Monolayer Studies of the Interactions between Histidyl Polypeptides and Metal Ions. II. The Effects of Copper, Zinc, Cobalt and Calcium Ions on the Monolayer Properties of Poly-1-benzyl-L-histidine

By Takuya YAMASHITA* and Toshizo ISEMURA

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The reactivity of the imidazole group of the histidyl residue in protein has a great importance in the understanding of interactions between proteins and several metal ions.[1] In the preceding paper,[2] the interactions of the cupric ion with poly-1-benzyl-L-histidine and with copoly-2 : 2 : 2 : 1-(glycine, *O*-benzyl-DL-serine, β-benzyl-L-aspartate, 1-benzyl-L-histidine) have been studied by using monolayer techniques at the air/water interface. It was found that the cupric ion has a strong interaction with the poly-1-benzyl-L-histidine monolayer at pH 5.6, while it interacts with the copolypeptide only with difficulty. For the present study, the effects of copper, zinc, cobalt and calcium ions on the film properties of polybenzyl-1-L-histidine were investigated at the air/water interface when the pH of the subsolution was varied and at the oil/water interface at pH 5.6.

Experimental

Poly-1-benzyl-L-histidine was spread from a solution in 0.05 N hydrochloric acid containing 20% of isopropyl alcohol, as in the previous study.[2] The desired pH value of the subsolution was obtained with a 0.1 M acetate buffer (acetic acid + sodium acetate) in the range of 4.8–6.2, and with a 0.1 M citrate buffer (sodium citrate + sodium hydroxide) at 6.7. The pH was measured with a Horiba M-3 glass electrode pH-meter.

The surface pressure, the surface viscosity and the surface potential at the air/water interface were measured by the methods described in the preceding paper.[2] The surface moment was calculated from the surface potential by utilizing the Helmholtz formula. The compression of the film was started 20 min. after the polypeptide had been spread.

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

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

Results

Figures 1, 2, 3 and 4 illustrate, at various pH values, the surface viscosity-area (η_s-A) curves of poly-1-benzyl-L-histidine on subsolutions containing cupric sulfate (0.01 M), zinc sulfate (0.01 M, 0.05 M), cobaltous sulfate (0.01 M, 0.05 M) and calcium chloride (0.05 M) respectively. Figure 5 shows the areas per residue at 0.05 surface poise in the presence and absence of these divalent metal ions against the pH. As can be seen in these figures, the cupric ion has a remarkable effect on the η_s-A relation of this polypeptide over a wide pH range, while the effects of the zinc, cobaltous and

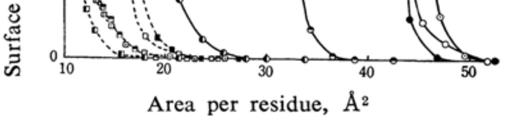

Fig. 1. Effect of the cupric ion on the surface viscosity-area relation of poly-1-benzyl-L-histidine at 24°C. Circles refer to 0.01 M $CuSO_4$ subsolutions; squares refer to control buffer subsolutions: ◓, ⬒, pH 4.8; ⊙, ⊡, 5.1; ○, □, 5.6; ●, ■, 6.2; ◐, ◧, 6.7.

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* Present address: Department of Chemistry, Faculty of Science, Osaka University, Kita-ku, Osaka.

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

[2] T. Yamashita and T. Isemura, This Bulletin, 37, 742 (1964).

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

[4] E. G. Cockbain, K. J. Day and A. I. McMullen, "Proc. 2nd Int. Cong. Surface Activity," Vol. 1. Butterworths Scientific Publications, London (1957), p. 56.

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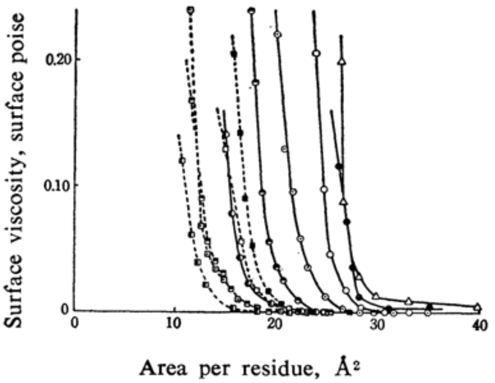

Fig. 2. Effect of the zinc ion on the surface viscosity-area relation of poly-1-benzyl-L-histidine at 24°C. Circles and triangles refer to 0.01 M and 0.05 M $ZnSO_4$ subsolutions, respectively; squares refer to control buffer subsolutions: ◓, ⬒, pH 4.8; ⊙, ⊡, 5.1; ○, △, □, 5.6; ●, ■, 6.2; ◐, ◧, 6.7.

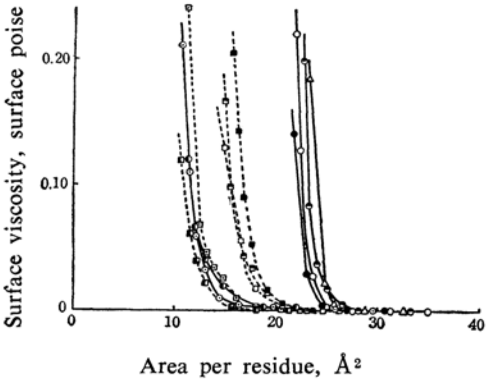

Fig. 3. Effect of the cobaltous ion on the surface viscosity-area relation of poly-1-benzyl-L-histidine at 24°C. Circles and triangles refer to 0.01 M and 0.05 M $CoSO_4$ subsolutions, respectively; squares refer to control buffer subsolutions: ⊙, ⊡, pH 5.1; ○, △, □, 5.6; ◓, ⬒, 5.9; ●, ■, 6.2; ◐, ◧, 6.7.

calcium ions are much less than that of the cupric ion.

The surface pressure-area (Π-A) curves of poly-1-benzyl-L-histidine on subsolutions at various concentrations of zinc sulfate are shown in Fig. 6 at pH 5.6. The presence of 0.001 M zinc sulfate does not practically affect the shape of the Π-A curve, while the values of 0.01 M and 0.05 M are high enough to cause the disappearance of the plateau of the Π-A curve found on the control subsolution.

Figure 7 shows the surface potential-area (ΔV-A) and the surface moment-area (μ-A) curves of poly-1-benzyl-L-histidine at pH 5.6 in the presence and absence of the zinc ion. In the presence of 0.01 M zinc sulfate, the surface potential and the surface moment are higher by ca. 50 mV. throughout the measured areas, and higher by 52 mD in the linear portion of the μ-A curve than on the control subsolution.

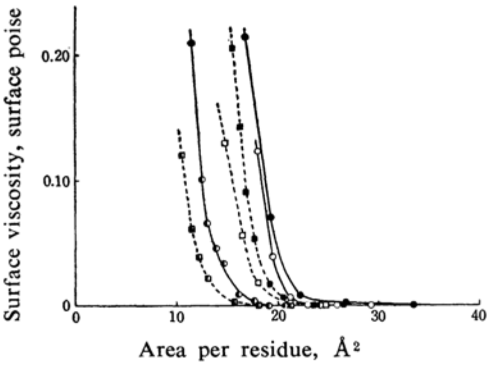

Fig. 4. Effect of the calcium ion on the surface viscosity-area relation of poly-1-benzyl-L-histidine at 24°C. Circles refer to 0.05 M $CaCl_2$ subsolutions; squares refer to control buffer subsolutions: ○, □, pH 5.6; ●, ■, 6.2; ◐, ◧, 6.7.

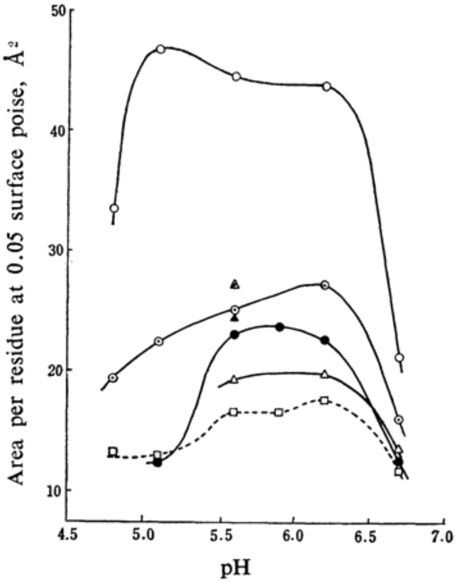

Fig. 5. Areas per residue at 0.05 suface poise against pH at 24°C: ○, 0.01 M $CuSO_4$; ⊙, 0.01 M $ZnSO_4$; ◬, 0.05 M $ZnSO_4$; ●, 0.01 M $CoSO_4$; ▲, 0.05 M $CoSO_4$; △, 0.05 M $CaCl_2$; □, control (without divalent metal ions).

Figure 8 shows the effects of cobaltous sulfate and calcium chloride on the Π-A relation of poly-1-benzyl-L-histidine at pH 5.6. In the presence of the cobaltous ion, film expansion was observed. However, even 0.05 M cobaltous sulfate did not cause the disappearance of the plateau of the Π-A curve found on the control

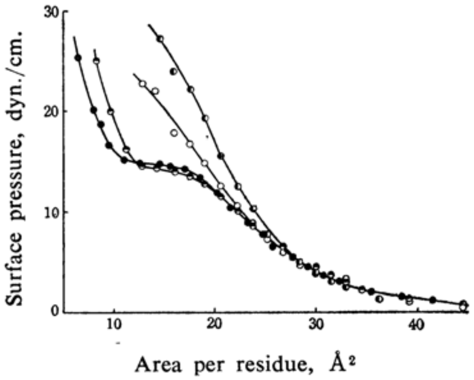

Fig. 6. Effect of the zinc ion on the surface pressure-area relation of poly-1-benzyl-L-histidine at pH 5.6 (0.1 M acetate buffer) : ●, control (20°C) ; ◓, 0.001 M ; ○, 0.01 M ; ◐, 0.05 M (ZnSO$_4$ the last three, at 24°C).

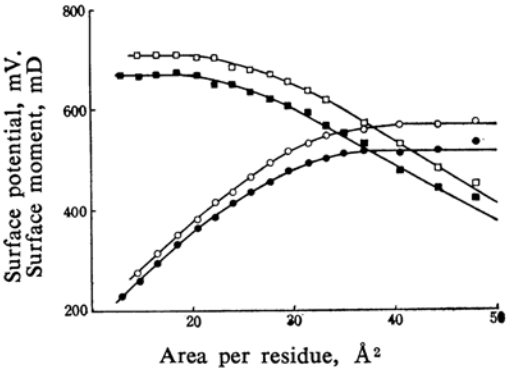

Fig. 7. Surface potential-area and surface moment-area curves of poly-1-benzyl-L-histidine on 0.1 M acetate buffer (pH 5.6 ; ■, ●) and on 0.1 M acetate buffer containing 0.01 M zinc surfate (pH 5.6 ; □, ○) : 12°C.

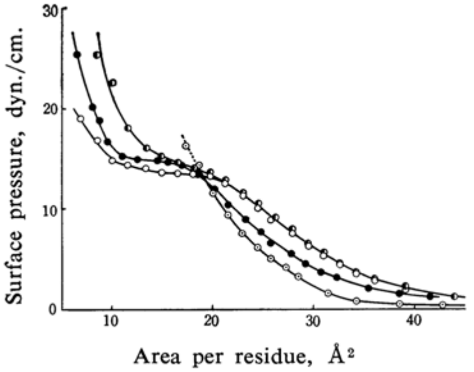

Fig. 8. Effect of the cobaltous and calcium ions on the surface pressure-area relation of poly-1-benzyl-L-histidine at pH 5.6 (0.1 M acetate buffer) : ●, control (20°C) ; ○, 0.01 M CoSO$_4$; ◐, 0.05 M CoSO$_4$; ⊙, 0.05 M CaCl$_2$ (the last three, at 24°C).

subsolution. On the other hand, 0.05 M calcium chloride resulted in film condensation. The film was unstable over areas smaller than 19 Å^2 per residue.

The interfacial pressure-area (Π_i-A) curves of poly-1-benzyl-L-histidine at the interfaces between oil and water when various salts were dissolved are shown in Fig. 9. The Π_i-A curves were nearly identical with one another on the 0.1 M potassium chloride subsolution and on the acetate buffer subsolutions at pH 5.6 with and without 0.01 M zinc or cobaltous sulfate. The cupric ion had a remarkable effect as well as at the air/water interface.[2)]

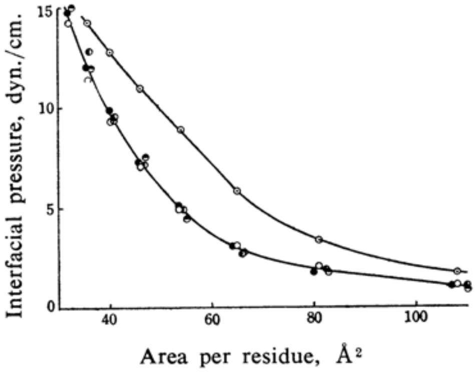

Fig. 9. Monolayers of poly-1-benzyl-L-histidine at the oil/water interface at 21°C : ○, 0.1 M KCl ; ●, 0.1 M Acetate buffer (pH 5.6) ; ⊙, 0.01 M CuSO$_4$; ◐, 0.01 M ZnSO$_4$; ◓, 0.01 M CoSO$_4$ (the last three salts were dissolved in 0.1 M acetate buffer, pH 5.6).

Discussion

Air/Water Interface. — *The Effect of the Cupric Ion.*—It has been found that the various metal ions form complexes with imidazole,[6),7)] 4-methylimidazole,[8)] and poly-L-histidine[9)] as model substances of the histidyl residue in protein. In the preceding work,[2)] a strong interaction of the cupric ion with the poly-1-benzyl-L-histidine monolayer has been found at pH 5.6 at the air/water interface. In the presence of the cupric ion in the subsolution, the surface pressure increased remarkably throughout the measured areas, and the η_s-A curve shifted to a much larger area than on the control subsolution, the surface pressure being quite low at the area where the surface viscosity began to rise. This finding was interpreted in terms of the cross-linkage formation

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

[7)] R. B. Martin and J. T. Edsall, ibid., **80**, 5033 (1958).

[8)] Y. Nozaki, F. R. N. Gurd, R. F. Chen and J. T. Edsall, ibid., **79**, 2123 (1957).

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

between molecules at the surface through the complex formation of copper with the side chains of poly-1-benzyl-L-histidine.

In the present study, the effect of the cupric ion on the η_s-A relation of poly-1-benzyl-L-histidine was investigated in the 4.8—6.7 pH range. As Fig. 1 shows, in the presence of the cupric ion, the η_s-A curves shift to much larger areas than on the control subsolutions at the pH's studied. The effect of the cupric ion is remarkaly large compared with those of zinc, cobaltous and calcium ions, as Fig. 5 shows. The cross-linked structure should be formed between poly-peptide molecules at the surface in these pH range as a result of complex formation between the cupric ions and the side chains. The optimum pH for the copper-monolayer interaction is in the 5.1—6.2 range.

The Effect of the Zinc Ion.—As Figs. 2 and 5 show, the zinc ion interacts considerably with the monolayer of poly-1-benzyl-L-histidine in the pH range of 5.1—6.2, where the areas per residue at the 0.05 surface poise are higher by 8—10 Å^2 than on the control subsolutions. This value is much less than in the case of the cupric ion. The cupric ion causes the increment of 26—34 Å^2 in the optimum pH range. At pH 5.6, which seems to be an optimum pH for the zinc-monolayer interaction, the plateau of the Π-A curve found on the control subsolution disappears in the presence of 0.01 M and 0.05 M zinc sulfate. This might be caused by the cross-linkage formation in the monolayer. Such a cross-linked structure has also been found with tanned protein monolayers.[10-12]

The rise in the surface potential or surface moment in the presence of the zinc ion might be caused by the increment in the net positive charge of the film as a result of the combination of zinc ions with the interacting sites of the side chains of polypeptide, as in the case of copper ions,[2] and so may be another evidence of the complex formation. A similar increase in the surface potential has been reported for a gelatin monolayer tanned with basic chromium sulfate.[11]

The Effect of the Cobaltous Ion.—The effect of the cobaltous ion on the η_s-A relation of poly-1-benzyl-L-histidine was investigated in the 5.1—6.7 pH range. The optimum pH for the cobalt-monolayer interaction is found to be in the 5.6—6.2 range, and the cobalt ion does not interact practically at the 5.1 and 6.7 pH's, as Figs. 3 and 5 show. The interaction of the cobalt ion with the monolayer is somewhat weaker than that of the zinc ion in the pH range studied. Such a weaker interaction of the cobalt ion than those of the zinc and copper ions has also been found in bulk with bacterial-α-amylase,[13] the interacting sites of which are histidyl groups, and with imidazole.[3]

The effect of the cobaltous ion on the Π-A relation of poly-1-benzyl-L-histidine was investigated at a pH in the optimum pH range. At pH 5.6, in the presence of the cobaltous ion, the film expanded more than on the control subsolution. However, even 0.05 M cobaltous sulfate does not cause the disappearance of the plateau of the Π-A curve observed on the control subsolution unlike zinc ion. This fact may indicate that the cross-linked structure caused by the cobalt ion is somewhat looser than that caused by the zinc ion because of its weaker interaction, even though such a structure is formed.

The Effect of the Calcium Ion.—The effect of the calcium ion on the η_s-A relation of poly-1-benzyl-L-histidene is quite small. Even a 0.05 M calcium chloride subsolution causes merely an increase of 2—3 Å^2 in areas per residue at a 0.05 surface poise in the 5.6—6.7 pH range. At pH 5.6, the calcium ion reduces the surface pressure more than on the control subsolution, unlike copper,[2] zinc and cobalt ions. This finding resembles the facts found concerning the metal-stearic acid monolayer interactions;[14] it might be caused by the difference in modes of interactions between the alkaline earth metals and the copper, zinc and cobalt ions. The calcium ion might form the cross-linked stucture in the monolayer only with difficulty.

The Estimation of the Extent of Metal-Monolayer Interactions.—On the basis of the preceding[2] and the present works, we conclude that the cupric ion interacts especially strongly with the monolayer of poly-1-benzyl-L-histidine, and

TABLE I. THE FIRST ASSOCIATION CONSTANTS FOR IMIDAZOLE AND 4-METHYLIMIDAZOLE WITH VARIOUS METAL IONS AT 25°C

	Cu^{2+}	Zn^{2+}	Co^{2+}	Ca^{2+}
Imidazole	4.33[a]	2.57[a]	2.42[b]	0.08[d]
4-Methylimidazole	4.13[c]	2.44[c]		

Ionic strength; 0.15—0.16.
a) Ref. 6; b) Ref. 7; c) Ref. 8; d) Ref. 15.

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

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

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

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

14) T. Sasaki and R. Matuura, This Bulletin, **24**, 274 (1951).

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

TABLE II. AREAS OF POLY-DL-PHENYLALANINE[5] AND POLY-1-BENZYL-L-HISTIDINE UNDER VARIOUS INTERFACIAL PRESSURES

Polypeptide	Area per residue, Å^2			
	1 dyn./cm.	5 dyn./cm.	10 dyn./cm.	15 dyn./cm.
Poly-DL-phenylalanine*	47	20	15	13
Poly-1-benzyl-L-histidine**	100	54	40	32

* Oil/distilled water interface (16.5°C).

** Oil/0.1 M acetate buffer interface (pH 5.6, 21°C).

that the decreasing order of the metal-monolayer interactions is Cu^{2+}, Zn^{2+}, Co^{2+}, Ca^{2+}. As Table I shows, this order corresponds to the first association constants of imidazole or 4-methylimidazole, which are measures of the extent of interaction.

The Oil/Water Interface.—At the oil/water interface, the poly-1-benzyl-L-histidine monolayer expands much more than at the air/water interface as a result of the release of van der Waals force between side chains. The extent of the expansion is considerably larger than the film of poly-DL-phenylalanine which has benzyl groups directly attached to the backbone carbon atoms. The areas occupied by poly-1-benzyl-L-histidine film are compared with those by poly-DL-phenylalanine under various interfacial pressures in Table II. Such a large expansion of the former film suggests that the imidazole groups, which are hydrophilic to some extent because of the effect of nitrogen atoms, lie at the interface.

Unlike at the air/water interface, no appreciable effects of zinc and cobalt ions on the monolayer of poly-1-benzyl-L-histidine are found at the concentrations of 0.01 M at pH 5.6. This fact might be caused by the restriction of the accessibility of these metal ions to the interacting sites by the oil. On the other hand, the effect of the cupric ion is quite large, as at the air/water interface.[2] Accordingly, complexes of cupric ions with poly-1-benzyl-L-histidine should be formed even at the oil/water interface.

Interestingly, the plateau of the Π-A curve found on the 0.1 M acetate buffer subsolution at pH 5.6 disappears at the oil/water interface. The Π_i-A curve practically agrees with that at the oil/0.1 M potassium chloride interface. At the oil/water interface, the lateral cohesion between side chains is released by the intervening of oil between them. This may be the cause of the lack of a difference between the Π_i-A curves at the interfaces between oil and the 0.1 M potassium chloride solution and between oil and the 0.1 M acetate buffer.

Summary

The effects of metal ions on the monolayer properties of poly-1-benzyl-L-histidine, a model substance of the histidyl residue in protein, have been investigated at the air/water interface when the subphase pH is varied, and at the oil/water interface at pH 5.6.

At the air/water interface, it was found from the measurements of the surface viscosity that the cupric ion interacts especially strongly with the monolayer of poly-1-benzyl-L-histidine over a wide pH range and that the interaction of the calcium ion is quite weak. The decreasing order of metal-monolayer interactions is Cu^{2+}, Zn^{2+}, Co^{2+}, Ca^{2+}. The surface pressure-area isotherms suggest that the cobalt ion forms a somewhat looser cross-linked structure in the monolayer than does the zinc ion because of its weaker interaction.

At the oil/water interface, the film expands remarkably because of the release of van der Waals force between side chains. No appreciable effects on the interfacial pressure-area relation were observed with the zinc and cobaltous ions, because the accessibility of these ions to the interacting sites of side chains would have been restricted by the oil. The cupric ion had a considerable effect here as well as at the air/water interface.

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Institute for Protein Research
Osaka University
Kita-ku, Osaka