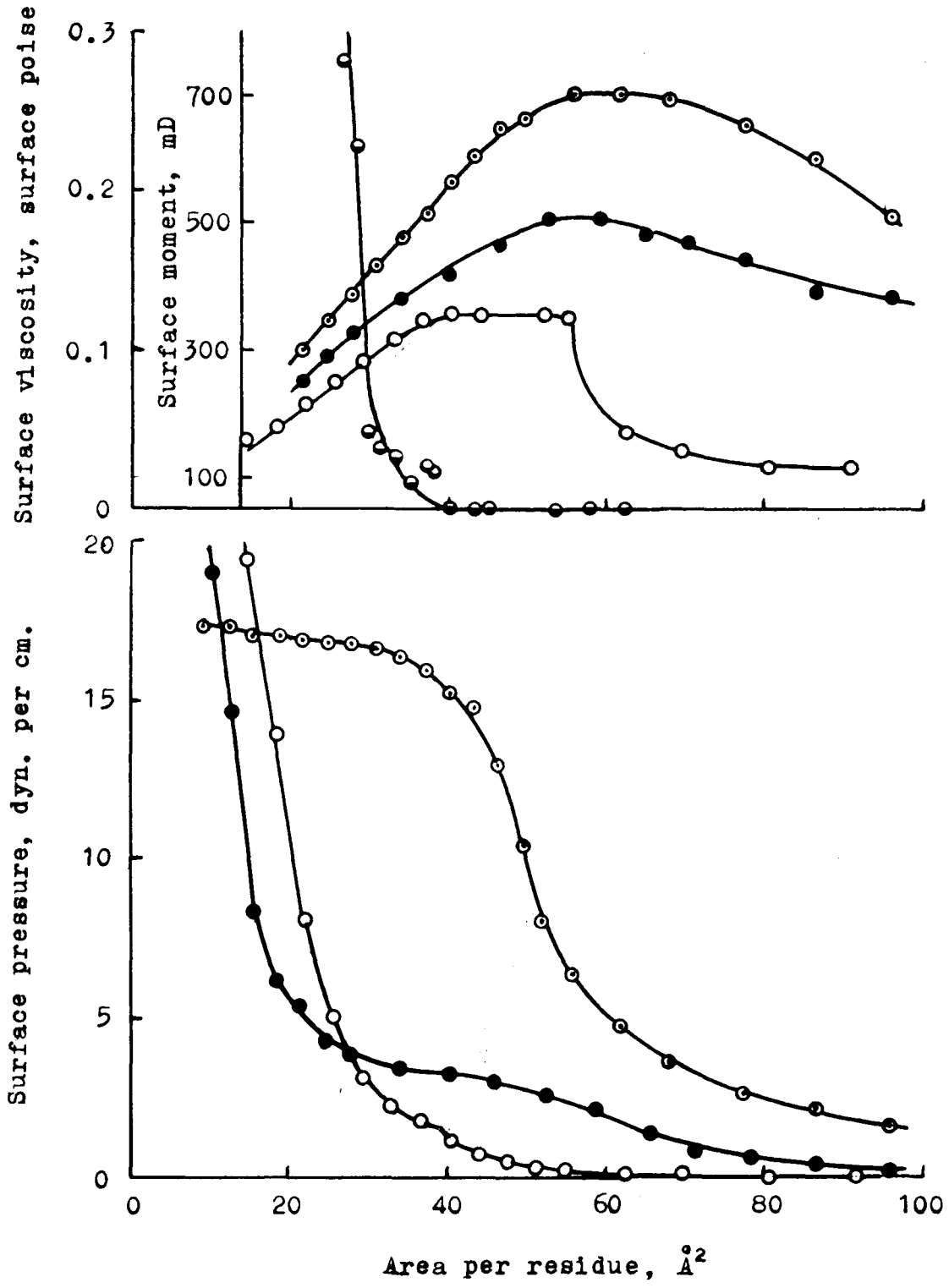

Fig. 3. Surface pressure-area and surface moment-area curves of Nylon 7 on distilled water (○) and on sulfuric acid (3N, ● ; 6N, ⊙) at 21°C, and its surface viscosity-area curve on distilled water (◒) at 17°C.

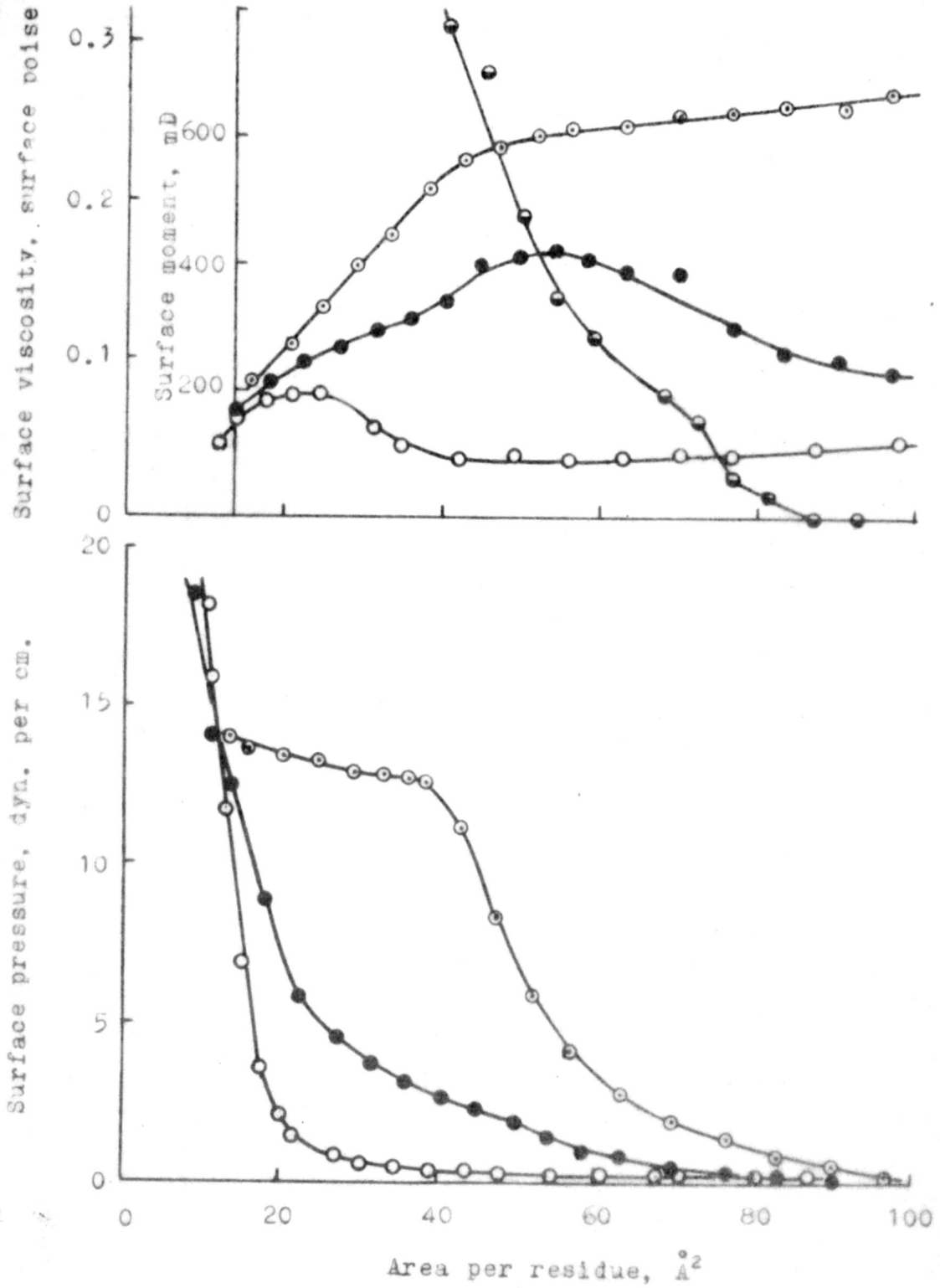

Fig. 4. Surface pressure-area and surface moment-area curves of Nylon 8 on distilled water (○) and on sulfuric acid (3N, ● ; 6N, ⊙), and its surface viscosity-area curve on distilled water (◒) at 21°C.

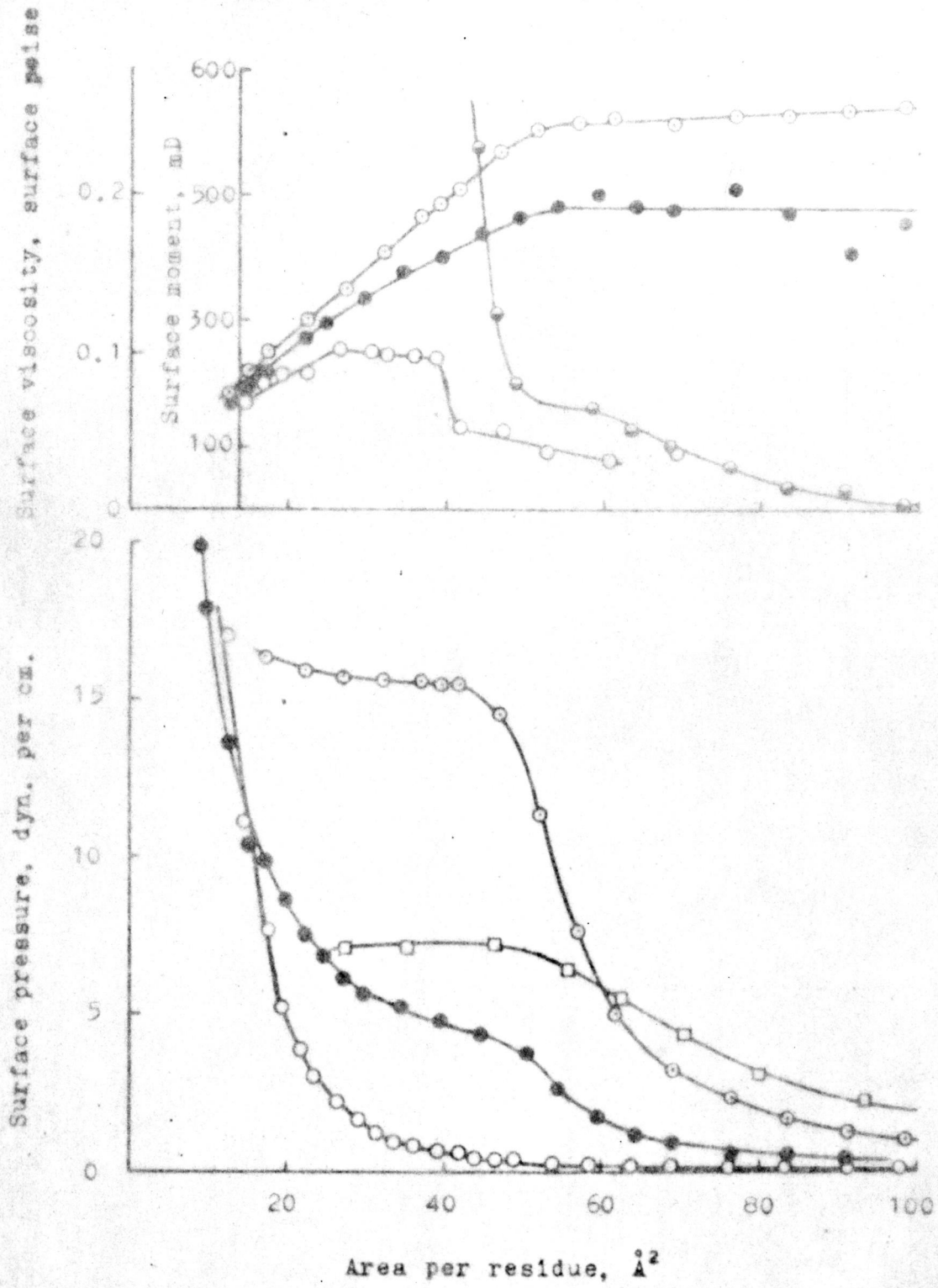

Fig. 5. Surface pressure-area and surface moment-area curves of Nylon 9 on distilled water (○) and on sulfuric acid (3N, ● ; 6N, ⊙) at 24°C, and its surface viscosity-area (◒, 24°C) and interfacial pressure-area (□, 18°C) curves on distilled water.

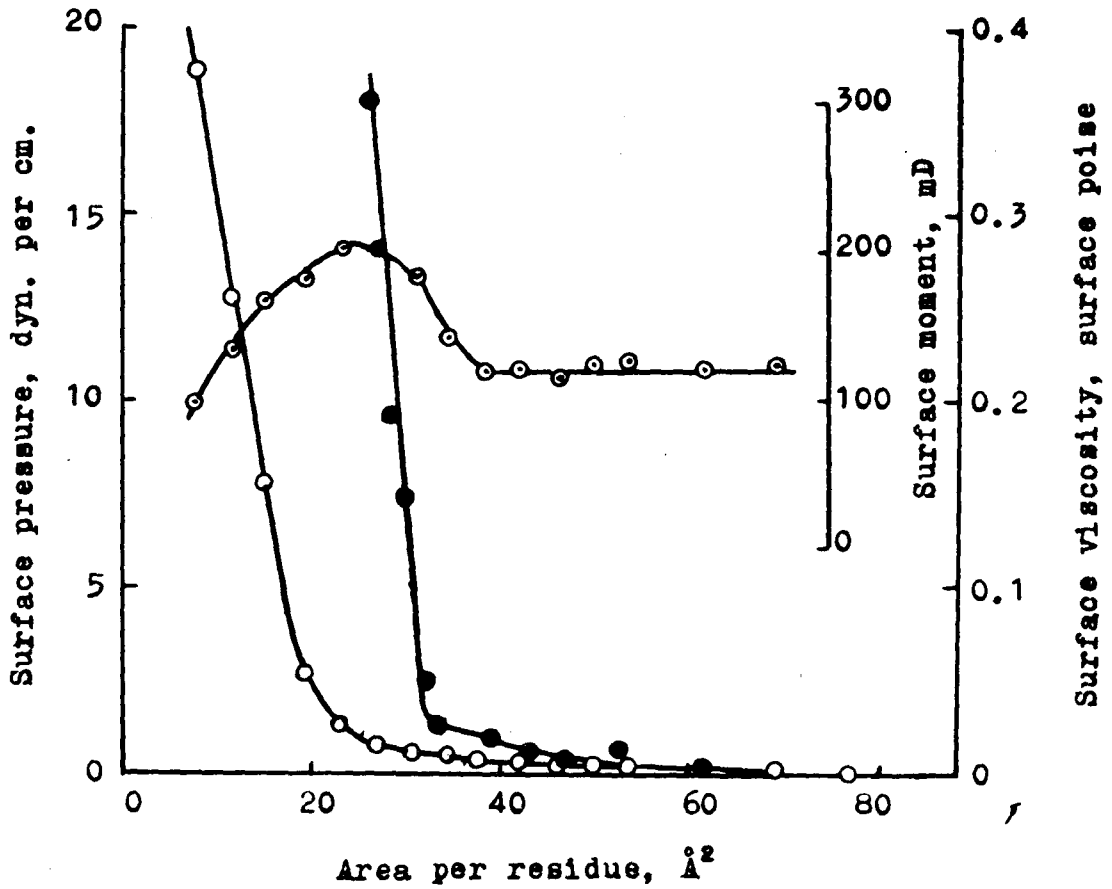

Fig. 6. Surface pressure-area (○), surface moment-area (⊙) and surface viscosity-area (●) curves of Nylon 10 on distilled water at 21°C.

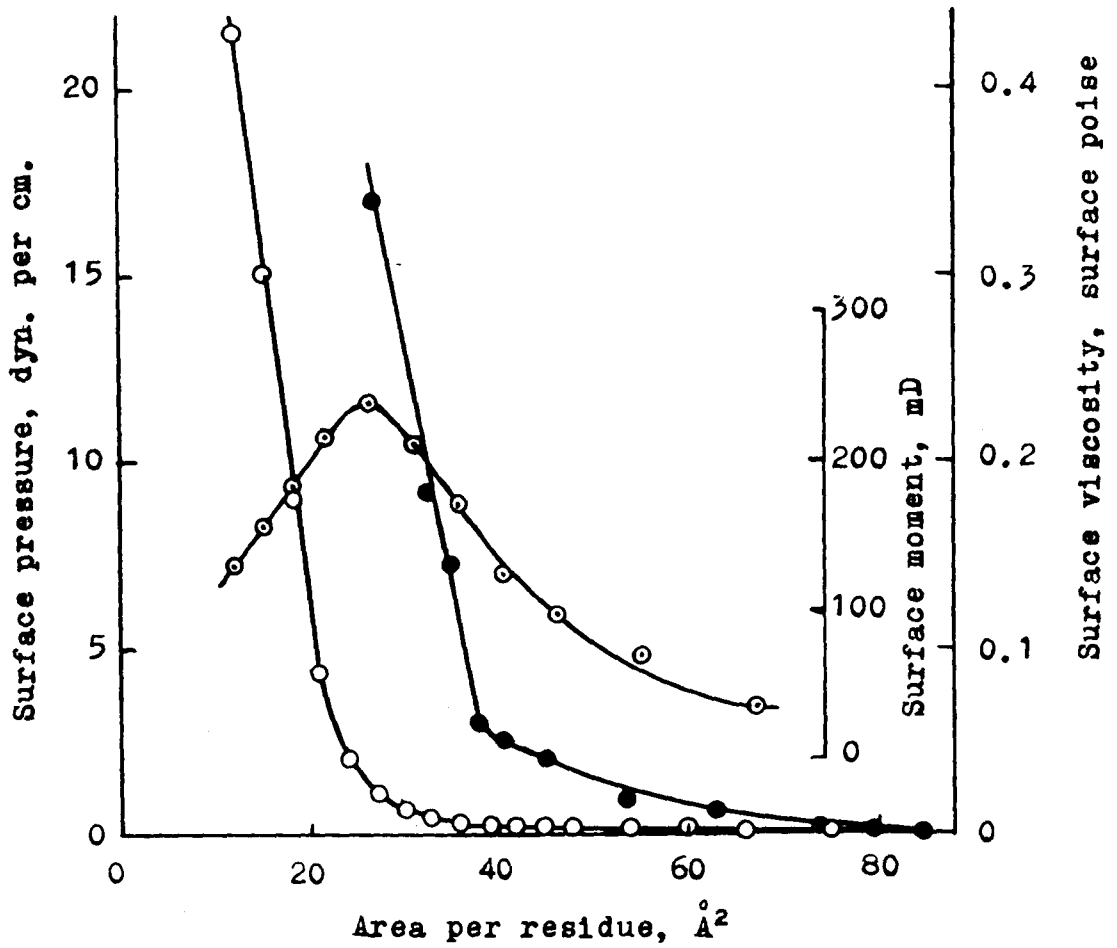

Fig. 7. Surface pressure-area (○), surface moment- area (⊙) and surface viscosity-area (●) curves of Nylon 11 on distilled water at 21°C.

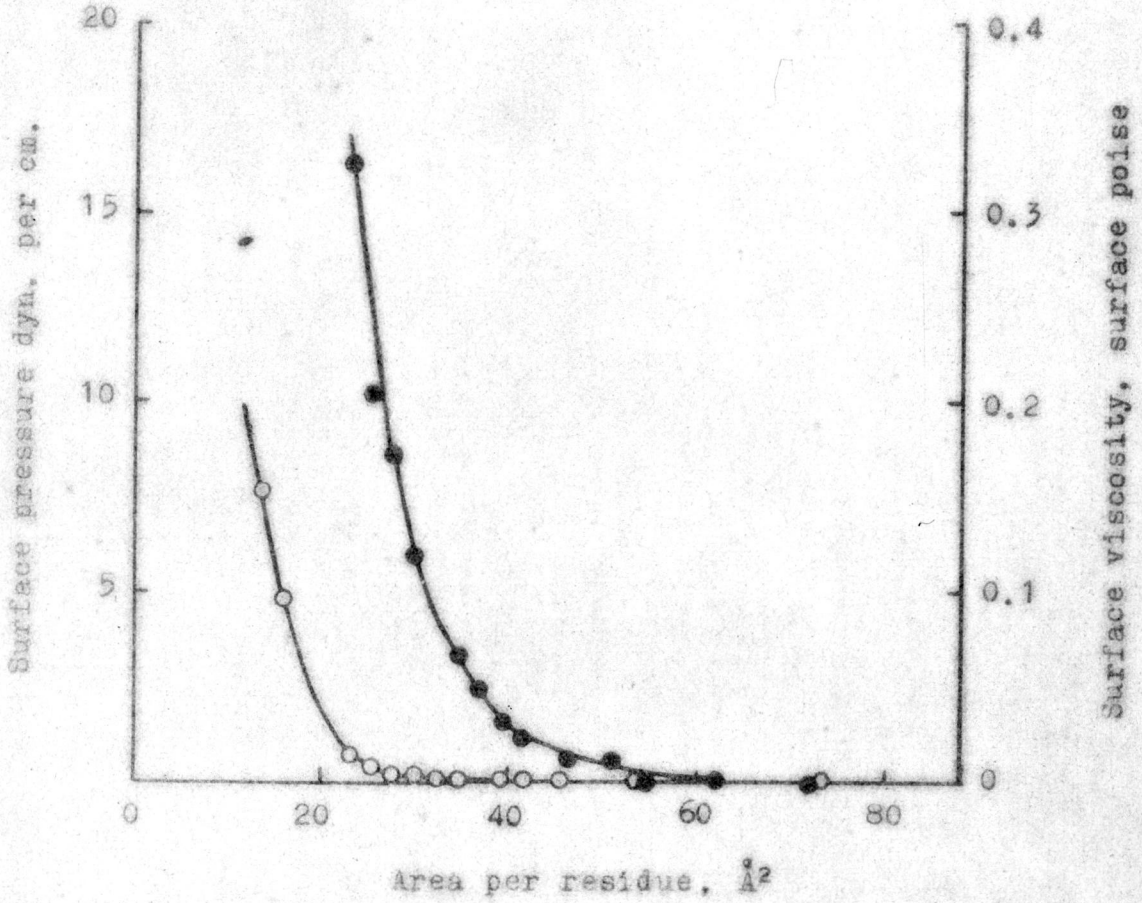

Fig. 8. Surface pressure-area (○), surface viscosity-area (●) curves of Nylon 12 on distilled water at 17.5°C.

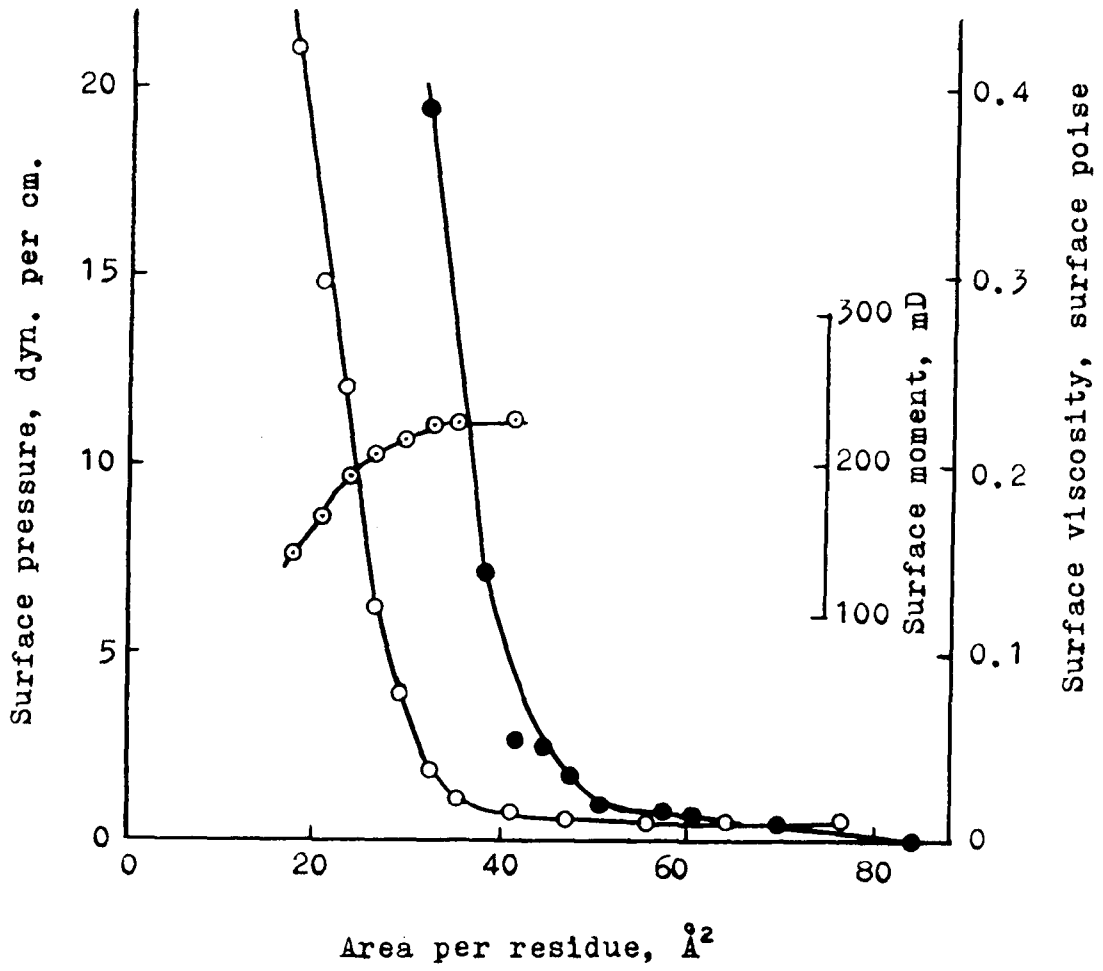

Fig. 9. Surface pressure-area (○), surface moment-area (⊙) and surface viscosity-area (●) curves of polyurea on distilled water at 20°C.

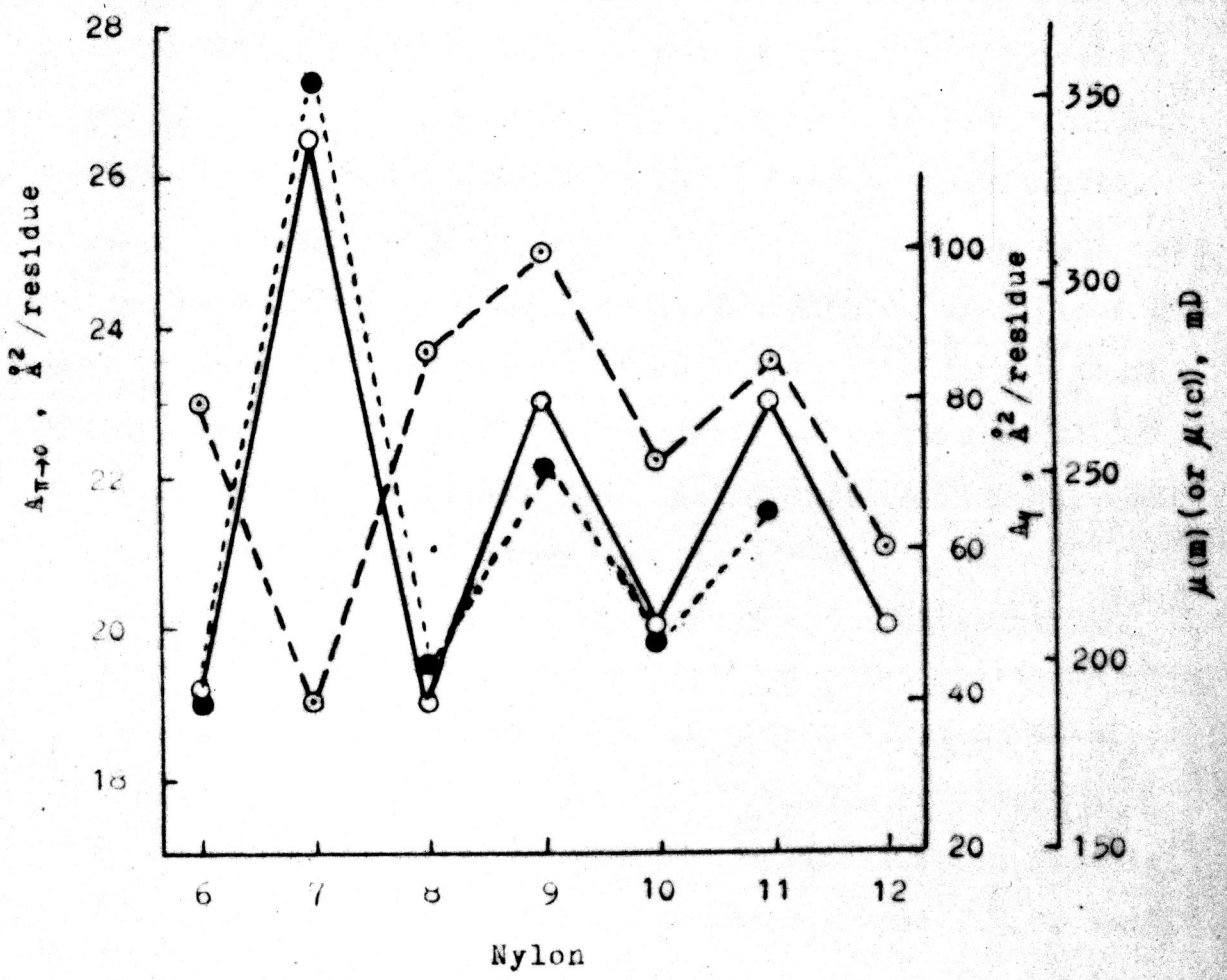

Fig. 10. Plots of $A_{\pi\to0}$ (○), A_γ (⊙) and μ(m) (or μ(c)) (●) values of nylons against the number of carbon atoms.

VI. Effect of Cupric Ion on the Monolayer Properties of Histidyl Polypeptides

Histidyl residue in protein is a very important binding site for metal ions through the reactivity of its imidazole group[1]. It was found in our laboratory that bacterial-α-amylase interacts strongly with metal ions such as copper, zinc and mercury, and the interaction of cobalt ion with this enzyme is rather weaker[2]. The combination of metal ions has been studied with imidazole[3,4] and 4-methylimidazole[5] as model substances of histidyl residue. Patchornik et al.[6] synthesized poly-L-histidine and found that it forms insoluble complexes with various heavy metals.

Monolayer technique appears to be a useful means to

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1) F.R.N. Gurd and P.B. Wilcox, "Advance in Protein Chemistry", Vol. 11, Academic Press, New York (1956), p. 311.

2) K. Kakiuchi, S. Kato and T. Isemura, Unpublished results.

3) J.T. Edsall, G. Felsenfeld, D.S. Goodman and F.R.N. Gurd, J. Am. Chem. Soc., 76, 3054 (1954).

4) R.B. Martin and J.T. Edsall, ibid., 80, 5053 (1958).

5) Y. Nozaki, F.R.N. Gurd, R.F. Chen and J.T. Edsall, J. Am. Chem. Soc., 79, 2123 (1957).

6) A. Patchornik, A. Berger and E. Katchalski, ibid., 79, 5227 (1957).

investigate the interaction of protein with metal ions as has been shown with the monolayer studies of mineral tanning of proteins[7,8]. Schulman and Dogan[7] have found that copper ion does not react with carboxyl group but with imidazole in the protein monolayer. We have attempted to study the interaction of poly-L-histidine with cupric and other biologically important metal ions using a monolayer technique in order to investigate the metal binding property of histidyl residue in protein. Poly-L-histidine, however, could not be spread as a monolayer even on a subphase containing 40 % ammonium sulfate and or on subsolutions of cupric sulfate at various concentrations probably owing to the strong hydrophility of its side chains. Thus, poly-1-benzyl-L-histidine as a model substance was chosen in the present experiments for studying interaction of metal ions with histidyl group, as it could form stable films at air/water interface. The monolayer of copoly-2:2:2:1-(glycine, O-benzyl-DL-serine, β-benzyl-L-aspartate, 1-benzyl-L-histidine) was also studied.

Experimental

Poly-1-benzyl-L-histidine and copoly-2:2:2:1-(glycine, O-benzyl-DL-serine, β-benzyl-L-aspartate, 1-benzyl-L-histidine), which were synthesized by the polymerization of

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7) J.H. Schulman and M.Z. Dogan, Disc. Faraday Soc., 16, 158 (1954).

8) K.G.A. Pankhurst, "Surface Phenomena in Chemistry and Bioloty", Pergamon Press, London and New York (1958), p. 100.

N-carboxyanhydrides of their corresponding amino acids, were supplied by Professor J. Noguchi of Hokkaido University. The spreading solutions were made by dissolving the former in 0.05 N hydrochloric acid and the latter in the mixture of dichloroacetic acid and benzene (1:4, v/v). The concentration of these polymers in solutions was about 0.25 mg./ml.

The Wilhelmy hanging plate method was inadequate for the present experiments because the surface of glass plate became remarkably water-repellent owing probably to the adsorption of poly-1-benzyl-L-histidine onto the plate. Therefore, the surface pressure was measured using a float type surface balance. In order to avoid the contamination by metal ion from the trough, a trough made of polymethyl methacrylate was used, the rim of which was coated with purified paraffin.

The surface viscosity was measured by the rotatory oscillation of a disk on the surface of liquid[9].

The surface potential was measured by the vibrating electrode method. The surface moment, μ was calculated from the surface potential, ΔV utilizing the Helmholtz formula.

The compression of the film was started 20 minutes after spreading the polypeptides. All the experiments were carried out at room temperature. The fluctuation of temperature, however, never exceeded one degree during the course of experiment. The pH of the subsolution was measured using a Horiba M-3 glass electrode pH-meter.

Acetate buffer consisting in an appropriate ratio of 0.1 M sodium acetate and 0.1 M acetic acid was used as the subsolution

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[9]) Part I of this series.

in some cases.

Results

The effects of potassium chloride, sodium sulfate and sodium acetate (0.1 M acetate buffer, pH 5.6) on the surface pressure-area (π-A) relationship of the monolayer of poly-l-benzyl-L-histidine are shown in Fig. 1. Potassium sulfate (0.01 M, not shown in the figure) gave the identical π-A curve with that on 0.01 M sodium sulfate. The limiting area per residue which was determined from the minimum compressibility of the film was found to be 16.0 Å for the film on distilled water, the value being similar to that for nonionic polypeptides such as poly-DL-phenylalanine (15.6 $Å^2$)[10]. The presence of salts in the subsolution resulted in the expansion of the film, which suggests more horizontal orientation of side chains. The effect of acetate ion is very specific. The film expands on acetate subsolution more than on other salts such as potassium chloride and sodium sulfate. There appears a plateau region between 12 and 18 $Å^2$ per residue in the π-A curve. The addition of 0.1 M potassium chloride (not shown in Fig. 1) to 0.1 M acetate buffer gave no influence upon π-A relationship on 0.1 M buffer solution.

The effect of cupric sulfate on poly-l-benzyl-L-histidine monolayer is shown in Fig. 2 together with those of potassium chloride and sodium sulfate. On 0.01 M cupric sulfate, the area per residue is much larger than on 0.1 M potassium chloride though the film is somewhat more condensed than on

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10) T. Yamashita and T. Isemura, Bull. Chem. Soc. Japan, 35, 929 (1962).

0.01 M cupric sulfate.

Fig. 3 shows the surface viscosity-area (η_s-A) curves of this polypeptide on distilled water, 0.01 M potassium sulfate and 0.01 M cupric sulfate containing 0.01 M potassium sulfate. The influence of cupric sulfate is markedly different from that of potassium sulfate. The area where surface viscosity appears is considerably large compared with those on distilled water and potassium sulfate. The results shown in Fig. 2 and 3 suggest that the influence of cupric ion on the monolayer properties of poly-l-benzyl-L-histidine differs from that of alkali salts.

The π-A curves of poly-l-benzyl-L-histidine on the subsolutions at various concentrations of cupric sulfate in the absence and presence of acetate ion are shown in Figs. 4 and 5, respectively. In both cases, the π-A relations are affected markedly by changing concentration. The plateau of π-A curve found on the acetate buffer disappeared by addition of cupric ion. The maximum effect of cupric ion was found at the concentration of 0.01 M whether acetate ion is present or not.

The effect of the concentration of cupric sulfate in subphase of 0.1 M acetate buffer (pH 5.6) on the η_s-A relation of poly-l-benzyl-L-histidine monolayer is represented in Fig. 6. The surface viscosity was detected at much larger area where surface pressure is considerably lower than in the absence of cupric ion. The maximum effect of cupric sulfate was found at the concentration of 0.01 M, same as in the case of π-A relation.

The surface potential-area (ΔV-A) and surface moment-area

(μ-A) curves of poly-1-benzyl-L-histidine on 0.1 M acetate buffer (pH 5.6) containing 0.01 M cupric sulfate are compared with those on copper-free subsolution in Fig. 7.

In the presence of cupric ion, the surface potential and surface moment increased more than in its absence in the higher area region. The surface moment of the film on the subsolution containing copper ion was higher by about 50 mD in the horizontal portion of the μ-A curve than that on the acetate buffer without cupric ion.

The effect of cupric ion on the film properties of this polypeptide is summarized in Table I. It is evident that the interaction of cupric sulfate with poly-1-benzyl-L-histidine differs from that of alkali salts.

The π-A curves of copoly-2:2:2:1-(glycine, O-benzyl-DL-serine, β-benzyl-L-aspartate, 1-benzyl-L-histidine) on distilled water and on 0.01 M cupric sulfate are shown in Fig. 8. No effect was found with cupric sulfate. 0.05 M potassium chloride and 0.01 M hydrochloric acid also did not affect the π-A relation of this copolypeptide.

Table I. The effect of cupric ion on the surface viscosity, surface potential and surface moment of poly-1-benzyl-L-histidine

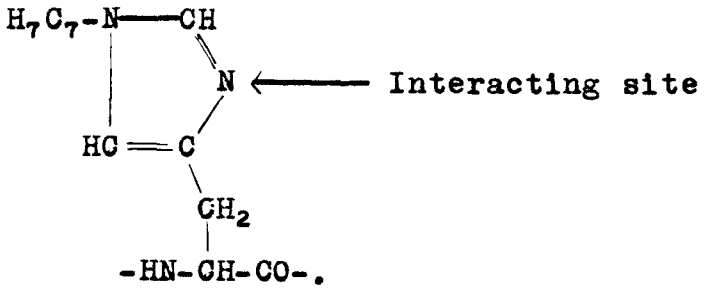
Subphase	Area at 0.05 surface poise	at 0.05 surface poise	ΔV 35 Å²/res.	ΔV 45 Å²/res.	μ**
	Å²/res.	dyn./cm.	mV	mV	mD
Distilled water	13.0	13.6			
0.01 M K_2SO_4	16.6	26.1			
0.01 M K_2SO_4 + 0.01 M $CuSO_4$	37.0				
0.1 M acetate (pH 5.6)	20.4	12.0	546	428	516
$CuSO_4$* 0.001 M	45.0	0.85			
$CuSO_4$* 0.01 M	50.6	0.86	580	484	568
$CuSO_4$* 0.05 M	41.9	1.0			

* Dissolved in 0.1 M acetate buffer (pH 5.6)

** Values at the linear portion of Π-A curves

Discussion

As has been described in the preceeding section, there exists a significant difference between cupric sulfate and univalent alkali salts in the nature of interaction with histidine-containing polypeptide, poly-1-benzyl-L-histidine, although in both cases the interacting site is at the nitrogen atom in the 3-position of benzylimidazole group,



In the presence of cupric sulfate in the subsolution, the film expands much more than in the presence of alkali salts. The difference between the effect of alkali salts and cupric sulfate is pronounced in the surface viscosity-area relation. If cupric ion is present in the subsolution, surface viscosities appear at higher areas and accordingly lower surface pressures than in its absence. Therefore, there might be two types of interaction between poly-1-benzyl-L-histidine and salts; (1) interaction with alkali salts, and (2) interaction with divalent metal salts.

Interaction with alkali salts. ----- From the study on the properties of long chain amines on aqueous solutions at different pH, Adam[11] pointed out that the pH's of the subsolution seems to be of little importance in the acid

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[11] N.K. Adam. Proc. Roy. Soc., A126, 526 (1930).

subsolution, and the films were very much affected by anions in buffer solution. He concluded that the amines form salts with anions. The similar fact was also found by Hoffman et al.[12].

Patchornik et al.[6] found that a dilute aqueous solution of poly-1-benzyl-L-histidine containing hydrochloric acid gave precipitates of polyvalent salts with various alkali salts. This fact suggests combination of poly-1-benzyl-L-histidine monolayer with chloride, sulfate and acetate ion likely as in the case of long chain amine monolayers. The salts would be formed between positively charged benzylimidazolium groups of this polypeptide and anions. Consequently, the film expands on the alkali salt subsolutions more than on distilled water because the side chains assume probably more horizontal orientation owing to the decrease in their hydrophility.

The effect of acetate ion is anomalous. Adam[11] found that the monolayer of long chain amine acetate has much less lateral adhesion than that of amine chloride. The monolayer of the salt of poly-1-benzyl-L-histidine with acetate ion might have much less lateral adhesion than those with chloride and sulfate, which cause the more expansion and the reorientation of its side chains resulting in the plateau in the Π-A curve.

Interaction with divalent metal salts. ----- The pronounced effect of cupric ion on the film properties of

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12) E.J. Hoffman, G.E. Boyd and A.W. Ralston, J. Am. Chem. Soc., 64, 498 (1942).

poly-1-benzyl-L-histidine can hardly be interpreted by mere interaction between film and anion. Strong interaction exists between the monolayer and cupric ion. It has been found that zinc, cobaltous and calcium ions also interact with poly-1-benzyl-L-histidine monolayer. The results will be reported in the subsequent paper.

Schulman and Dogan[7] have found a stronger interaction of copper ion with methemoglobin than with serum albumin in the monolayer, the former having more histidyl groups in its molecule. Patchornik et al.[6] reported that poly-L-histidine forms insoluble complexes with copper, silver, magnesium, mercury, zinc and cobalt. It thus seems to be reasonable to assume a formation of complexes of poly-1-benzyl-L-histidine with cupric ions through the nitrogen atoms in the 3-position of benzylimidazole groups. The assumption could possibly be supported by the fact that the surface viscosity of the film appears at much larger area and lower surface pressure in the presence of cupric ion than in its absence.

It has been found in mineral or vegetable tanning of protein monolayers that the cross-linkage structure is formed between molecules at surface, accompanying the remarkable increase in surface viscosity[13]. A similar structure can be considered between poly-1-benzyl-L-histidine molecules as a result of complex formation, being responsible for the large area where surface viscosity is first detected. The formation of cross-linkage is reported also for protein monolayer

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[13]) S.C. Ellis and K.G.A. Pankhurst, Disc. Faraday Soc., 16, 170.

reacted with silicic acid[14,15].

The fact that the surface potential and surface moment increase more in the presence of cupric ion than in its absence seems to be another evidence of the formation of complexes. The cationic groups are revealed in the interaction of cupric ions with poly-1-benzyl-L-histidine monolayer. The similar fact is found with gelatin monolyaer tanned with basic chromium sulfate[13].

The maximum effect of cupric ion was found at 0.01 M, above which the area of monolayer decreased whether acetate ion was present or not, as shown in Figs. 4 and 5. It has been found that the coordination ratio of imidazole[3] or 4-methylimidazole[5] to copper decreases from the maximum (4:1) with increasing concentration of cupric ion. By analogy with this, the number of benzylimidazole groups which associate with a copper ion in subsolution seems to be four at its maximum value and to be decreased with increasing the ion concentration. The decrease may result in the weakening of the cross-linkage structure and account for the decreases in areas in π-A and γ_s-A relations.

In contrast with poly-1-benzyl-L-histidine, the monolayer of copoly-2:2:2:1-(glycine, O-benzyl-DL-serine, β-benzyl-L-aspartate, 1-benzyl-L-histidine) was not affected in the presence of cupric sulfate in the subsolution. This copolymer

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14) S.G. Clark, P.F. Holt and C.W. Went, Trans. Faraday Soc., 53, 1500 (1957).

15) S.G. Clark and P.F. Holt, ibid., 53, 1509 (1957).

contains only 11 % of benzyl-histidyl residue, so the polynuclear complexes with copper may hardly be formed.

Summary

Effects of salts on the monolayer properties of poly-1-benzyl-L-histidine and copoly-2:2:2:1-(glycine, O-benzyl-DL-serine, β-benzyl-L-aspartate, 1-benzyl-L-histidine) have been investigated.

In the presence of potassium chloride, sodium sulfate, and sodium acetate (pH 5.6), the film expansion has been observed, which was interpreted by the salt formation between side chains and anions. Addition of cupric sulfate to subsolution caused the expansion of film and increased the surface viscosity and potential, for which cross linkage by complex formation between side chains and metal ions might be responsible.

On the other hand, the monolayer of the copolymer was not affected in the presence of cupric sulfate owing to the less content of histidyl residue.

The author wishes to express his gratitude to Prof. Toshizo Isemura of Osaka University for his kind guidance throughout the present work, and to Prof. Junzo Noguchi of Hokkaido University for the supply of samples.

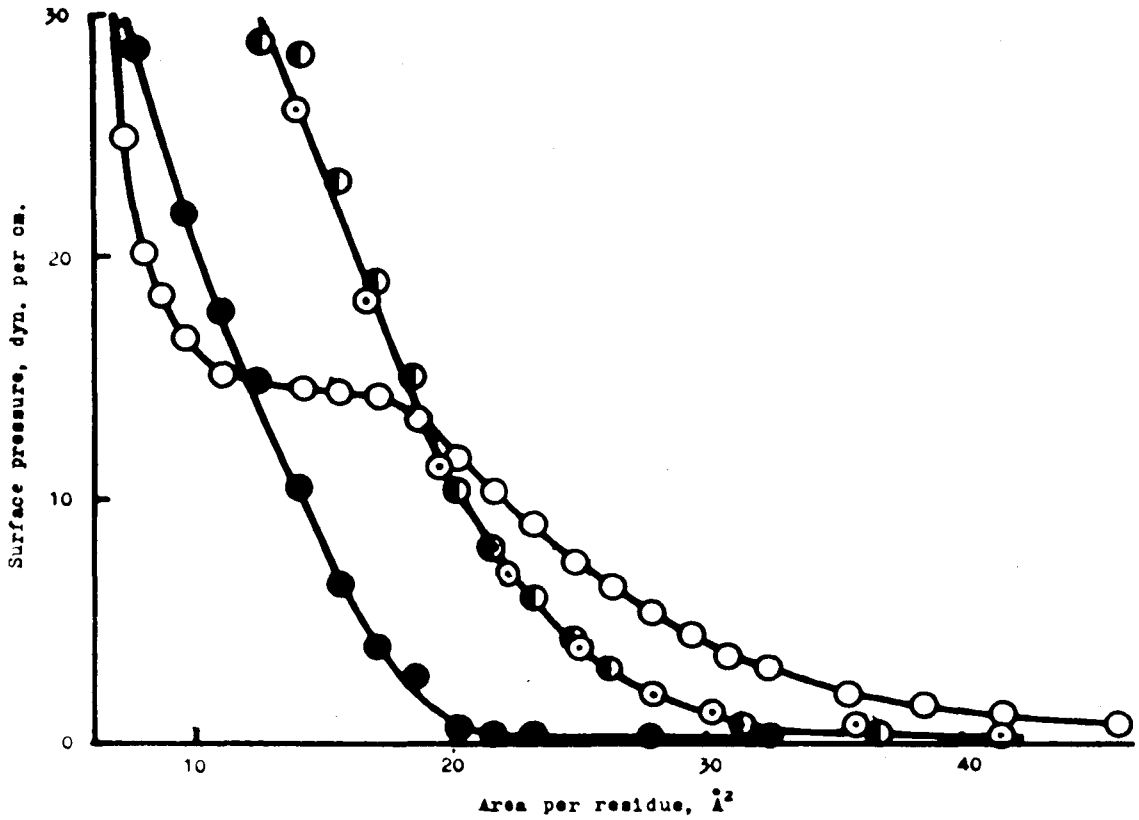

Fig. 1. Surface pressure-area curves of poly-1-benzyl-L-histidine on distilled water (●), 0.1 M potassium chloride (◐), 0.01 M sodium sulfate (⊙) and 0.1 M acetate buffer (pH 5.6, ○): 20° C.

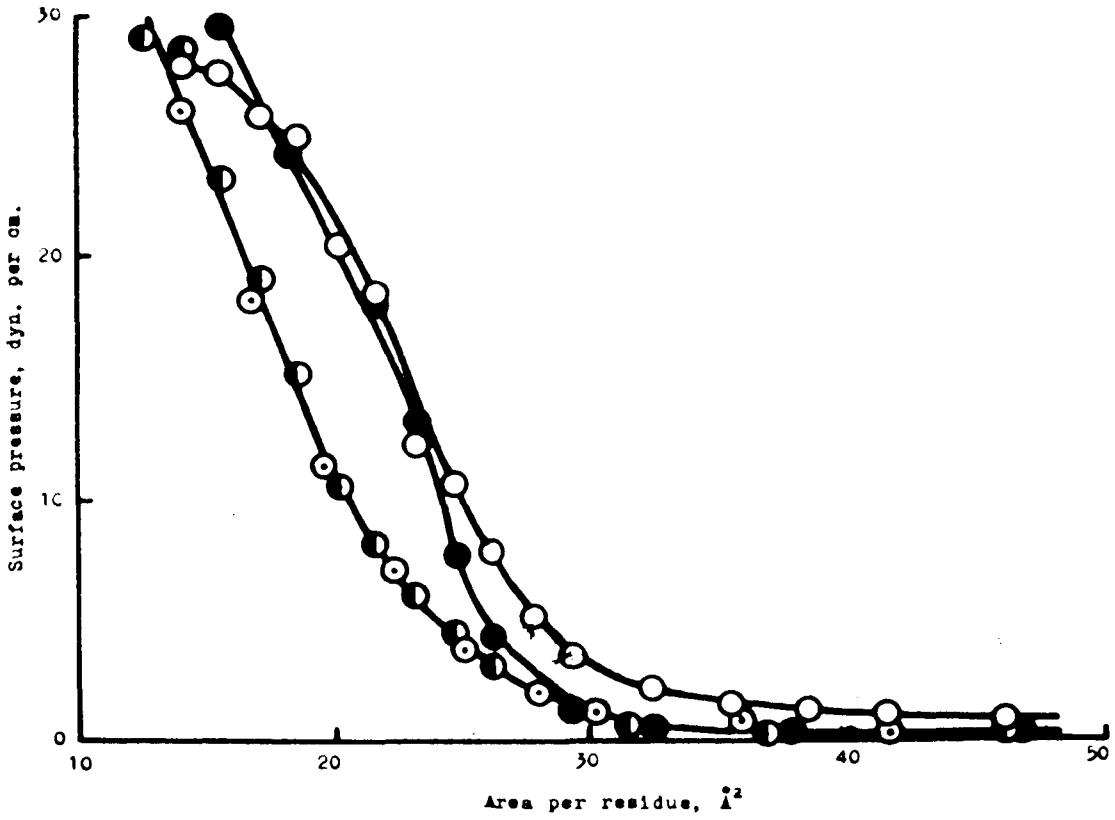

Fig. 2. Effect of cupric sulfate on poly-1-benzyl-L-histidine monolayer at 20° C: ○ , 0.01 cupric sulfate; ● , 0.1 M potassium chloride containing 0.01 M cupric sulfate; ◐ , 0.1 M potassium chloride; ⊙ , 0.01 M sodium sulfate.

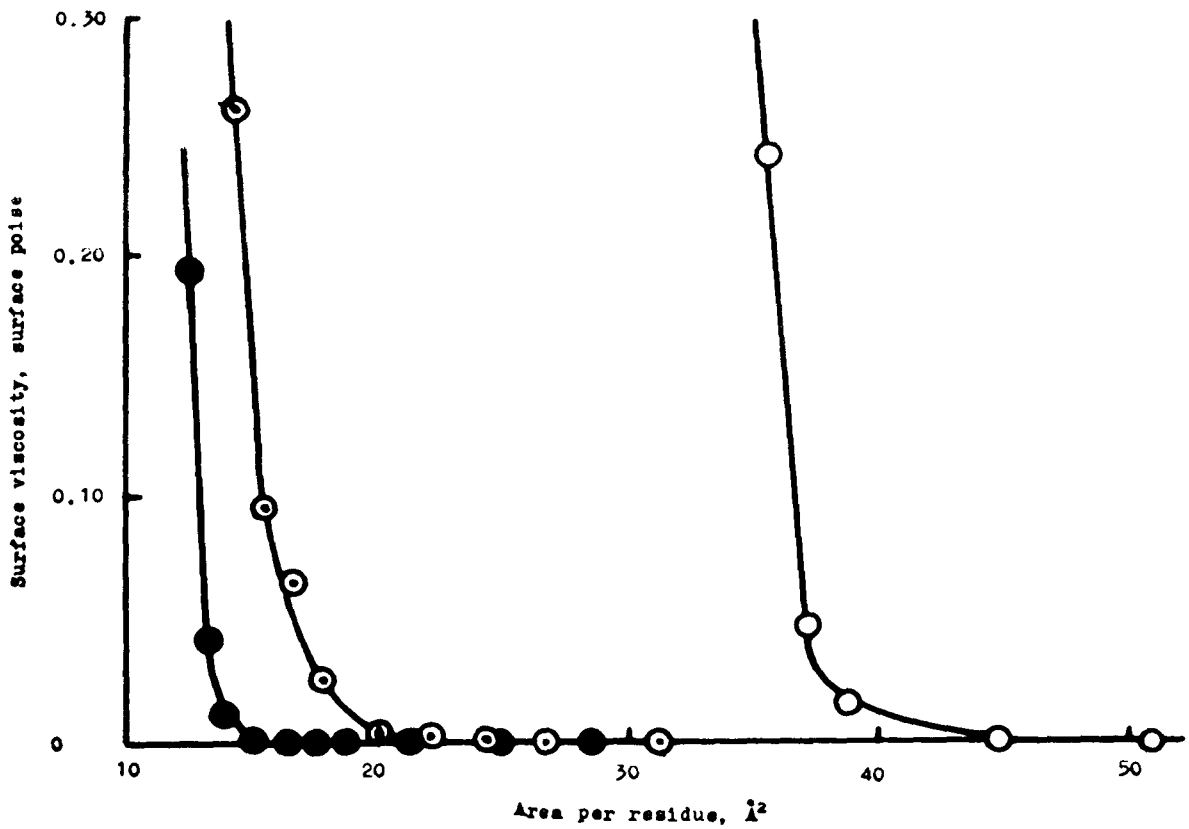

Fig. 3. Surface viscosity-area curves of poly-1-benzyl-L-histidine on distilled water (●), 0.01 M potassium sulfate (⊙) and 0.01 M potassium sulfate containing 0.01 M cupric sulfate (○): 16°C.

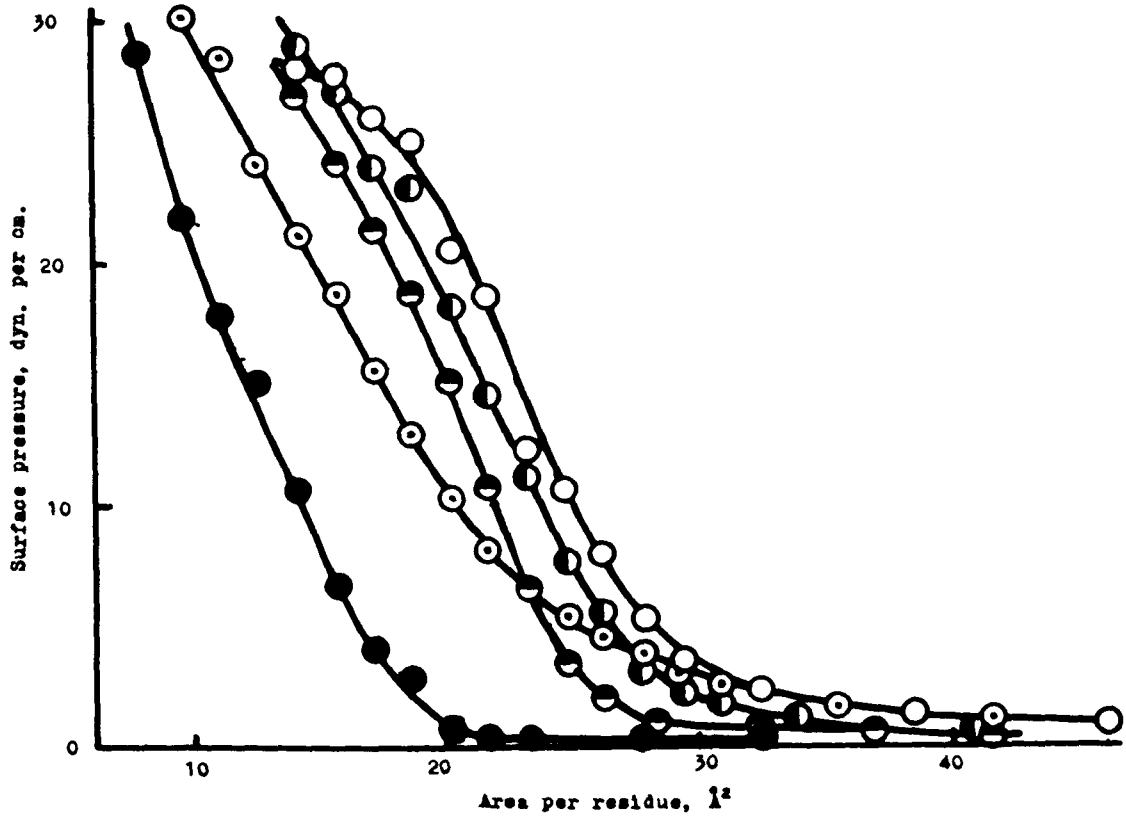

Fig. 4. Effect of concentration of cupric sulfate on the surface pressure-area relation of poly-1-benzyl-L-histidine at 20°C: ●, distilled water; ◒, 0.0001 M; ◐, 0.001 M; ○, 0.01 M; ⊙, 0.05 M.

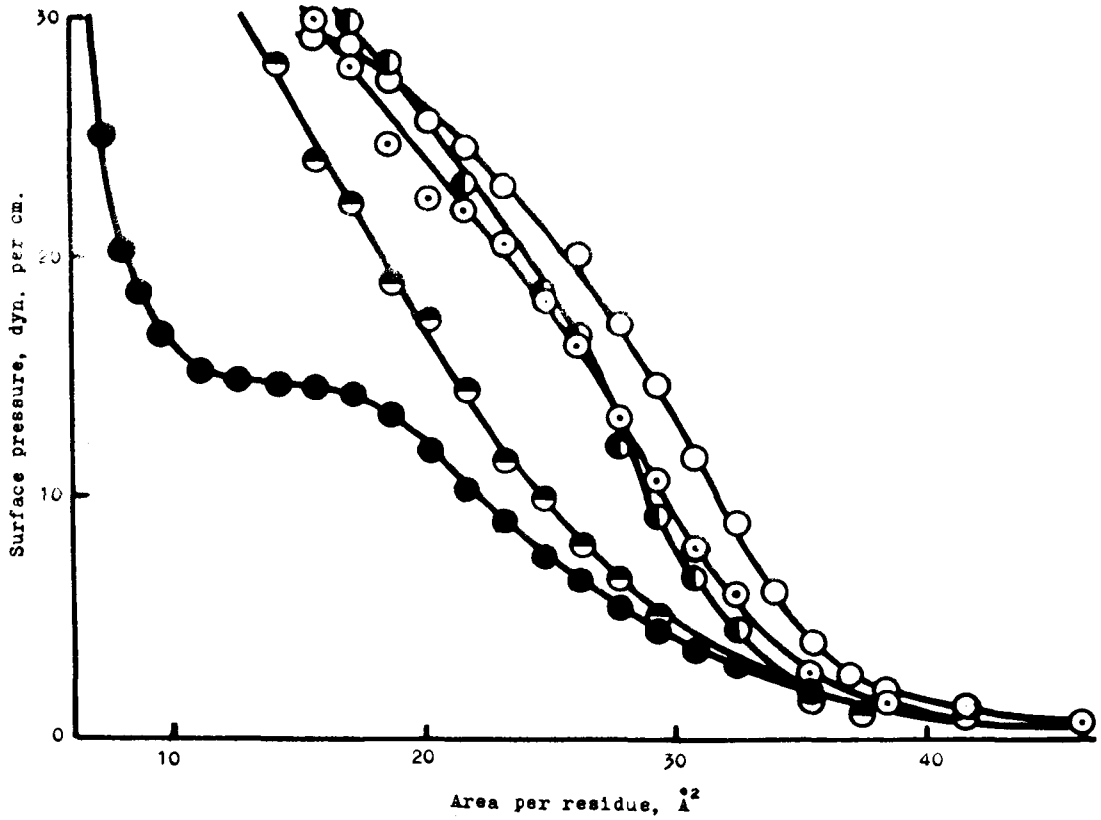

Fig. 5. Effect of the concentration of cupric sulfate dissolved in 0.1 M acetate buffer (pH 5.6) on the surface pressure-area relation of poly-1-benzyl-L-histidine at 20° C: ●, 0 M (control); ◓, 0.0001 M; ◐, 0.001 M; ○, 0.01 M; ⊙, 0.05 M.

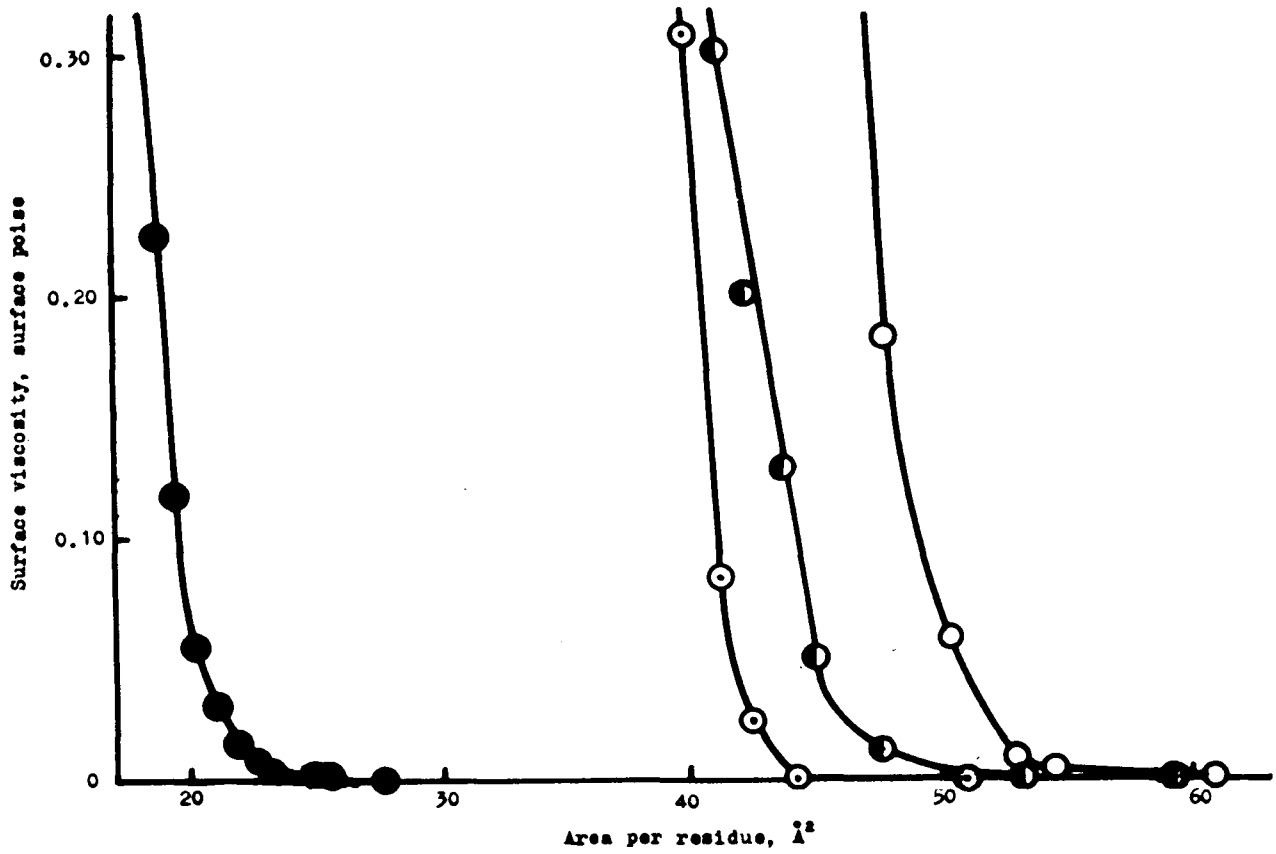

Fig. 6. Effect of the concentration of cupric sulfate dissolved in 0.1 M acetate buffer (pH 5.6) on the surface viscosity-area relation of poly-1-benzyl-L-histidine at 13.5° C: ●, 0 M (control); ◐, 0.001 M; ○, 0.01 M; ⊙, 0.05 M.

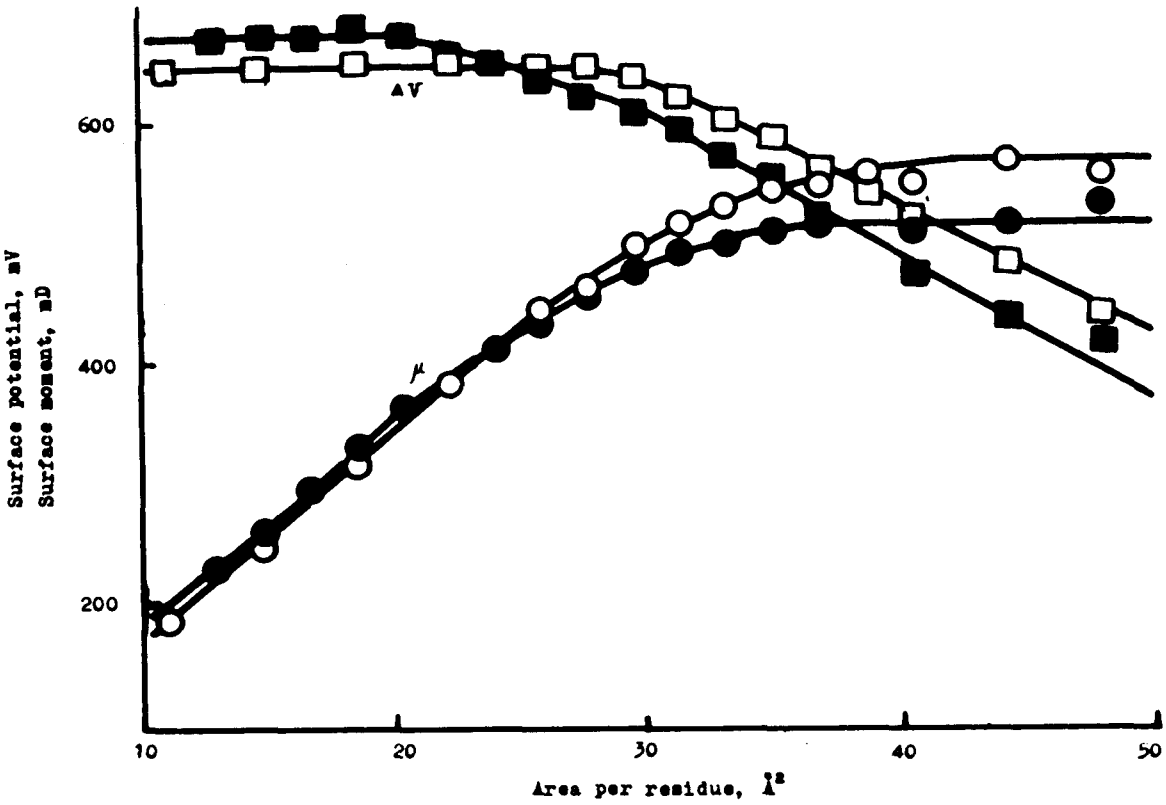

Fig. 7. Surface potential-area and surface moment-area curves of poly-1-benzyl-L-histidine on 0.1 M acetate buffer (pH 5.6; ■ , ●) and on 0.1 M acetate buffer containing 0.01 M cupric sulfate (pH 5.6; □ , ○): 12°C.

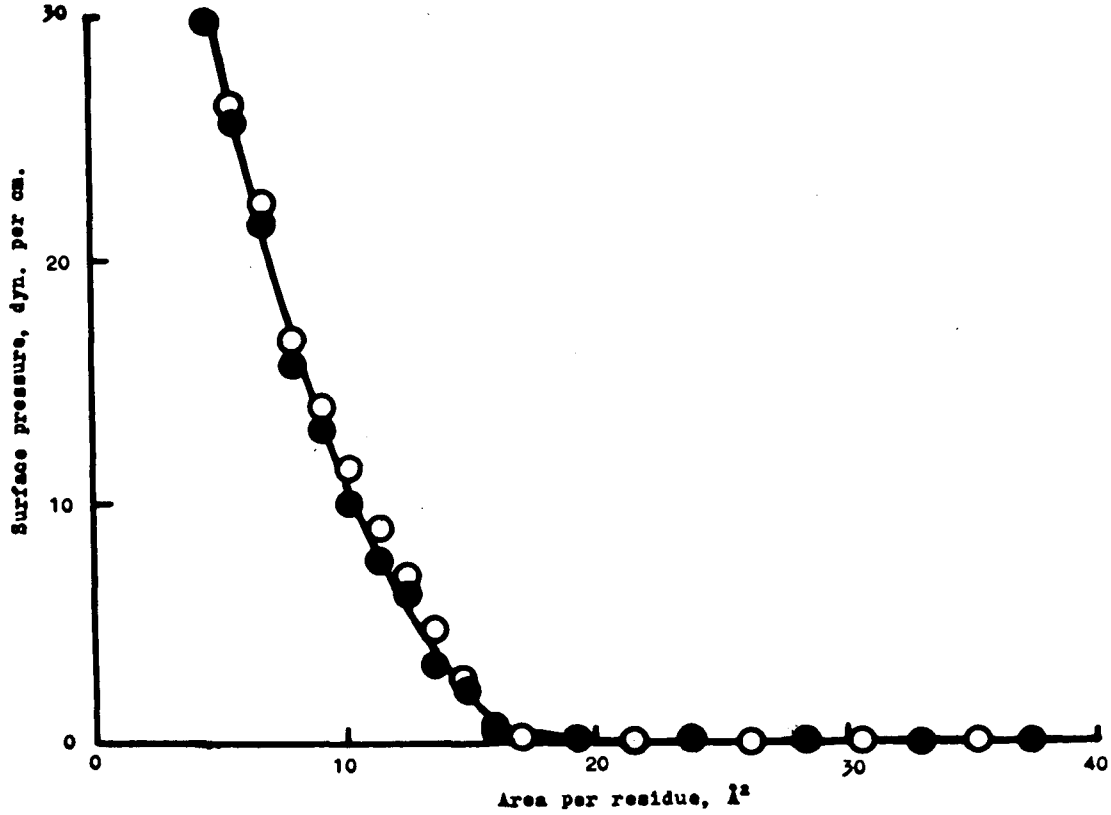

Fig. 8. Surface pressure-area curves of copoly-2:2:2:1-(glycine, O-benzyl-DL-serine, β-benzyl-L-aspartate, 1-benzyl-L-histidine) on distilled water (○) and on 0.01 M cupric sulfate (●) at 16°C.

VII. Effects of Zinc, Cobalt and Calcium Ions on the Monolayer Properties of Poly-1-benzyl-L-histidine

The reactivity of imidazole group of histidyl residue in protein has a great importance for the understanding of interactions between proteins and several metal ions[1]. In the previous paper[2], the interactions of cupric ion with poly-1-benzyl-L-histidine and copoly-2:2:2:1-(glycine, O-benzyl-DL-serine, β-benzyl-L-aspartate, 1-benzyl-L-histidine) have been studied using monolayer technique at air/water interface. It was found that cupric ion has a strong interaction with poly-1-benzyl-L-histidine monolayer, while it does not interact with copolypeptide. In the present study, the effects of zinc, cobalt and calcium ions, and the acidity on the film properties of poly-1-benzyl-L-histidine were investigated at air/water interface. The effects of these metal ions were also studied together with that of cupric ion at oil/water interface.

Experimental

The preparation of the spreading solution of poly-1-

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1) F.R.N. Gurd and P.B. Wilcox, "Advance in Protein Chemistry", Vol. 11, Academic Press, New York (1956), p. 331.

2) Part VI of this series.

benzyl-L-histidine was described in the previous paper[2]. The surface pressure, surface viscosity and surface potential at air/water interface were measured according to the same methods as reported in the preceeding paper.

At oil/water interface, the interfacial pressure was measured by the ring method. The diameter of the ring used was 2.77 cm. Petroleum ether of which boiling point is 85-115°C was used as an oil phase. The interfacial concentration of the polypeptide was changed by the successive injection method using a Agla micrometer syringe, being corrected by the Thomas theoretical correction formula[3-5]. The measurement of the interfacial pressure was carried out five minutes after every injection.

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

Results

The surface pressure-area (π-A) and surface viscosity-area (η_s-A) relations of poly-1-benzyl-L-histidine on 0.1 M acetate buffer (pH 5.6, acetic acid + sodium acetate) containing various concentrations of zinc sulfate are shown in Figs. 2 and 3, respectively. In the presence of 0.001 M

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3) A.G. Thomas, Nature, 179, 776 (1957).

4) E.G. Cockbain, K.J. Day and A.I. MucMullen, "Proc. 2nd Int. Congress of Surface Activity", Vol. 1, Butterworth Scientific Publication, London (1957), p. 56.

5) T. Yamashita and T. Isemura, Bull. Chem. Soc. Japan, 35, 929 (1962).

zinc sulfate in the subsolution, the plateau which was found on the zinc-free solution was present in the π-A curve. On 0.01 M and 0.05 M zinc sulfate, however, the plateau disappeared. Zinc sulfate caused the increase in area where surface viscosity appears with its concentration. The extent was much less than in the case of cupric sulfate[2].

The surface potential-area (ΔV-A) and surface moment-area (μ-A) curves of poly-l-benzyl-L-histidine on 0.1 M acetate buffer with and without 0.01 M zinc sulfate are illustrated in Fig. 3. In the presence of zinc ion, the surface potential and surface moment are higher by 50 mV in all the areas, and by 52 mD in the linear portion, respectively.

The effect of zinc ion on the film properties of poly-l-benzyl-L-histidine is summarized in Table I. In the presence of zinc ion, the areas where surface viscosities become 0.05 surface poises increased accompanying the decrease in surface pressures with its concentration.

The π-A curves on the acetate buffer subsolutions containing cobaltous sulfate and calcium chloride are shown in Fig. 4. In the presence of cobalt ion, the plateau appeared in the π-A curve, although the film much more expanded than on the control acetate buffer solution. On the other hand, calcium chloride caused a slight condensation of film and the plateau disappeared.

Fig. 5 shows the π-A curve of poly-l-benzyl-L-histidine on 0.001 N hydrochloric acid together with that on distilled water which has already been reported in the previous paper[2]. On 0.001 N hydrochloric acid, the surface pressures at the

same areas are much lower than on distilled water, the film being of expanded type. On 0.1 N hydrochloric acid, the film dissolved completely in aqueous phase.

The interfacial pressure-area (π_i-A) curves of poly-l-benzyl-L-histidine at the interfaces of oil and water dissolving various salts are shown in Fig. 6. On the subsolutions of 0.1 M potassium chloride and 0.1 M acetate buffer (pH 5.6) with and without 0.01 M zinc or cobaltous sulfate, the π_i-A curves were nearly identical with each other. Cupric ion gave a stronger interaction with this polypeptide as well as at air/water interface.

Table I. The effect of zinc ion on the surface viscosity, surface potential and surface moment of poly-l-benzyl-L-histidine

Subphase		Area at 0.05 surface poise Å²/res.	π at 0.05 surface poise dyn./cm.	ΔV 35 Å²/res. mV	ΔV 45 Å²/res. mV	$\mu_{\Delta V}$**
0.1 M acetate		20.4	12.0	546	428	516
$ZnSO_4$*	0.001 M	23.6	8.5			
	0.01 M	26.8	6.0	594	484	568
	0.05 M	29.6	4.0			
$CuSO_4$*	0.001 M-0.05 M	40	< 1			

* Dissolved in 0.1 M acetate buffer (pH 5.6)

** Values at the linear portion of π-A curves

Discussion

Air/water interface. ------ Effect of zinc ion. -----

It has been found that various metal ions form complexes with imidazole[6,7] and 4-methylimidazole[8] as model substances of histidyl residue in protein. Further, Patchornik et al.[9] found that poly-L-histidine forms insoluble complexes with heavy metal ions in solution. The extent of the complex formation of cobalt with poly-L-histidine was much less than those of copper, silver and zinc.

In the preceeding paper, it was reported that cupric ion interacts markedly with poly-l-benzyl-L-histidine monolayer at air/water interface. The film was expanded and became highly viscous in the presence of cupric ion. This finding was interpreted by the closs-linked structure between polypeptide molecules through the complex formation of copper with side chains.

In the presence of 0.01 M and 0.05 M zinc sulfate in the subsolution, the plateau of π-A curve found on the zinc-free acetate buffer (pH 5.6) disappeared and the area where surface viscosity can be detected increased with the increase in concentration of zinc ion. As shown in Table I, zinc ion

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6) J.T. Edsall, G. Felsenfeld, D.S. Goodman and F.R.N. Gurd, J. Am. Chem. Soc., 76, 3054 (1954).

7) R.B. Martin and J.T. Edsall, ibid., 80, 5053 (1958).

8) Y. Nozaki, F.R.N. Gurd, R.F. Chen and J.T. Edsall, ibid., 79, 5227 (1957).

9) A. Patchornik, A. Berger and E. Katchalski, ibid., 79, 5227 (1957).

~~As shown in Table I, zinc ion~~ affects the film properties of poly-1-benzyl-L-histidine. The effect of zinc ion, however, is much less than that of cupric ion.

The facts shown above suggest that the complexes of zinc with this polypeptide are formed although the extent is much less than in the case of cupric ion. As a result of complex formation, a cross-linkage structure would be caused between polypeptide molecules at the surface as found with vegetable and mineral tannings of protein monolayers[10-12].

The fact that the surface potential and moment increase more in the presence of zinc ion than in its absence is another evidence of complex formation of zinc with this polypeptide. The increases are comparable to those in the presence of cupric ion.[2] Gelatin monolayer tanned with basic chromium sulfate also gives the increase of about 50 mV in its surface potential. The increases in surface potential and surface moment indicate that cationic groups as a result of complex formation are revealed in the interaction of zinc ion with the film of this polypeptide.

Effects of calcium and cobaltous ions.----- It has already been reported[2] that the addition of 0.1 M potassium chloride to the subsolution of 0.1 M acetate buffer (pH 5.6)

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10) J.H. Schulman and M.Z. Dogan, Disc. Faraday Soc., 16, 158 (1954).

11) S.C. Ellis and K.G.A. Pankhurst, ibid., 16, 170 (1954).

12) K.G.A. Pankhurst, "Surface Phenomena in Chemistry and Biology", Pergamon Press, London and New York (1958) p. 100.

gives no influence upon the π-A relationship of poly-l-benzyl-L-histidine[2]. On the other hand, the condensation of film is observed on 0.1 M acetate buffer subsolution containing 0.005 M calcium chloride compared with the film on the control acetate buffer (pH 5.6). The plateau of π-A curve, however, disappeared as well as in the presence of copper or zinc ion. This fact might be caused by a cross-linkage structure between polypeptide molecules as a result of complex formation of calcium ion with the side chains.

The effect of cobaltous ion on the monolayer of poly-l-benzyl-L-histidine is different from those of cupric and zinc ions. On acetate buffer subsolution containing cobaltous sulfate (pH 5.6), the plateau of π-A curve found on the control acetate buffer is also found, although the film expansion is observed. The interaction might be caused by either of the following mechanisms; (i) a very weak cross-linkage is formed between polypeptide molecules through the complex formation with cobaltous ion, or (ii) only one benzylimidazole group combines with one cobaltous ion and further association does not take place, and no network structure is formed, the film being expanded owing to the repulsion between charged groups. Such a weak interaction of cobaltous ion than of cupric or zinc has also found with bacterial-α-amylase[13], the interacting sites of which are histidyl groups, and with imidazole[3] and poly-L-histidine[9].

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13) K. Kakiuchi, S. Kato and T. Isemura, Unpublished results.

As has been described in the previous and present papers, in general, at air/water interface divalent metal ions seem to form complexes with poly-1-benzyl-L-histidine monolayer more or less at the pH studied (5.6) besides the salt formation between positively charged benzylimidazolium groups and anions such as sulfate, chloride and acetate ions. The decreasing order of the interactions excepting calcium ion is found to be Cu^{++}, Zn^{++}, Co^{++}. This order corresponds to the first association constants of imidazole and 4-methylimidazole with these metal ions as shown in Table II, which are the measures of the extent of interactions.

Table II. First association constants for imidazole and 4-methylimidazole with various metal ions

	Cu^{++}	Zn^{++}	Co^{++}	Ca^{++}
imidazole	4.33[a]	2.57[b]	2.42[b]	0.08[d]
4-methylimidazole	4.13[c]	2.44[c]		

Ionic strength; 0.15, 25°C.

a, Ref. 3; b, Ref. 4; c, Ref. 8; d, Ref. 14.

Effect of acidity ----- In acid pH region, the areas under the same surface pressures were decreased with increase in acidity. On 0.1 N hydrochloric acid, the film dissolved completely in the subphase. On 0.001 N solution (pH 2.8), the film was of expanded type. These facts might be caused by the increased hydrophility of side chains and the repulsion

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14) J. Schubert, J. Am. Chem. Soc., 76, 3442 (1954).

between charged groups due to the protonation to side chains.

Oil/water interface.----- At oil/water interface, poly-l-benzyl-L-histidine monolayer expands much more than at air/water interface owing to the release of van der Waals force between side chains. The extent of the expansion is considerably larger than in the film of poly-DL-phenylalanine which has benzyl groups directly attached to the backbone atoms. The areas of poly-l-benzyl-L-histidine film at definite interfacial pressures are compared with those of poly-DL-phenylalanine in Table III. Such a large expansion of poly-l-benzyl-L-histidine film suggests that histidyl groups which are hydrophilic at the nitrogen atoms lie near the interface. However, the accessibility of the metal ions is restricted by oil. Unlike at air/water interface, no appreciable effects of zinc and cobaltous ions on the poly-l-benzyl-L-histidine monolayer were observed at oil/water interface. The π_c-A curves on 0.01 M zinc sulfate and 0.01 M cobaltous sulfate were coincident with that on the controle acetate buffer solution (0.1 M, pH 5.6). On the other hand, cupric sulfate has a remarkable effect on the film property of this polypeptide as in the case of the film at air/water interface. At air/water interface, cupric ion interacts much more strongly than zinc, cobalt and calcium ions. Accordingly, the complexes of poly-l-benzyl-L-histidine monolayer with cupric ions would be formed even at oil/water interface.

It is interesting that the plateau of π-A curve found on 0.1 M acetate buffer (pH 5.6) at air/water interface is

absent at oil/water interface, and the π_c-A curve was coincident with that at oil/0.1 M potassium chloride interface. At air/water interface, the salt of poly-1-benzyl-L-histidine with acetate ion has much less lateral cohesion than that with chloride ion, causing the plateau in π-A curve. At oil/water interface, however, the lateral cohesion between polypeptide side chains at interface is released by intervening of oil between them. Then, the plateau of π-A curve observed at air/water interface disappears, and the identical π_c-A curves are obtained at oil/acetate buffer and oil/potassium chloride interfaces.

Table III. Areas of poly-DL-phenylalanine[5] and poly-1-benzyl-L-histidine under various interfical pressures

	Area (Å^2/residue)			
Polypeptide	1 dyn./cm.	5 dyn./cm.	10 dyn./cm.	15 dyn./cm.
Poly-DL-phenylalanine*	47	20	14.9	13
Poly-1-benzyl-L-histidine**	100	54	39.6	32

* Oil/distilled water interface

** Oil/0.1 M acetate buffer interface (pH 5.6)

Summary

The effects of metal ions on the monolayer properties of poly-1-benzyl-L-histidine as a model substance of histidyl polypeptide have been studied at air/water and oil/water

interfaces.

Zinc ion was found to interact with the monolayer of this polypeptide, although the extent is much less than in the case of cupric ion. In the presence of zinc ion, the plateau of surface pressure-area curve found on the controle acetate buffer solution (pH 5.6) disappeared and the film became more viscous. This finding was attributed to the complex formation of zinc ion with this polypeptide, which causes a cross-linkage structure between molecules at surface. It was found that cobaltous and calcium ions also form complexes with this polypeptide. The decreasing order of the interaction was found to be Cu^{++}, Zn^{++}, Co^{++}.

At oil/water interface, the film was expanded remarkably owing to the release of van der Waals force between side chains. The effects of metal ions were not observed with the exception of cupric ion because the accessibility of metal ions to the interacting sites of side chains of this polypeptide is restricted by oil.

The author wishes to express his gratitude to Prof. Toshizo Isemura of Osaka University for his kind guidance throughout the present work, and to Prof. Jinzo Noguchi of Hokkaido University for the supply of samples.

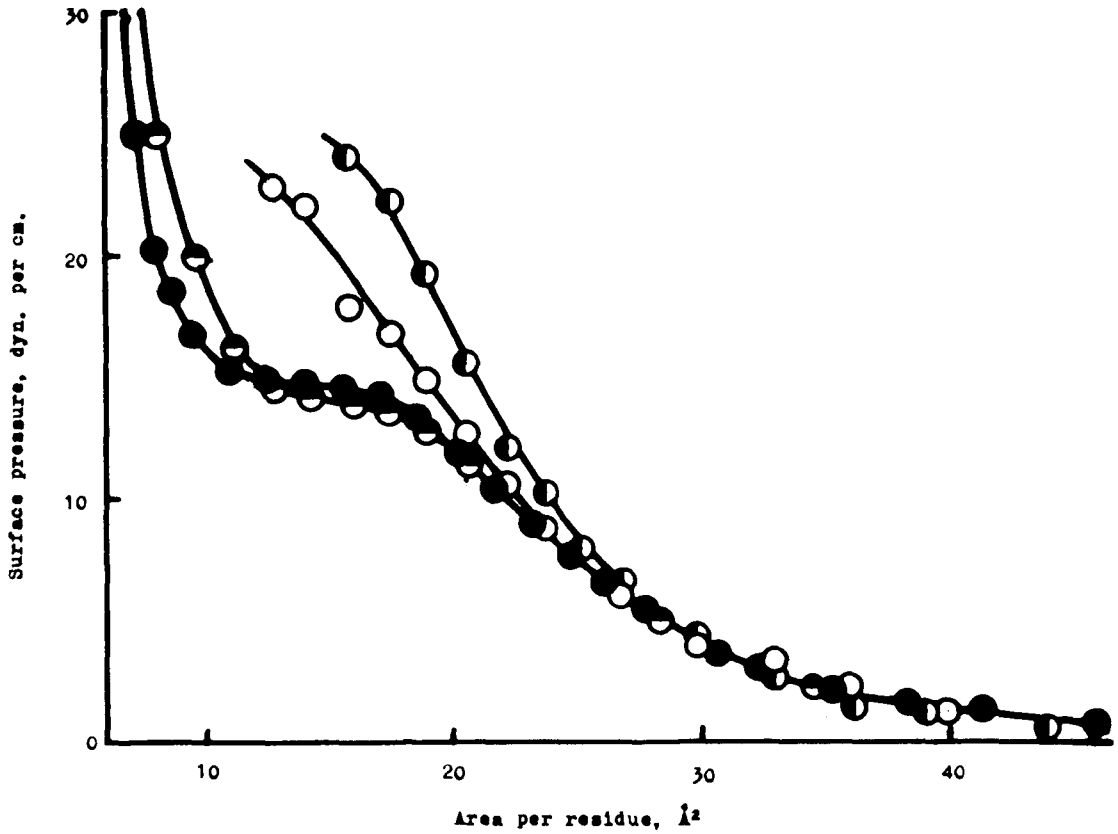

Fig. 1. Effect of zinc sulfate (dissolved in 0.1 M acetate buffer, pH 5.6) on the surface pressure-area relation of poly-1-benzyl-L-histidine at 24°C: ●, 0.1 M acetate buffer (control), 20°C; ◓, 0.001 M; ○, 0.01 M; ◐, 0.05 M.

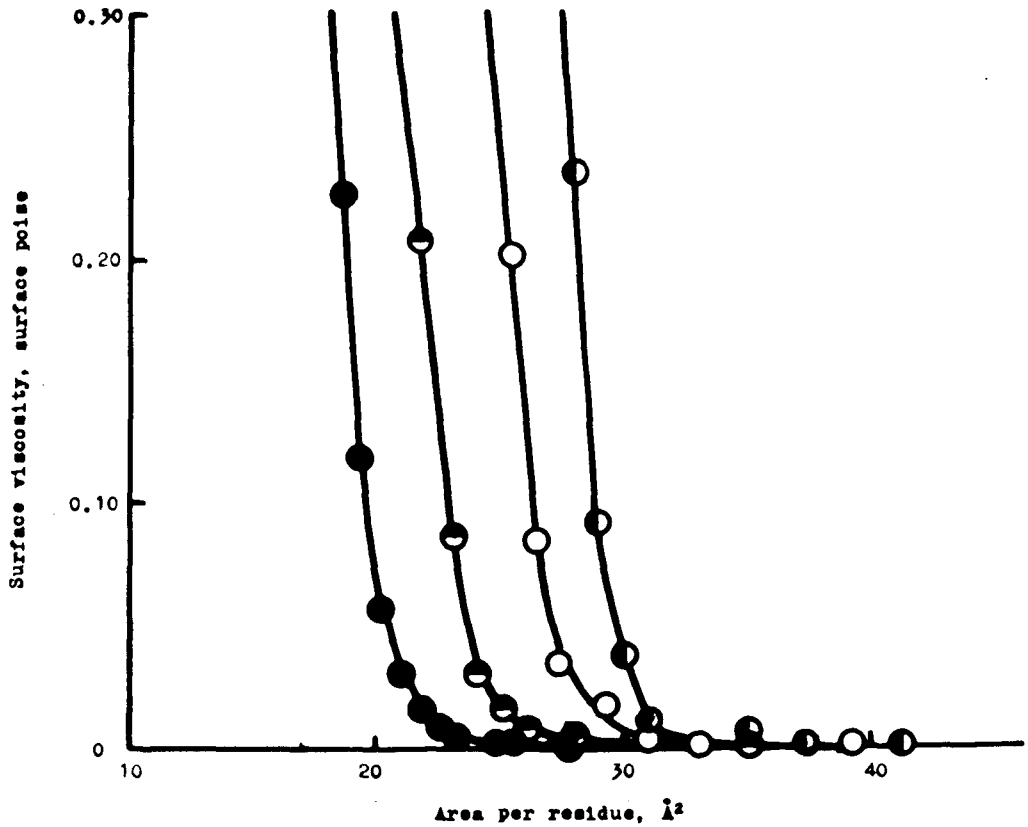

Fig. 2. Effect of zinc sulfate (dissolved in 0.1 M acetate buffer, pH 5.6) on the surface viscosity-area relation of poly-1-benzyl-L-histidine at 16°C: ●, 0.1 M acetate buffer (control); ◓, 0.001 M; ○, 0.01 M; ○, 0.05 M.

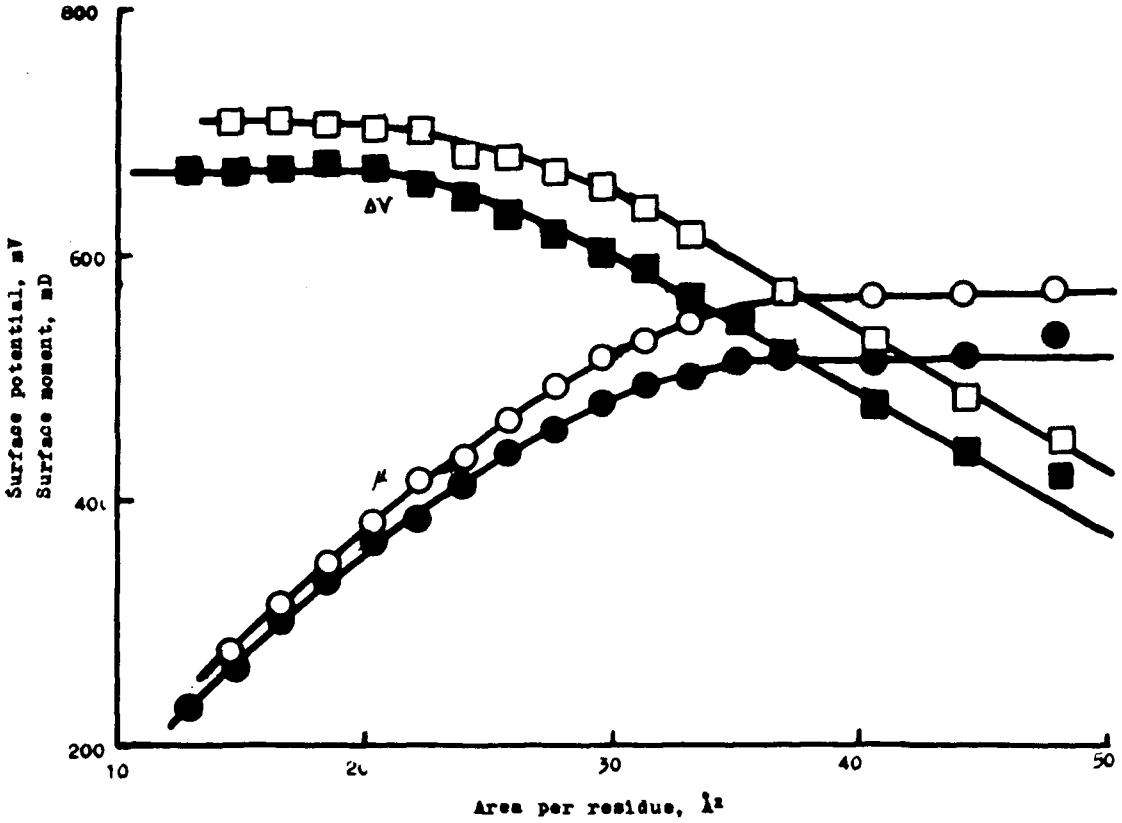

Fig. 3. Surface potential-area and surface moment-area curves of poly-l-benzyl-L-histidine on 0.1 M acetate buffer (pH 5.6; ■ , ●) and on 0.1 M acetate buffer containing 0.01 M zinc sulfate (pH 5.6; □ , ○): 12° C.

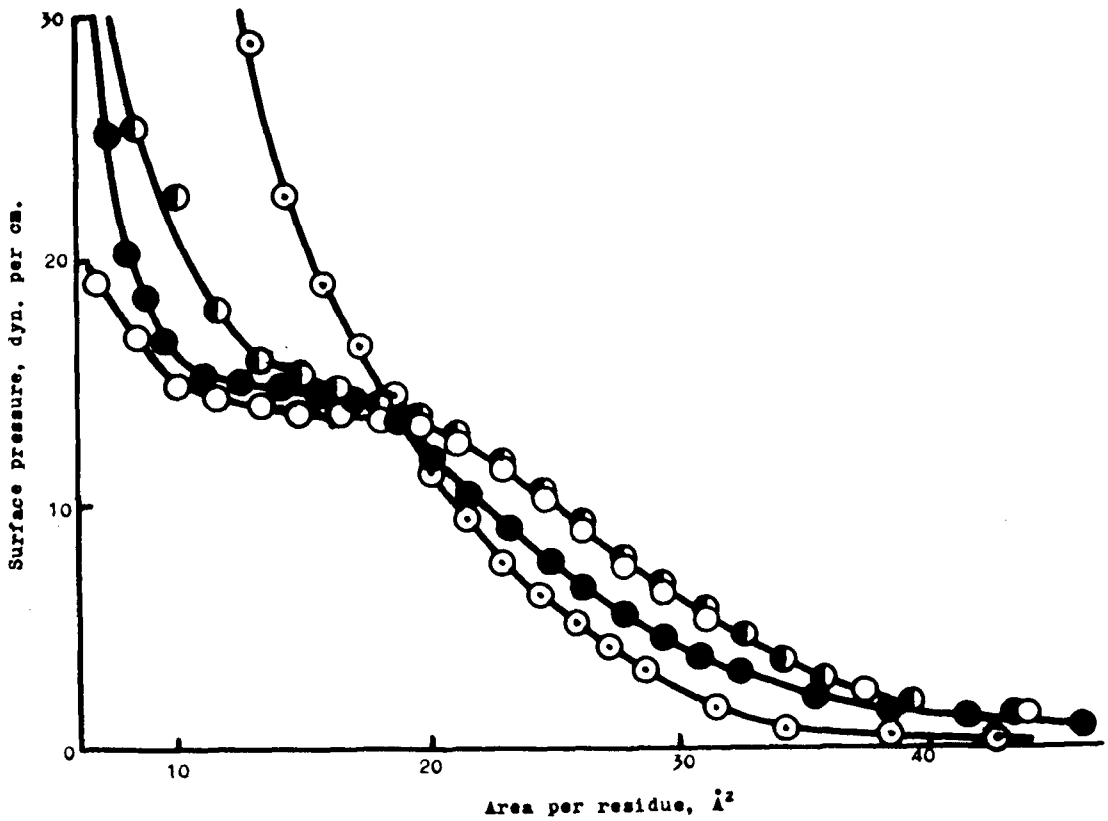

Fig. 4. Effects of cobaltous sulfate and calcium sulfate (dissolved in acetate buffer, pH 5.6) on the monolayer of poly-l-benzyl-L-histidine at 24° C: ● , 0.1 M acetate buffer (control), 20° C; ○ , 0.01 M $CoSO_4$; ◐ , 0.05 M $CoSO_4$; ⊙ , 0.05 M $CaCl_2$.

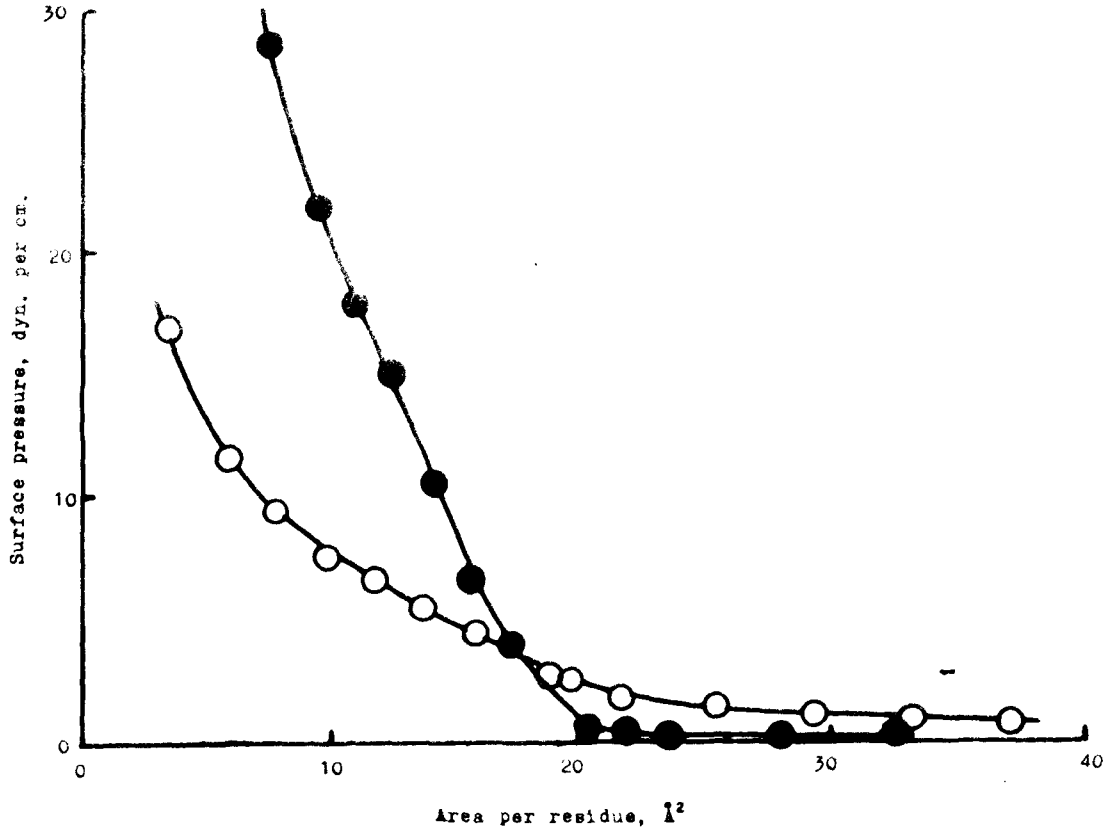

Fig. 5 Surface pressure-area curves of poly-1-benzyl-L-histidien on distilled water (●) and on 0.001 M hydrochloric acid (O) at 20° C.

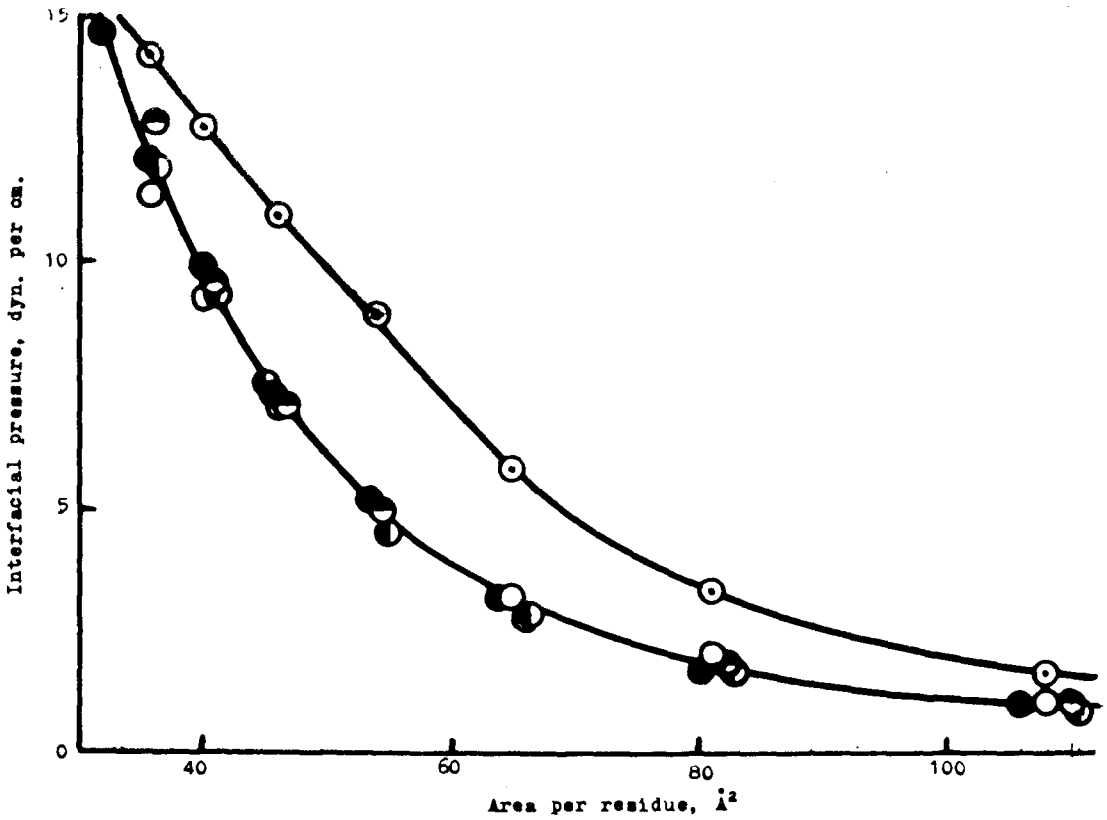

Fig. 6. Monolayers of poly-1-benzyl-L-histidine at oil/water interface (21° C): ●, 0.1 M acetate buffer (pH 5.6); O, 0.1 M KCl; ⊖, 0.01 M $ZnSO_4$ (0.1 M acetate, pH 5.6); ◐, 0.01 M $CoSO_4$ (0.1 M acetate, pH 5.6); ⊙, 0.01 M $CuSO_4$ (0.1 M acetate, pH 5.6).