Surface Chemistry of Synthetic Protein Analogues. I. Surface Pressure-Area Relation of Synthetic Polypeptides as the Model of Proteins

By Toshizo ISEMURA and Kozo HAMAGUCHI

(Received October 8, 1951)

Introduction

Many investigations have been made of the monolayers of proteins at air-liquid or liquid-liquid interfaces to obtain some information about the nature of protein fiims.[1] The surface chemistry of proteins involves many problems to be solved, because of the fact that the proteins are of a rather complicated structure and consisted of various amino-acid residues which are not arranged in orderly manner with uniform distribution along the polypeptide chain. Recently, it became possible to synthesize simple protein analogues of the known structure and of the high molecular weight, by polymerization of N-carboxy-anhydride of α-amino acid.[2] It is well convinced that these synthetic protein analogues play a very important role as a model of natural protein, because of these simple constitutions having one or two known amino acids, though it is unfortunate that the molecular weight distribution is not so uniform as that of natural proteins. It is our aim to obtain some information of natural protein from the surface chemical study of these polymers and to approach to the structure of proteins.

Recently, some interesting studies by Ambrose,[3] Astbury[4] and their collabrators on the synthetic polypeptides by x-ray and infrared analysis have appeared. Whether the results obtained by the monolayer technique would coincide with these by x-ray and infrared data, or not, is also an interesting problem to be investigated.

The synthetic protein analogues examined in the present investigation were poly DL-α-aminocaproic, poly DL-α-aminocaprylic, poly DL-α-aminocapric, poly DL-α-aminolauric acid, and poly γ-methyl-L-glutamate. Poly ε-aminocaproic acid(amilan) was also examined in comparison with the polymers of α-amino acid.

At the first step, surface pressure-area (F-A) relations of the monolayers of these protein analogues on water were examined, and some results obtained were reported in the previous notes.[5],[6] It was found that the F-A curve of poly DL-α-aminocaproic acid which has shorter side chain and those given by natural proteins such as ovalbumin or pepsin resembled very closely in shape. The region which has a very high compressibility appeared in the F-A curves of the polypeptides of which side chains have more than six carbon atoms. It is believed that poly DL-α-aminocaproic, poly DL-α-aminocaprylic, poly DL-α-aminocapric, and poly DL-α-aminolauric acid have a structure of β-keratin type on the surface of water since the area which was found for every polymer to be in the closest packing of polypeptide backbone, was all 14.7 Å.^{2}/amino acid residue. This value is in good agreement with the area calculated by x-ray data for an

(1) J. S. Mitchell, *Trans. Faraday Soc.*, **33**, 1129 (1937); I. Langmuir and V. J. Schaefer, *Chem. Rev.*, **24**, 181 (1939).

(2) R. B. Woodward and C. H. Schramm, *J. Am. Chem. Soc.*, **69**, 1551 (1947); H. Tani, J. Noguchi, *et al.*, *Chem. High Polymers, Japan*, **8**, 51, 53, 57, 62, 65 (1951).

(3) E. J. Ambrose and W. E. Hanby, *Nature*, **163**, 483 (1949); C. H. Bamford, W. E. Hanby and F. Happey, *ibid.*, **164**, 138 (1949); C. H. Bamford, W. E. Hanby and F. Happey, *ibid.*, **164**, 752(1949); A. Elliot and E. J. Ambrose, *ibid.*, **165**, 921 (1950); C. H. Bamford, W. E. Hanby and F. Happey, *ibid.*, **166**, 829 (1950).

(4) W. T. Astbury, C. E. Dagliesh, S. E. Darmon and G. B. B. M. Sutherland, *Nature*, **162**, 596 (1948); W. T. Astbury, *ibid.*, **163**, 722 (1949); W. T. Asthury, *ibid.*, **164**, 439 (1949).

(5) T. Isemura, K. Hamaguchi, H. Tani, J. Noguchi and H. Yuki, *Nature*, **168**, 165 (1951).

(6) T. Isemura and K. Hamaguchi, *Kagaku (Science)*, **22**, 87 (1952).

arrangement of β-configuration. Polyγ-methyl-L-glutamate occupied less area per residue than any other polypeptides. It may have a structure of α-keratin configuration. This supposition was comfirmed by the transformation of the film into a film constituted of the molecules of β-configuration by adding some acids to the substrate water.

Experimental

Experimental Method and Materials.—Surface pressure was registered by Sasaki's modification[7] of Harkins' method using the Wilhelmy balance.

The synthetic polypeptides used were prepared by Dr. H. Tani, Dr. J. Noguchi and their collabrators and the samples of amilan were presented by Toyo Rayon Co. The polypeptides were used without any further purification. Amilan was purified by the precipitation from the solution in formic acid by adding water.

Poly DL-α-aminocaproic, poly DL-α-aminocaprylic, poly DL-α-aminocapric, and poly DL-α-aminolauric acids were spread from benzene solution, poly γ-methyl-L-glutamate from pyrididine solution and amilan from a solution in a mixed solvent of cresol and benzene. The molecular weight of the poly DL α-aminolauric acid determined by the osmotic pressure measurement was 172,000. The molecular weight of poly α-aminocaproic acid by the surface technique was 1,060,000.

Results.—The *F-A* curves obtained with the five polypeptides described above on distilled water are shown in Figs. 1 and 2. The abscissa is the area (m.^{2}) per milligram of polypeptide in Fig. 1 and the area (Å.^{2}) per amino acid residue in Fig. 2. These curves were obtained for the films allowed to stand on water prior to the experiment for six minutes after spreading.

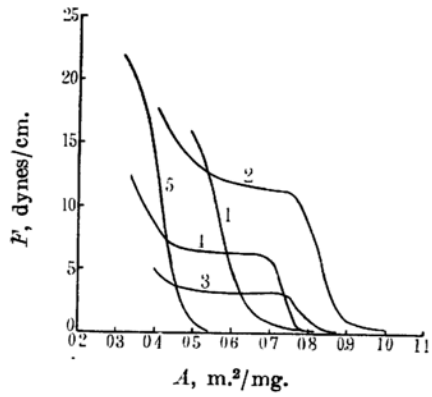

Fig. 1.—The surface pressure-area curves of polypeptide monolayers on distilled water (9°C.): Curve 1, poly DL-α-aminocaproic acid; curve 2, poly DL-α-aminocaprylic acid; curve 3, poly DL-α-aminocapric acid; curve 4, poly DL-α-aminolauric acid; curve 5, poly γ-methyl-L-glutamate.

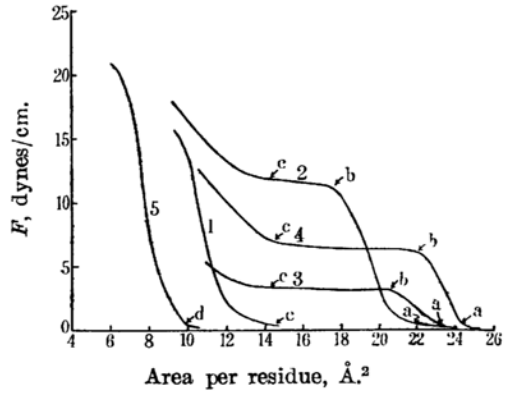

Fig. 2.—The surface pressure-area curves of polypeptide monolayers on distilled water (9°C.).

Poly DL-α-aminocaproic acid (curve 1) occupied the area of 0.68 m.^{2}/mg. (13 Å.^{2}/residue) at 1 dyne/cm., which is in good agreement with the area of 0.7 m.^{2}/mg. (about 14 Å.^{2}/residue) at 1 dyne for the egg albumin or pepsin.[8] The shape of the curve of this polymer was also very similar to that of these natural proteins.

The plateau which is a region of very high compressibility (from point b to c in Fig. 2), appeared in the *F-A* curves of poly DL-α-aminocaprylic (curve 2), poly DL-α-aminocapric (curve 3), and poly DL-α-aminolauric acid (curve 4). As far as our surface balance of hanging plate type was concerned, the plateau was not recognized for the curve of poly DL-α-aminocaproic acid, but was found to exist in very low pressure region (at about 0.05 dyne/cm.) by using the surface balance of Langmuir-Adam type which has the sensitivity of about 0.001 dyne/cm.

The areas at the points a, b, c, and d which are indicated by arrows for each curve in Fig. 2 are summarized in Table 1. The longer the side chain was, the larger the limiting area (at point a) became. The area at point c was almost the same irrespective of the monomer of α-amino acid. (about 14.7 Å.^{2}/residue). Poly γ-methyl-L-glutamate (curve 5) had a very small limiting area of 9.8 Å.^{2}/residue (at point d).

(7) T. Sasaki, *J. Chem. Soc. Japan*, **62**, 796 (1941).

(8) I. Langmuir and D. F. Waugh, *J. Am. Chem. Soc.*, **62**, 2771 (1940).

Table 1

Polypeptides	Side chain	Area, Å.²/residue Point a	b	c	d
α-Aminocaproic acid	$-(CH_2)_3CH_3$			14.6	
α-Aminocaprylic acid	$-(CH_2)_5CH_3$	22.0	18.0	14.7	
α-Aminocapric acid	$-(CH_2)_7CH_3$	23.0	21.0	14.8	
α-Aminolauric acid	$-(CH_2)_9CH_3$	24.2	22.2	14.7	
γ-methyl-L-glutamate	$-(CH_2)_2COOCH_3$				9.8

The *F-A* curves of poly DL-α-aminocaproic acid and poly γ-methyl-L-glutamate changed little with time after spreading, but the *F-A* curves of the other polypeptides which have longer side chains changed extremely with time. For instance, the effect of aging for poly DL-α-aminocaprylic and poly DL-α-aminolauric acid is shown in Figs. 3 and 4, respectively. The initial spreading concentrations of the former and the latter polymer were 0.51 and 0.48 mg./m.², respectively.

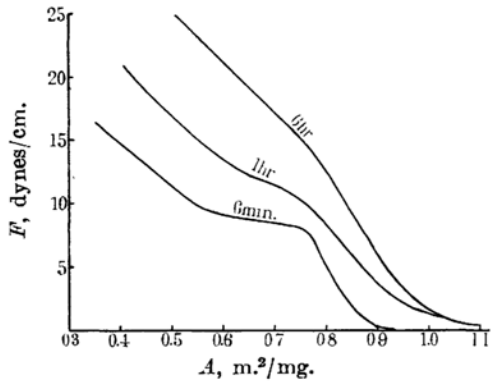

Fig. 3.—Poly DL-α-aminocaprylic acid aged on distilled water (16.5°C.).

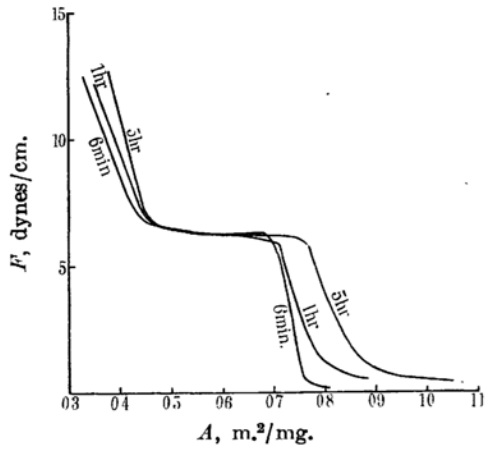

Fig. 4.—Poly DL-α-aminolauric acid aged on distilled water (9°C.).

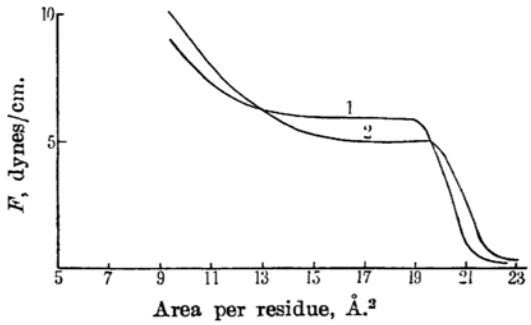

Fig. 5.—Effect of substrate on the acidity of monolayer of poly DL-α-aminocapric acid (21°C.): curve 1, on distilled water; curve 2, on 0.2 *M* formic acid.

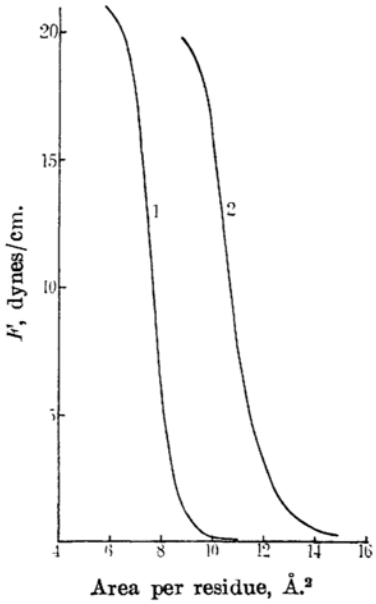

Fig. 6.—Effect of acidity of substrate on the monolayer of poly γ-methyl-L-glutamate (22°C.): curve 1, on distilled water; curve 2, on 0.02 *M* formic acid.

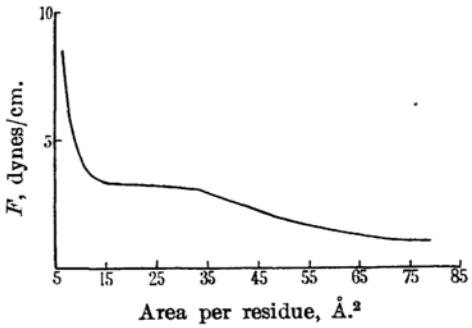

Fig. 7.—The surface pressure-area curve of poly ε-aminocaproic acid. (23°C.)

Acidic substrate exerted no detectable effect upon the monolayers of poly DL-α-aminocaprylic, poly DL-α-aminocapric and poly DL-α-aminolauric acid. For instance, the effect of 0.2 *M* formic acid on the monolayer of poly DL-α-aminocapric acid is shown in Fig. 5. The *F-A* curve of the monolayer on acidic substrate is shown by curve 2, together with the curve on distilled water (curve 1) in the figure.

On the other hand, when poly γ-methyl-L-glutamate was spread on acidic substrate, the limiting area was increased to larger area than on distilled water. Curve 2 in Fig. 6 shows *F-A* curve of the polymer on acidic substrate containing formic acid in concentration of 0.02 *M*. For comparison with the monolayer on acidic substrate, the curve of the monolayer on distilled water is shown by curve 1 in the figure. Using acetic or hydrochloric acid solution as substrate, the curves were practically the same as curve 2. The limiting area of the curve on these acid substrate was also 14.7 Å.^{2}/residue, corresponding to the area of the other polypeptides at point c in Table 1.

In Fig. 7, the *F-A* curve of amilan on distilled water is shown. The polymer of ε-amino acid gave the curve of an expanded type which was entirely different from those of the polymers of α-amino acid.

Discussion

As pointed out above, the *F-A* curve of poly DL-α-aminocaproic acid resembled those of natural proteins such as the egg albumin or pepsin, the reason of which may be that the carbon number of hydrocarbon side chain of the polymer (four) is very near to the average carbon number of side chain of natural proteins, that is, about three. The preliminary experiments on the surface viscosity of this polymer also bore a close resemblance to those of natural proteins.[9]

The plateau which is a region of very high compressibility was also found in the *F-A* curve of poly DL-α-aminocaproic acid at very low pressure. The results obtained by H. B. Bull from the experiments on the egg albumin[10] showed the existence of the plateau in very low pressure region. These facts also show the resemblance between the synthetic polypeptides having shorter side chains and natural proteins.

The curves for the other polypeptides having longer side chains had a plateau in higher pressure region, which is not found in the case of natural proteins. This fact may be attributed to the absence of longer side chains with more than six carbon atoms on the average.

At areas larger than that indicated by point a (Fig. 2), the polypeptide molecules are spread with chains flat on the surface. Between points a and b on the *F-A* curves, the surface pressure would be exerted mainly by the action of side chains. Surface viscosity measurement showed that the viscosity in this region was as large as that of the state of gel. From point b to c, the surface pressure was kept practically constant. It suggests that the molecules of polypeptide would be reorientated in this region by compression and the side chains would be compressed away from the surface, so that they might be forced upwards or downwards into the water. Thus, at point c, a film which is arranged in close packed polypeptide backbone of β-configuration would be formed, since the area at this point for every polymer was found to be 14.7 Å.^{2}/amino acid residue, and this value corresponds to the area calculated with the x-ray data for an arrangement of β-configuration. When the surface viscosity against area curve was registered for the monolayer of poly DL-α-aminocaproic acid, the sudden increase in slope of the curve occurred at 14.7 Å.^{2}/residue. The same value was found by Cumper and Alexander[11] from their investigation on the surface viscosity for polyalanine (at air-liquid and liquid-liquid interfaces) and polyphenylalanine (at air-liquid interface). From point c, the pressure was again increased by further compression. It might be considered that segments of the chain might be left the surface.

Polyorganosiloxanes,[12] Buna N,[13] and long chain urea derivatives[14] give the similar *F-A* curves as those of polypeptides with longer side chains, which have the plateau in the curve. The effect of temperature on these monolayers is also similar to that on the polypeptides. The higher the temperature of substrate is, the situation of the plateau passes to the lower pressure region. Fox, Taylor and Zisman[12] characterized the appearance of this plateau as the process of coiling of high linear

(9) M. Joly, *Research*, Supplement, "Surface Chemistry," Butterworth, 1949, p. 157.

(10) H. B. Bull, *J. Biol. Chem.*, **185**, 27 (1950).

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

(12) H. A. Fox, P. W. Taylor and W. A. Zisman, *Ind. Eng. Chem.*, **39**, 1401 (1947).

(13) K. Suzuki, *Koshitugaku-Ronso (Discussions Colloid Sci., Japan)*, **1**, 63 (1947).

(14) J. Glazer and A. E. Alexander, *Trans. Faraday Soc.*, **47**, 401 (1951).

polymer molecule by compression on the surface of water. Saraswat and Kalyanasundarm[15] presented the same idea. As far as the synthetic polypeptides are concerned, however, the process of coiling of the linear molecule would not be considered as the reason of the appearance of the plateau, because, contrary to polyorganosiloxanes, the longer the side chains of the polypeptides are, the more marked the existence of the plateau became and it would be impossible for polypeptide molecules of which side chains have six, eight, or ten carbon atoms to be coiled by compression. Merely, from the resemblance of the shape of *F-A* curves, it can hardly be concluded that the properties of the monolayers of polyorganosiloxanes and of polypeptides would be the same. From both points of view of chemical structure in which —CO—NH— group is contained and of the behaviors of monolayers, the most closely related films to that of polypeptide may be the films of hexadecyl and octadecyl urea which wer einvestigated by Glazer and Alexander[14]. The shape of *F-A* curves of these urea derivatives resembles closely those of polypeptides, and also it is the same for both substances that the physical state from point a to b is gel or solid. However, that the physical state of higher pressure region above point c is liquid in the case of urea derivatives, is different from those of polypeptides. They considered in the case of urea derivatives that the molecules are combined by hydrogen bonding with each other in the region from point a to b, and that in higher pressure region above point c, no interchain hydrogen bonding exists. Therefore, the appearance of the plateau was considered to be due to the breaking of hydrogen bonds. In the monolayer of synthetic polypeptides, however, there occurred reorientation of the molecules in the region from point b to c, being accompanied by the formation of inter-chain hydrogen bonds. The molecules are bound strongly by inter-chain hydrogen bond if the monolayer was sufficiently compressed, so that polypeptide could be drawn out as a string from the surface of water by a glass rod. This is in good agreement with the experimental results that the area at point c at which interchain hydrogen bonding may be completed are always 14.7 Å.²/amino acid residue, regardless of the lengths of the side chains.

As pointed out above, the monolayer of poly γ-methyl-L-glutamate gave much smaller limiting area than the other polypeptides. It gave the limiting area of 9.8 Å.²/residue on distilled water but this limiting area was increased to 14.7 Å.² on acidic substrate. The difference of the limiting area dependent on the nature of the substrate may be due to the change of the configuration of the molecule. Some considerations were presented by us previously.[6]

It is known from the results of infra-red analyis by Ambrose *et al.* that poly γ-methyl-L-glutamate has a structure of α-configuration and is not transformed to β-configuration even if it is forcibly stretched.[16] Protein molecules having a folded structure would be generally unfolded on the surface of water probably transforming to β-type, since interface such as the surface of water exerts a strong force on the molecules. If the model of α-configuration for proteins, presented by Shimanouchi and Mizushima[17] and by Ambrose *et al.*[16] independently, is accepted, the area per amino acid residue when molecules are arranged in α-configuration must be two thirds of the area occupied by the molecule arranged in β-configuration. On the other hand, if the model of α-configuration presented by Astburty,[18] is accepted, the area occupied by the molecule of α-type must be half the area in the case of β-configuration. The experimental results obtained with this polymer are in good agreement with the former case (14.7 Å.²×2/3=9.8 Å.²). It is known that poly γ-methyl-L-glutamate having α-configuration is transformed to β-configuration by the treatment with formic or acetic acid.[19] When poly γ-methyl-L-glutamate was spread on the substrate containing hydrochloric, formic, or acetic acid, the surface pressure against the area curve shown by curve 2 in Fig. 6 was obtained, and the limiting area became to 14.7 Å.²/residue, *i. e.*, the limiting area of the polypeptides having β-configuration. These results correspond to the α→β transformation due to the destruction of the intra-chain hydrogen bonding by the interaction of the polypeptide with the acids in substrate. The results obtained with the surface viscosity measurement, the details of which will be reported elsewhere, led to the same conclusion. On distilled water the surface viscosity of the monolayer formed by polypeptide of α-configuration is considerably greater than that of proteins even at lower pressure region and suddenly increased at the area of about 9.8

(15) H. C. Saraswat and A. Kalyanasundarm, *J. Polymer Sci.*, **6**, 82 (1951).

(16) E. J. Ambrose and W. E. Hanby, *Nature*, **163**, 483 (1949).

(17) T. Shimanouchi, and S. Mizushima, *Science (Japan)*, **17**, 24, 52 (1947); This Bulletin, **21**, 1 (1948).

(18) W. T. Astbury and F. O. Bell, *Nature*, **147**, 696 (1941).

(19) C. H. Bamford, W. E. Hanby and F. Happey, *Nature*, **166**, 829 (1950).

Å.²/residue. On acidic substrate, however, the surface viscosity increased steeply at about 14.7 Å.²/residue and at the area larger than this, the polymer exhibited the viscosity of the same order of magnitude as that of any other polypeptides. Moreover, acidic substrates exerted little effect on the monolayer of poly DL-α-aminocapric acid, which spread as a film of β-configuration as shown in Fig. 5. These facts must be the surface-chemical indications of the $\alpha \rightarrow \beta$ transformation of poly γ-methyl-L-glutamate.

The reason for the change of *F-A* curve of the monolayers of polypeptides having longer side chains as a function of time after spreading is not yet clear. This phenomena, however, may probably be caused by the decrease of the entanglement of the side chains of polypeptide molecules. The effect of aging on the monolayer of polyorganosiloxanes observed by Fox, Solomon and Zisman[20] are apparently analogous to those of the polypeptides. They considered that this effect may be due to the hydrolysis of polyorganosiloxanes by the interaction with substrate. The reason for the aging considered by them, however, may not be valid for the present case.

As previously mentioned, the behaviors of monolayers of the synthetic polypeptides are much influenced by inter and intra-chain hydrogen bonding. From this reason, the monolayer of poly ε-aminocaproic acid, was also examined, in which five methylene groups are placed between NH- and CO-groups. The *F-A* curve of this polymer is of very expanded type and is entirely different from that of the polymers of α-amino acid. It is concluded from this point of view that the poly ε-aminocaproic acid is inadequate as a model of natural proteins. Detailed consideration about this polymer will be reported elsewhere.

Summary

The surface pressure against area (*F-A*) curves of the monolayers of poly-DL-α-aminocaproic, poly DL-α-aminocaprylic, poly DL-α-aminocapric, poly DL-α-aminolauric acids, poly γ-methyl-L-glutamate and poly ε-aminocaproic acid (amilan) was measured. The results obtained were as follows:

(1) The *F-A* curve of poly DL-α-aminocaproic acid having four carbon atoms in the side chain resembled in shape very closely to those given by natural proteins such as the egg albumin or pepsin. This fact is due to the resemblance of the carbon number in the side chains of both of the polypeptide and proteins.

(2) The plateau which is a region of very high compressibility appeared in the *F-A* curves of the polypeptides having more than six carbon atoms in the side chains. The curves of these polypeptides were entirely different from those of natural proteins.

(3) The area per amino acid which was found for every polypeptide to be in the closest packing of polypeptide backbone was all 14.7 Å.² This value is in good agreement with the area calculated from x-ray data for an arrangement of β-keratin configuration.

(4) Poly γ-methyl-L-glutamate occupied the limiting area of 9.8 Å.²/amino acid residue on distilled water. This value can be sufficiently accounted for, if this polymer has a structure of α-configuration on water surface and the model of α-keratin configuration presented by Shimanouchi and Mizushima and by Ambrose *et al.* is accepted. On substrates containing hydrochloric, formic, or acetic acid, however, this polypeptide occupied the limiting area of 14.7 Å.²/residue, this value corresponding to the area of the other polypeptides which are arranged in close packed β-configuration. The $\alpha \rightarrow \beta$ transformation of this polypeptide could be demonstrated by monolayer technique.

(5) The monolayer of the polymer of ε-aminocaproic acid is inadequate as a model of protein films.

The authors wish to express their sincere thanks to Dr. H. Tani in Faculty of Science, Osaka University and to Dr. J. Noguchi at Kanazawa University who gave them the valuable samples of synthetic polypeptides. A part of the expense for the experiment has been defrayed from a grant given by the Ministry of Education, to which the authors' thanks are due.

The Institute of Scientific and Industrial Research, Osaka University, Sakai, Osaka

(20) H. W. Fox, E. M. Solomon and W. A. Zisman, *J. Phys. Coll. Chem.*, **54**, 723 (1950).