Surface Chemistry of Synthetic Protein Analogues. III. On the Surface Viscosity of Monolayers of Non-electrolytic Synthetic Polypeptides

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Introduction

In the preceding papers of this series,[1),2)] the properties of spread monolayers of some synthetic polypeptides on water investigated by surface pressure measurements were reported in relation to the models of proteins. It was found that poly-DL-α-aminocaproic acid showed very close resemblance of force-area relation to natural proteins such as pepsin and egg albumin among other polypeptides such as poly-DL-α-aminocaprylic acid, poly-DL-α-aminocapric acid and poly-DL-α-aminolauric acid. The latter polypeptides having longer side chains than six carbon atoms in length, gave quite different force-area relation. In F–A curves of these polypeptides, plateau of high compressibility appeared. Poly-γ-methyl-L-glutamate gave condensed film on distilled water. In this case, the polypeptide chain assumed presumably the structure of α-type. This film was expanded on the acidified substrate, and the polypeptide chain may be transformed into β-keratine type. The appearance of the plateau in the F–A curve was attributed to the reorientation of the side chain of the polypeptide. The surface pressure at the plateau depends on temperature and also on the number of carbon atoms in the side chains. From the temperature dependence of the transition pressure and the difference of the areas before and after transformation, the energy needed for reorientation was estimated experimentally.

It is generally accepted that the force-area curves of various natural proteins are not profoundly changed by the species of the proteins. Consequently, it is very difficult to know some characteristics of various proteins from their force-area relations of spread monolayers. On the other hand, it is well-known that the proteins are widely different from each other in their surface viscosity.[3)] Accordingly, the measurements of surface viscosity of the spread monolayers of synthetic polypeptides seemed to be very significant for the comprehension of the natural proteins. In the present paper, the results of the

1) T. Isemura and K. Hamaguchi, This Bulletin, **25**, 40 (1952).
2) T. Isemura and K. Hamaguchi, ibid., **26**, 424 (1953).
3) M. Joly, *J. chim. phys.*, **36**, 285 (1939).

measurements of surface viscosity of polypeptide monolayers and also some consideration of the results will be described.

Experimental

Surface viscosity was measured by the damping of the oscillatory motion of rotating disc on the surface of liquid. The method was essentially the same with that adopted by Langmuir and Schaefer[4] and by Joly[5]. The surface pressure was measured simultaneously by the hanging plate method. The moment of inertia of the oscillatory disc (I) was 66.51, and the radius of the disc (R_1) 1.27 cm. The torsion constant of the torsion wire was 42.0. The radius of the film surrounding the oscillatory disc (R_2) was 5.04 cm. The surface viscosity was calculated by the following formula,

$$\eta = \Delta\lambda_{10} \frac{2.3I}{2\pi P}\left(\frac{1}{R_1^2} - \frac{1}{R_2^2}\right)$$

where P is the period of the oscillatory pendulum and $\Delta\lambda_{10}$ is the difference between logarithmic decrements of oscillation both in the presence of film and in the absence of film. The polypeptides examined in the present experiments and their solvents used for the spreading are tabulated in Table I. Side chains and residue weight for amino acid are also shown in the Table.

TABLE I

Polypeptides	Side chains	Residue weight	Solvents
Poly-DL-α-aminocaproic acid	$-(CH_2)_3 \cdot CH_3$	113.1	benzene
Poly-DL-α-aminocaprylic acid	$-(CH_2)_5 \cdot CH_3$	141.1	benzene
Poly-DL-α-aminocapric acid	$-(CH_2)_7 \cdot CH_3$	169.1	benzene
Poly-DL-α-aminolauric acid	$-(CH_2)_9 \cdot CH_3$	197.1	benzene
Poly-γ-methyl L-glutamate	$-(CH_2)_2COOCH_3$	143.1	pyridine
Copolypeptide of glycine and alanine			dichloroacetic acid + benzene (1:4)
Poly-γ-benzyl-DL-and L-glutamate	$-(CH_2)COOCH_2C_6H_5$	219.1	chloroform
Amilan		99.1	cresol + benzen (1:3)

Results and Discussion

(I) Viscosity-area Curves of Poly-DL-α-aminocaproic Acid, Poly-DL-α-aminocaprylic acid, Poly-DL-α-aminocapric Acid and Poly-DL-α-aminolauric Acid.

The η–A curves of these polypeptides are shown in Fig. 1. From this figure, it can be

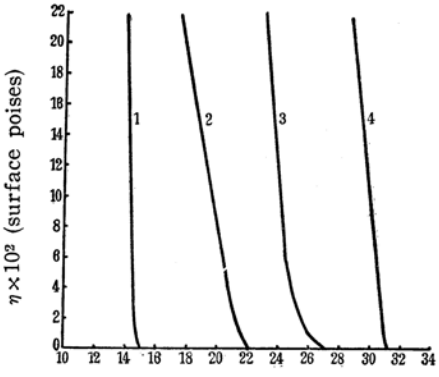

Fig. 1. The surface viscosity-area curves of poly-DL-α-aminocaproic acid (1), poly-DL-α-aminocaprylic acid (2), poly-DL-α-aminocapric acid (3) and poly-DL-α-aminolauric acid (4) (25°C.).

readily seen that the area where surface viscosity was first detected, was related to the side chains. However, the limiting area of the amino-acid residue obtained from the η–A curve for poly-DL-α-aminocaproic acid was 14.7Å². This is an area occupied by an amino-acid residue if the polypeptide of β-keratine configuration was packed closely and it also corresponds to the area per residue estimated from X-ray data as already reported in the previous paper.[1] Cumper and Alexander[6] also found 14.7 Å² as the limiting area per residue for the monolayer of poly-alanine at liquid/liquid and air/water interfaces and for the monolayer of poly-phenylalanine at air/water interface. However, if the length of the side chain becomes longer than six carbon atoms, as in the case of poly-DL-α-aminocaprylic acid, the limiting area becomes larger with the length of the side chain. Consequently, it seems that the increase of the length of the side chains laid on the surface profoundly affects the increase of surface viscosity. The surface viscosity of these polypeptides with longer side chains reaches considerably high values even under the extremely low surface pressure. So, we can not interpret the η–A curve in relation to the F–A curve of the respective polypetide. When the surface fluidity, namely, the reciprocal of surface viscosity was plotted as a function of the area per residue, the change of the nature of film could be readily detected from the relation of the fluidity and the area per residue, even at low surface pressure and

4) I. Langmuir and V. J. Schaefer, *J. Am. Chem. Soc.*, **59**, 2400 (1937).
5) M. Joly, *Kolloid-Z.*, **89**, 26 (1939).
6) C. W. N. Cumper and A. E. Alexander, *Trans, Faraday Soc.*, **46**, 235 (1950).

rather high surface viscosity region where any change can hardly be detected from F–A and η–A relations.

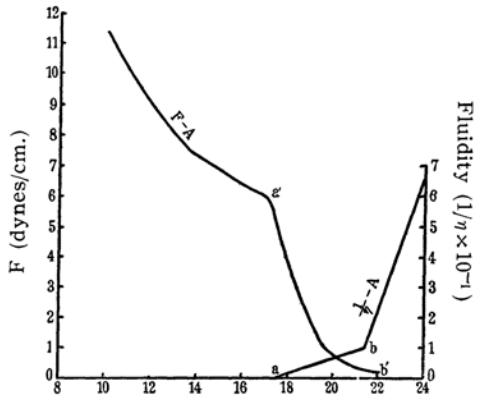

Fig. 2. The force-area and fluidity-area curves of poly-DL-α-aminocaprylic acid (24°C.).

F–A and $1/\eta$–A curve for poly-DL-α-aminocaprylic acid are shown in Fig. 2 and those for poly-DL-α-aminocapric acid in Fig. 3. In these $1/\eta$–A curves, there exist definite kink points b and c. a is the intercept of $1/\eta$–A

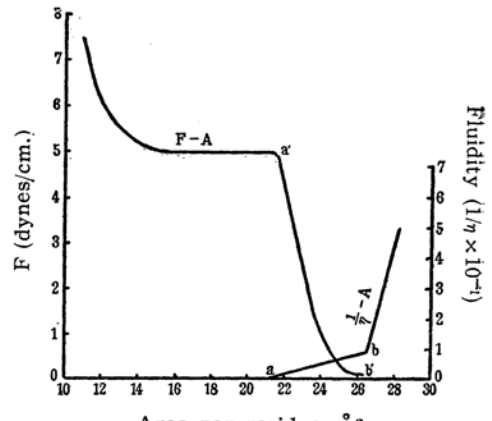

Fig. 3. The force-area and fluidity-area curves of poly-DL-α-aminocapric acid (23°C.).

curve, with area axis extrapolated to zero fluidity. On the other hand, b′ is the area where the surface pressure becomes detectable in the F–A curve and a′ is the area where the plateau begins. These areas are listed in Table II. From the table it will be seen that a is in good agreement with a′ and b with b′.

TABLE II

Polypeptides	Area (Å²/residue) a	a′	b	b′	c	
Poly-DL-α-aminocaprylic acid	17.3	17.2	31.5	21.5	25.3	(24°C.)
Poly-DL-α-aminocapric acid	21.0	22	26.5	24.5	28	(22°C.)
Poly-γ-benzyl-DL-glutamate	19.0	19.5	23.7	24		(9°C.)

(II) Change of Configuration of Poly-γ-methyl-L-glutamate in the Monolayer.

As already reported in the previous paper[1], the monolayer of poly-γ-methyl-L-glutamate behaves differently from other polypeptides, and has a limiting area of 9.8 Å²/residue which was determined by the F–A relation. However, when it was spread on the substrate acidified by formic acid or acetic acid, the monolayer gave the F–A curve analogous to those for other polypeptides with shorter side chain as poly-DL-α-aminocaproic acid and its limiting area per residue was found to be 14.7 Å². The change was interpreted in the light of the results of the infra-red observation of this polypeptide by Ambrose and Hanby[7] and by Bamford, Hanby and Happey[8]. It was concluded that on aqueous substrate the polypeptide assumes the α_{II} configuration proposed by Shimanouchi and Mizushima,[9] and by Ambrase and Hanby[7] independently, and on formic acid or acetic acid solution it turns

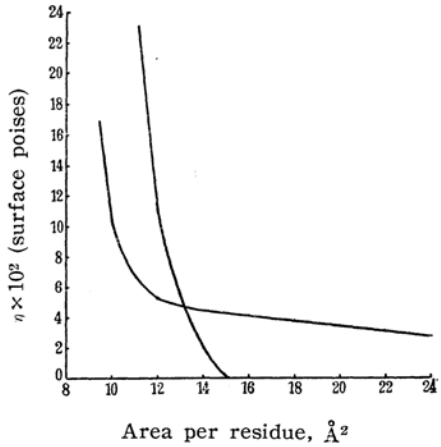

Fig. 4. The surface viscosity-area curves of poly-γ-methyl-L-glutamate. (10°C.): curve I, on distilled water; curve II, on 0.02 N formic acid.

7) E. J. Ambrose and W. E. Hanby, *Nature*, **163**, 483 (1949).
8) C. H. Bamford, W. E. Hanby and F. Happey, *Nature*, **166**, 829 (1950).
9) T. Shimanouchi and S. Mizushima, *Science (Japan)*, **17**, 24, 52 (1947), This Bulletin, **21**, 1 (1948).

to β-keratine type. The difference between both films spread on aqueous and on formic or acetic acid substrate was definitely demonstrated from the results of the surface viscosity measurement. The results are shown in Fig. 4. Curve I in the figure is the η–A curve on the distilled water and Curve II is that obtained on aqueous formic acid substrate (0.02 N). The film, assumed to be as of α-modification on the water surface, was viscous even in low surface concentration which exerts no detectable surface pressure with our surface balance. This is the distinct difference with films of other polypeptides examined. Below the area of 9.8 Å²/residue, the surface viscosity was rapidly increased with small reduction of area. On the other hand, the viscosity of the film spread on formic acid substrate increases below the area of 14.7 Å²/residue as other polypeptides of shorter side chains such as poly-DL-norleucine. It is very interesting that this may be the surface chemical indication of the $\alpha \rightarrow \beta$ transformation of poly-γ-methyl-L-glutamate by formic acid.

Recently, our conclusion that the α_{II}-structure proposed by Shimanouchi and Mizushima and by Ambrose and Hanby is plausible for the structure of α-keratine from our result of film area measurement, was criticised by Low[10]. He insisted that our conclusion is invalid because the area was reduced in proportion to the reduction of the length of the main chain, and no consideration for inter-chain packing and orientation was paid. However, in our opinion, when the polypeptide chain was compressed, the polypeptide chain is closely packed so as to orientate the side chain of one side in air and the other in water, irrespective of the structure of the polypeptide in either α- or β-configuration. The thickness of polypeptide chain lying flat should be the same, in the case of both α- and β-structure. This could lead to our conclusion that the area occupied by the closely packed polypeptide chain depends only on the length of the main chain.

(III) Surface Viscosity-area Relation of Copolymer of Glycine and Alanine.

The polypeptides so far investigated were of more than four carbon atoms in length. It is very interesting to investigate how the polypeptides behave at the surface if the polypeptides have no side chains or very short ones. When the polypeptides have long side chains, the coiling of the main chains might be difficult on account of the mutual hindrance of the side chains. In this connection the glycine-alanine copolymer was studied. This copolymer may be easily coiled. It is interesting to compare the behaviour of such a polypeptide with a linear polymer having no side chains such as amilan, on which we will discuss in the later part.

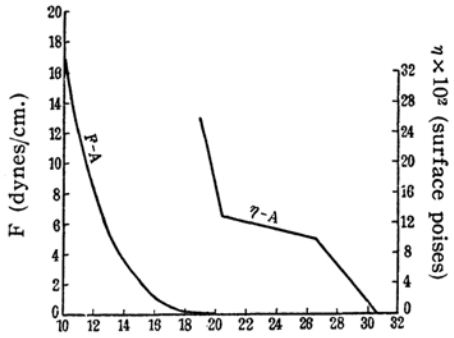

Fig. 5. The surface pressure-area and surface viscosity-area curves of glycine-alanine copolymer (22°C.).

The F–A curve and η–A curve of the glycine-alanine copolymer are shown in Fig. 5. The present investigation of F–A curve, the limiting area per glycine-alanine residue was about 20 Å², namely, about 10 Å² per amino-acid residue. This fact suggests that this polymer may assume also the structure of folded type as in the case of poly-γ-methyl-L-glutamate. The F–A curve for this copolymer film is, however, different from that for poly-γ-methyl-L-glutamate in its behaviour against the nature of substrate. Although the latter film expands on the acidic substrate considerably, the former film gave the same F–A curve on the acidic substrate as on the distilled water. On the other hand, the surface viscosity of this copolymer film was detected at the area of 30 Å² per glycine-alanine residue, namely at the area of 15 Å² per amino-acid residue, and at the area of 20 Å² per glycine-alanine residue, the surface viscosity was again suddenly increased. This area corresponds to the area from which the surface pressure was suddenly exerted. When the main chain of polypeptide in β-keratine type was packed closely, the residue of amino-acid in polypeptide occupies 15 Å² on the surface. On the other hand, the residue of amino-acid occupied 10 Å² on the surface, when the main chain of the polypeptide takes the folded structure of α_{II}-type and was packed closely. Accordingly, we might conclude that the copolymer was spread as a film assuming β-keratine type. However, the polypeptide chain will be readily trans-

10) B. W. Low, "The Proteins" ed. by H. Neurath and K. Bailey, Vol. I, Part A, p. 376 (1953).

formed into α-type by slight compression that this transformation cannot be detected by the surface pressure measurement. In the region of this transformation the film may be heterogeneous and the surface viscosity was somewhat fluctuated. From this result mentioned above the polypeptides having very short side chains or none tend to assume readily folded structure. This inference seems plausible from the recent observation of A. Elliott[11] on the infra-red spectrum of poly-DL-alanine. He described in his recent paper, that it is evident that in poly-DL-alanine the equilibrium between α- and β-forms is very nicely balanced, and the transformation from one to the other is readily induced. This conclusion might be applied generally to the polypeptide of very short side chain such as glycine-alanine copolymer. From this viewpoint, our results on the surface viscosity-area relation of this copolymer could be explained successfully.

(IV) The Difference between L-and DL-Polypeptides of γ-Benzyl Glutamate.

For the investigation of the difference between the behaviour of optical active and of racemic polypeptide, the film of poly-γ-benzyl-L-glutamate and of poly-γ-benzyl-DL-glutamate were studied. The F–A and η–A curves of these polypeptides are shown in Fig. 6. On

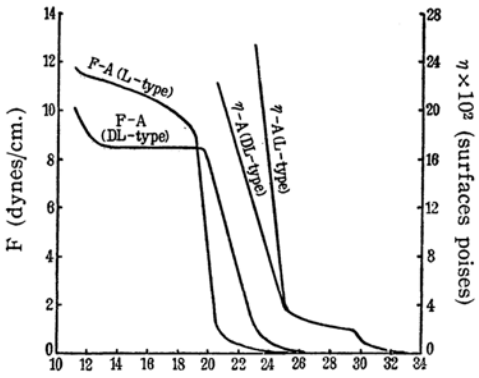

Fig. 6. The surface pressure-area and surface viscosity-area curves of poly-γ-benzyl L- and DL-glutamates (10°C.).

the one hand, F–A curves and η–A curves of these polypeptides showed generally similar trends respectively, and plateaus appeared in the F–A curves for both polypeptides. However, we can notice the following differences by careful inspection of these curves. (1) The limiting area determined from F–A curves were 24 Å/residue for DL-type polypeptide and 21 Å²/residue for L-type polypeptide. (2) The compressibility of the film up to the pressure at which the plateau appears was smaller for L-type polypeptide than for DL-type polypeptide. (3) The F–A curve of poly-γ-benzyl-DL-glutamate was strikingly influenced by temperature as reported in the previous paper.[2] The pressure where the plateau appearing in the F–A curve was decreased with the rise of temperature as in the cases of the films such as poly DL-α-aminolauric acid. On the contrary, in the case of poly-γ-benzyl-L-glutamate, temperature scarcely affected on the F–A relation and the pressure at which the plateau appears, was almost independent of temperature and nearly 8.5 dynes/cm. (4) The area at which the surface viscosity is suddenly increased, was about 25 Å²/residue irrespective of the type of polypeptide. However, the inclination of η–A curve, namely $\partial\eta/\partial A$ was smaller for L-polypeptide than for DL-polypeptide. To clarify the inter-relation between F–A curve and η–A curve, $1/\eta$–A curve was considered. (Fig. 7). a, b, a′ b′,

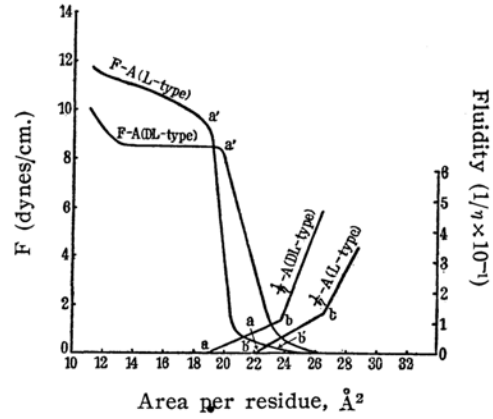

Fig. 7. The force-area and fluidity-area curves of poly-γ-benzyl-DL- and L-glutamates (10°C.).

for L-polypeptide and DL-polypeptide were summarized in Table III. The results shown

TABLE III

Poly-γ-benzyl glutamate	Area (Å²/residue) a	a′	b	b′
L-type	22.3	18.5	26.5	22
DL-type	19.0	19.5	23.7	24

in Table III were obtained as already described, with poly-γ-benyl-DL-glutamate. The area from which the plateau begins to appear, a′, coincides with the area found from extrapolation of $1/\eta$–A curve to $1/\eta=0$. Whereas, with the film of poly-γ-benzyl-L-glutamate, the area from which the surface pressure becomes appreciable, b′ (22 Å²) corresponded to the area found by extrapolation

11) A. Elliott, *Nature*, **170**, 1066 (1952).

of $1/\eta$-A curve to $1/\eta=0$ (21.5Å²).

From these experimental facts, it can be concluded that the films compressed up to the pressure where the plateau begins to appear, are entirely different for L- and DL-polypeptide, respectively. The arrangement of the L-polypeptide chains is far more closely packed. The film of L-polypeptide in such condition is in a nearly solid state.

(V) The Surface film of Amilan.

F–A curve of the monolayer of amilan was already reported in the previous paper[1]. In F–A curve, a kink point was found at about 32 Å²/residue. This area is in good agreement with the area occupied by the residue calculated in the basis of the molecular scale model.[6),12)] On the other hand, it was found that some change occurs at the area of 80 Å²/residue from the sudden change of surface viscosity as shown in Fig. 8. This is

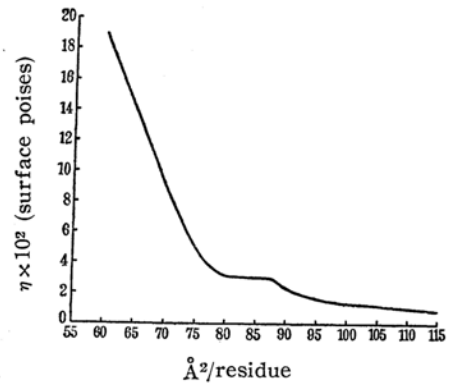

Fig. 8. The surface viscosity-area curve of amilan (22°C.).

entirely different from the films of poly-DL-α-aminocaproic acid and glycine-alanine copolymer. From the surface potential measurement it was found that the potential fluctuated down to the area 80~90 Å²/residue, below which it changes regularly as reported by Hotta[13)] in our Laboratory. Amilan was quite different from the polypeptides films. From this result, our conclusion that amilan is not adequate as the model of protein, was again confirmed.

12) D. J. Crisp, *J. Colloid Sci.*, **1**, 49 (1946).
13) H. Hotta, This Bulletin, **27**, 80 (1954).

Summary

The surface viscosity of the spread monolayers of synthetic polypeptides with non-electrolytic side chains was investigated in relation to the surface area and to the surface pressure. If the length of a side chain becomes longer than six carbon atoms, the limiting area becomes larger with the length of the side chain. Polypeptide with no side chains or very short ones such as glycine-alanine copolymer might be changed to α-configuration from "extended" β-configuration merely by compression. The ease of transition from one configuration to another corresponds to the discovery by Elliott in bulk solution with poly-alanine. $\alpha_{II} \rightarrow \beta$ transformation of poly-γ-methyl-L-glutamate which had been reported in the previous paper, was observed also by the surface viscosity measurements. The difference of the nature of films of optical active and of racemic polypeptide was investigated. The polypeptide consisting of single optical isomeride was able to be more closely packed in the film than that of racemic. Our discovery by surface pressure measurement that the polymer of ϵ-aminocaproic acid, namely, amilan is not adequate as the model of protein, was again confirmed from the study of surface viscosity.

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