Effects of Tyrosyl Residue on the Polypeptide Monolayers

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(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, $\delta = -dA/Ad\Pi$, 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).

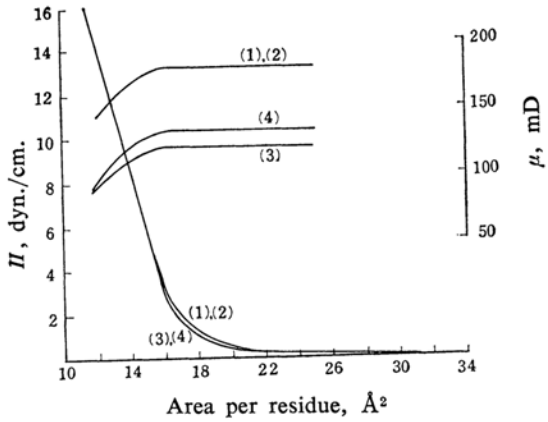

Fig. 1. Surface pressure-area and surface moment-area curves of poly-DL-phenylalanine (24°C): (1), on 0.01 N HCl; (2), on distilled water; (3), on 0.02 N K_2CO_3; (4), on 0.1 N K_2CO_3.

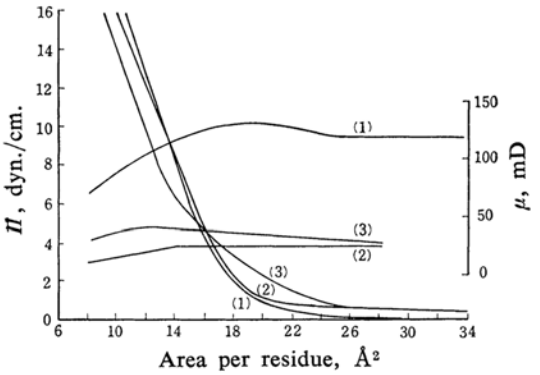

Fig. 2. Surface pressure-area and surface moment-area curves of poly-L-tyrosine (16°C): (1), on distilled water (Ref. 2); (2) on 0.02 N K_2CO_3; (3), on 0.1 N K_2CO_3.

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-

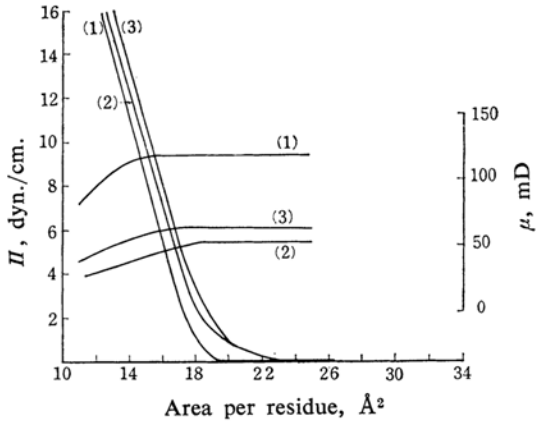

Fig. 3. Surface pressure-area and surface moment-area curves of copoly-1 : 1-(L-tyrosine, L-phenylalanine (19°C): (1), on distilled water; (2), on 0.02 N K_2CO_3; (3), on 0.1 N K_2CO_3.

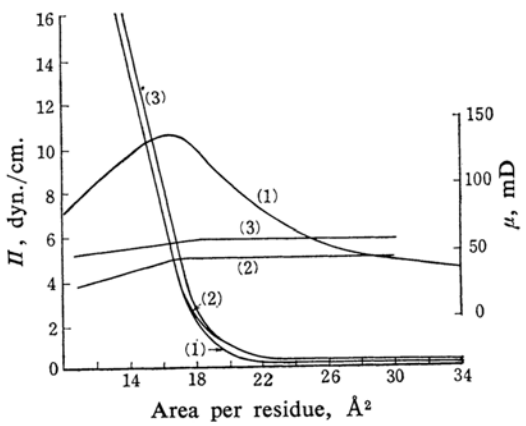

Fig. 4. Surface pressure-area and surface moment-area curves of copoly-1 : 1 : 2-(L-tyrosine, *o*-benzyl-L-tyrosine, L-phenylalanine) (10°C): (1), on distilled water; (2), on 0.02 N K_2CO_3; (3), on 0.1 N K_2CO_3.

(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

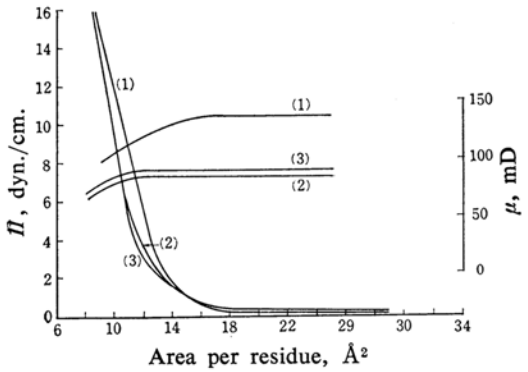

Fig. 5. Surface pressure-area and surface moment-area curves of copoly-1 : 1-(L-tyrosine, glycine) (10°C): (1), on distilled water; (2), on 0.01 N K_2CO_3; (3), on 0.1 N K_2CO_3.

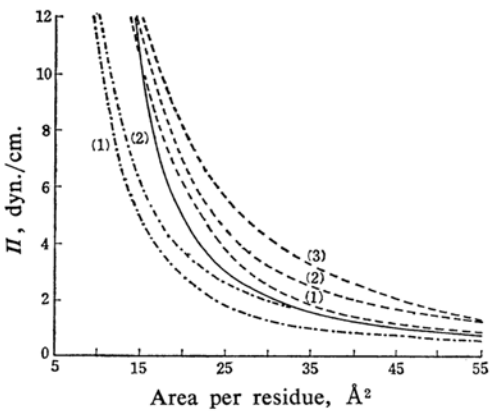

Fig. 6. Interfacial pressure-area curves of various polypeptides (16.5°C).
—— Poly-DL-phenylalanine, on distilled water.
---- Copoly-1 : 1-(L-tyrosine, L-phenylalanine): (1), on 0.01 N HCl; (2), on 0.02 N K_2CO_3; (3), on 0.1 N K_2CO_3.
-·-· Copoly-1 : 1-(L-tyrosine, glycine): (1), on 0.01 N HCl and 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_Π Å²/residue	A_μ Å²/residue	μ_c mD	$\Delta\mu$ mD
Poly-DL-	0.01 N HCl	24	15.6	16.0	182	
phenylalanine	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,	dw	19	17.0	15.2	120	
L-phenylalanine)	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,	dw	10	17.0	17.0	137*	
o-benzyl-L-	0.02 N K_2CO_3	10	17.0	17.2	46	91
tyrosine, L-	0.1 N K_2CO_3	10	17.2	18.2	62	75
phenylalanine)						
Copoly-1 : 1-	dw	10	11.5	16.0	138	
(L-tyrosine,	0.02 N K_2CO_3	10	10.9	12.0	85	53
glycine)	0.1 N K_2CO_3	10	10.9	11.5	90	48

A_Π: Limiting area (area at minimum compressibility)
A_μ: 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 *II-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 *II-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 *II-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 *II-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.

The authors wish to express their hearty thanks to Professor Junzo Noguchi of Hokkaido University, to Professor Hisaya Tani and Dr. Heimei Yuki of the Faculty of Science, Osaka University, and to Professor Ephraim Katchalski and Dr. Michael Sela of the Weizmann Institute of Science for supplying valuable samples. This work was partly supported by a grant given by the Ministry of Education, to which the authors' thanks are due.

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