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

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We have previously studied[1-6] the monolayers of synthetic polypeptides with nonelectrolytic 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

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[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**, 380 (1953).

[8] H. Hotta, ibid., **26**, 386 (1953).

[9] H. Hotta, ibid., **27**, 412 (1954).

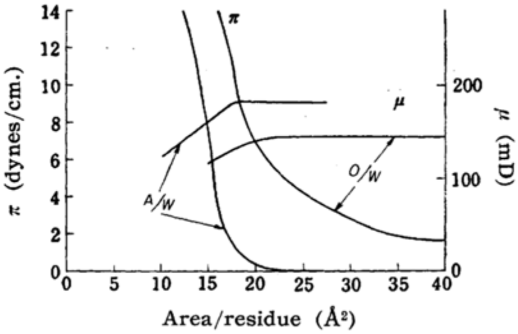

Fig. 1. Surface (or interfacial) pressure (π)-area and surface (or interfacial) moment (μ)-area curves of poly-DL-phenylalanine on distilled water. (A/W interface, 10°C and O/W interface, 13°C).

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 Å^2. This area is somewhat larger than that of the typical polypeptide with non-electrolytic paraffine side chains, namely 14.7 Å^2. 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 Å^2/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 Å^2).

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

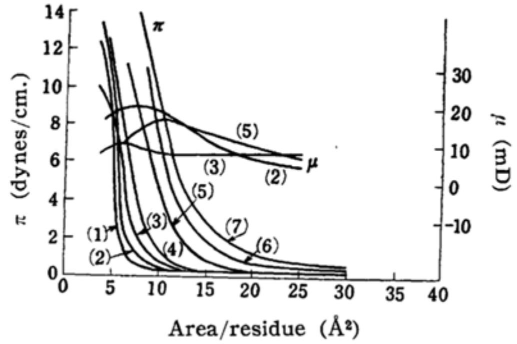

Fig. 2. Surface pressure (π)-area and surface moment (μ)-area curves of poly-L-tyrosine (spread from pyridine-isopropyl alcohol solution). (1) pH 2.0, (2) pH 5.6, (3) pH 7.2, (4) pH 9.4, (5) pH 11.0, (6) pH 11.3 and (7) pH 12.5. (26°C).

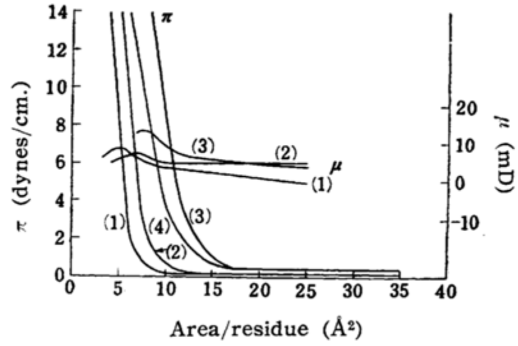

Fig. 3. Surface pressure (π)-area and surface moment (μ)-area curves of poly-DL-tyrosine. (1) pH 5.6, (2) pH 9.2, (3) pH 11.0 and (4) pH 12.5. (24°C).

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* 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).

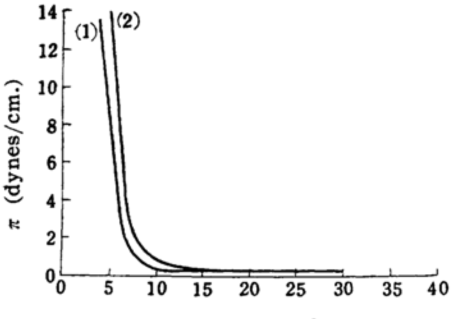

Fig. 4. Effect of the salt on poly-L-tyrosine (spread from pyridine-isopropyl alcohol solution) at pH 5.6. (1) no salt and (2) 1 mol. KCl. (26°C).

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

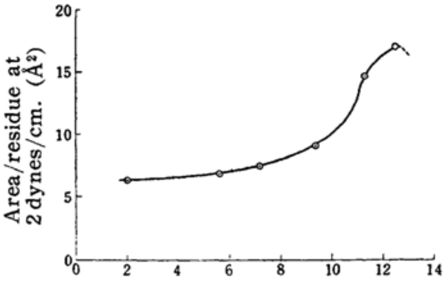

Fig. 5. Effect of pH of the substrate water on the area at 2 dynes/cm. of poly-L-tyrosine (spread from pyridine-isopropyl alcohol solution) at air/water interface.

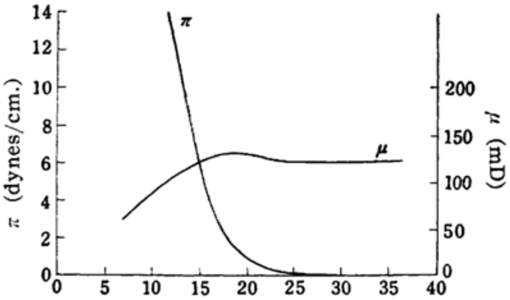

Fig. 6. Surface pressure (π)-area and surface moment (μ)-area curves of poly-L-tyrosine (spread from dichloroacetic acid and benzene solution) on distilled water. (16°C).

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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).

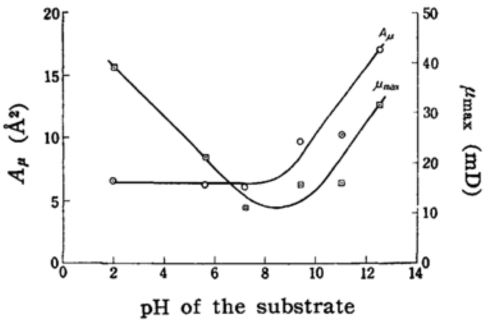

Fig. 7. Monolayers of poly-L-tyrosine (spread from pyridine-isopropyl alcohol solution). Area at maximum surface moment (A_μ)-pH and maximum surface moment ($\mu_{\max}$)-pH curves.

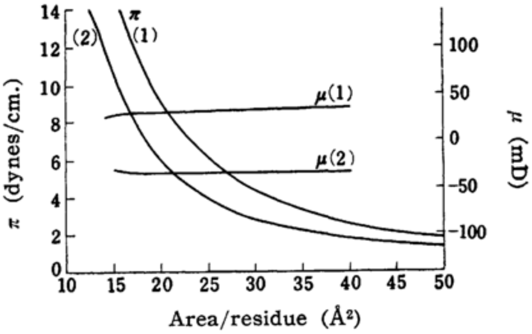

Fig. 9. Interfacial pressure (π)-area and interfacial moment (μ)-area curves of poly-DL-tyrosine. (1) pH 2.0 and (2) pH 11.0. (22°C).

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, $\mu_{\max}$ and the area where the maximum moment was found, A_μ, are plotted against pH. $\mu_{\max}$ 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 $\mu_{\max}$ 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. 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.

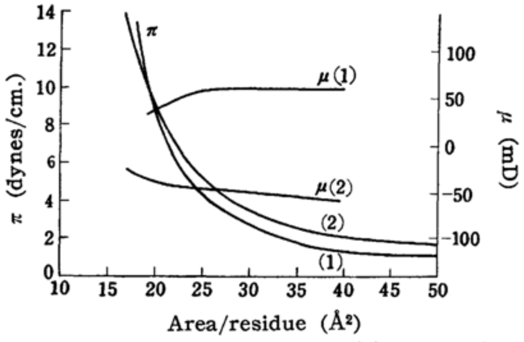

Fig. 8. Interfacial pressure (π)-area and interfacial moment (μ)-area curves of poly-L-tyrosine. (1) pH 2.0, 13°C and (2) 11.0, 15°C.

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

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

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 ($\mu_{\max}$) is found.

Poly-*o*-benzyl-DL-tyrosine. — Force-area and surface (interfacial) moment-area 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 Å^2 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

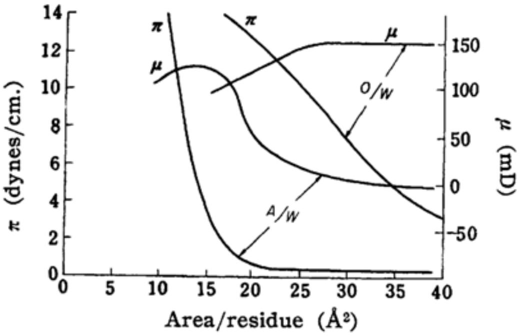

Fig. 10. Surface (or interfacial) pressure (π)-area and surface (or interfacial) moment (μ)-area curves of poly-*o*-benzyl-DL-tyrosine on distilled water. (24°C).

TABLE II

AREAS AND SURFACE AND INTERFACIAL MOMENTS FOR EACH POLYPEPTIDE

	Interface	Aqueous phase	pH	Temp. (°C)	A_π (Å^2/residue)	$A_{\pi=5}$ (Å^2/residue)	μ (mD)	A_μ (Å^2/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 Å^2)	
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 Å^2)	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.

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