J. Fac. Fish. Anim. Husb.,
Hiroshima Univ. (1975), **14** : 15～22

Zinc- Complexes of Histidine and the Related Compounds in Aqueous Solution

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(Figs. 1–2; Table 1)

Zinc is the constituent element of insulin and is an essential element for the function of some enzymes, such as carbonic anhydrase, carboxypeptidase and alcohol dehydrogenase. Deficiency of zinc in the media of the tissue culture of mammalian kidneys causes inhibition of the biosyntheses of thiamine kinase and deoxyribonucleic acid.

On the other hand, most of the amino acids have an ability to form metal complexes. In food technology, the ability of amino acids as sequestering agents for transitional metal elements is applied to the prevention of the autoxidation of glycerides. Among numerous amino acids, histidine is of great interest because the imidazole group forms π-bonds with metal elements.

Most of the researches on the zinc-histidine complexes so far published are concerned with equilibria in the complex formations[1)～4)] or with crystallographic analyses of the complexes.[5)6)] Such results are not available for considering the biochemical roles of zinc and histidine. Knowledges wanted for understanding biochemical phenomena, such as an enzyme action or a protein denaturation are the effects of pH on the formation of zinc-histidine complexes in aqueous solution. Accordingly, the formation of zinc-complexes with histidine and the related compounds in aqueous solution are dealt with in this paper.

MATERIALS AND METHODS

1. Materials

All the reagents used in this experiment were of guaranteed grade, and distilled-deionized water was used in the preparation of the various samples. A stock solution of $ZnSO_4$ was prepared so as to contain 1.8 or 3.6×10^{-3}M Zn and the Zn concentra-

tion was determined with a Hitachi 207 atomic absorption spectrophotometer. Each stock solution of ligands contained 3.6×10^{-3}M ligand. The ligand concentration was determined by the potentiometric titration method. As ligand, the following reagents were used: L-histidine monohydrochloride (Wako Pure Chemical Co., Ltd.), L-histidine dihydrochloride (Nakarai Chemicals Co., Ltd.), L-histidylglycine (SIGMA Chemicals Co., Ltd.), glycyl-L-histidine (SIGMA Chemicals Co., Ltd.) and glycylglycine (Wako Pure Chemical Co., Ltd.).

2. Potentiometric titration

Potentiometric titrations were carried out with a Hiranuma RAT-101S autorecording potentiometric titration apparatus using a combination electrode MC 30. The samples were mixed solutions in which Zn and a ligand were dissolved in the molar ratio of Zn : ligand as 1 : 1 or 1 : 2. The ionic strength (μ) of these solutions was adjusted to 0.1 with $NaClO_4$ solution before titration. Titrations were made with 0.1 N NaOH in a stream of N_2 gas at 25 ± 0.5°C. As a control, the ligand was titrated in the absence of Zn under the same conditions.

RESULTS

Titration curves obtained for Zn-histidine (His), -histamine (Hista), -histidine methylester (HisMe), -histidylglycine (HisGly), -glycylhistidine (GlyHis) and -glycylglycine (GlyGly) systems are shown in Fig. 1. Decreases of pH were observed in all the Zn-ligand systems. The lowering of pH was caused by a release of protons from donor groups and is regarded as an index of the Zn-complex formation. The dissociation constant of each ligand and the pH range of the lowering are indicated in Table 1.

DISCUSSION

1. Zn-His complexes

In the titration curves of Zn-His systems, there was a remarkable inflection at a = 2; a representing the number of equivalents, mole number, of NaOH added per mole of a ligand (Fig. 1–1). This fact indicates that two protons per mole of His are liberated in the Zn-His complex formation. His molecule has three donor groups, namely, α-NH_2, α-COOH and imidazole groups, and two of them contribute to the formation of the Zn-complex. For the chemical structure of Zn-His complex satisfying the above conditions, one of the Formulae I, II and III in Fig. 2 is assumed. The chelate rings of I, II and III are 7–, 5– and 6–membered, respectively. In general, stable chelate compounds have 5– or 6–membered rings.[7] The possibility of Formula I can thus be excluded. The chelate ring of II formed by the participation of α-NH_2 and α-COOH groups resembles that of Formula IV for the Zn-Gly complex. From the stability

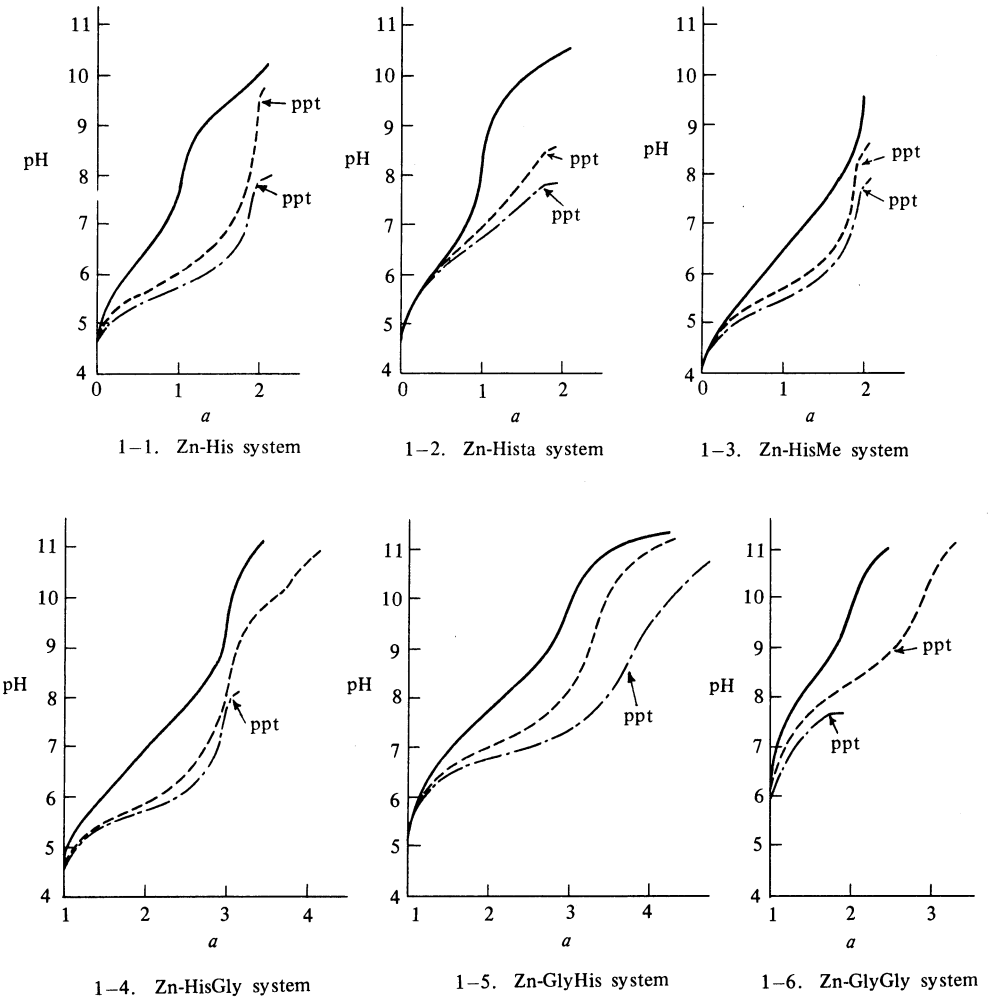

Fig. 1. Potentiometric titration curves of various Zn-ligand systems.
———— : Control solution contained ligand alone
—·—·— : Reaction solution contained Zn and a ligand in the molar ratio of 1 : 1
---------- : Reaction solution contained Zn and a ligand in the molar ratio of 1 : 2
a : Moles of NaOH added per mole of a ligand.

constants of Zn-His complex (12.00) and Zn-Gly complex (9.30), ALBERT[2] proposed Formula III for the Zn-His (1 : 2) complex and attributed the high stability of the Zn-His complex to the participation of the imidazole group. EDSALL *et al.*[8] reported that the binding ability of His to metal ions was due to the α-NH_2 and the imidazole groups. Formula III, accordingly, is postulated for the probable structure of the Zn-His (1 : 2) complex.

As above-mentioned, if the α-COOH group of His does not participate in the formation of the Zn-His complex, the titration curves of Zn-Hista systems would resemble those of Fig. 1–1, and the structure of the Zn-Hista complex would have a chelate ring

similar to that of the Zn-His complex as shown in Formula V. There were observed, however, two different points between their titration curves: the values of a and pH at the beginning of the protonations. In the case of the Zn-Hista systems, the protonation occurred at the middle point of $a = 0 \sim 1$ (Fig. 1–2). The pH value at the point is about 6 which is compatible with the pK_2 value of His, i.e., the acid-dissociation constant of the imidazole group. As to the Zn-His systems, the protonation occurred at the point of $a = 0$ and below pH 6 (Fig. 1–1). The protonation below pH 6 is considered to be due to the dissociation of the α-COOH group. Consequently, for the Zn-His complex in an aqueous solution below pH 6 other formulae than III may be postulated, where the participation of the α-COOH group should be taken into consideration. As an example for this Formula VII may be given.

Table 1. Protonation of histidine and the related compounds in the formation of zinc (II)-complexes.

Ligand	Acid-dissociation constant of ligand	Molar ratio of Zn : ligand	pH area of protonation	Mole number of OH^- consumed per mole of ligand
His	$pK_2 = 6.1 \pm 0.1$	1 : 1	4.6 ~ 8.0 (ppt)	2
	$pK_3 = 9.1 \pm 0.1$	1 : 2	4.6 ~ 9.8 (ppt)	2
Hista	$pK_1 = 6.2 \pm 0.1$	1 : 1	6.0 ~ 7.8 (ppt)	2
	$pK_2 = 9.7 \pm 0.1$	1 : 2	6.0 ~ 8.3 (ppt)	2
HisMe	$pK_1 = 5.3 \pm 0.1$	1 : 1	4.0 ~ 7.8 (ppt)	2
	$pK_2 = 7.3 \pm 0.1$	1 : 2	4.0 ~ 8.5 (ppt)	2
HisGly	$pK_2 = 5.9 \pm 0.1$	1 : 1	4.7 ~ 8.1 (ppt)	3
	$pK_3 = 7.7 \pm 0.1$	1 : 2	4.7 ~ (no ppt)	3
GlyHis	$pK_2 = 6.8 \pm 0.1$	1 : 1	5.0 ~ 8.5 (ppt)	3
	$pK_3 = 8.3 \pm 0.1$			
GlyGly	$pK_2 = 8.2 \pm 0.1$	1 : 1	5.0 ~ 7.6 (ppt)	3
		1 : 2	5.7 ~ 8.5 (ppt)	3
Gly	$pK_2 = 9.6 \pm 0.1$	1 : 1	5.6 ~ 7.7 (ppt)	1
		1 : 2	5.6 ~ 8.9 (ppt)	1

(t = 25°C, $\mu = 0.1$)

For further examinations on the contribution of the α-COOH group to the Zn-complex formation, potentiometric titrations on Zn-HisMe systems were carried out (Fig. 1–3). The reason for selecting HisMe is that the COOH group of HisMe is masked with CH_3. The titration curves of the Zn-HisMe systems resemble considerably those of the Zn-His systems. Therefore, the chemical structure of the Zn-HisMe complex is assumed to be illustrated by Formula VI in Fig. 2.

In the case of the Zn-His system, Formula VII was given as a possible structure for the complex in acidic solution. The structure is made up of 1 : 1 molar ratio of Zn to His: The Zn-His (1 : 2) complex seems improbable, since the coordination number of Zn is usually four.

2. Zn-His containing dipeptide complexes

As mentioned above, the behavior of His in free state provided some valuable information on the participation of the NH_2 or the COOH group in the formation of Zn-His complexes. It appeared desirable to examine the behavior of His in combined state, the suitable choice being His containing dipeptides of which the NH_2 or the COOH group of His took part in the peptide linkage formation. Accordingly, Zn-HisGly, -GlyHis and -GlyGly systems were examined and the results are shown in

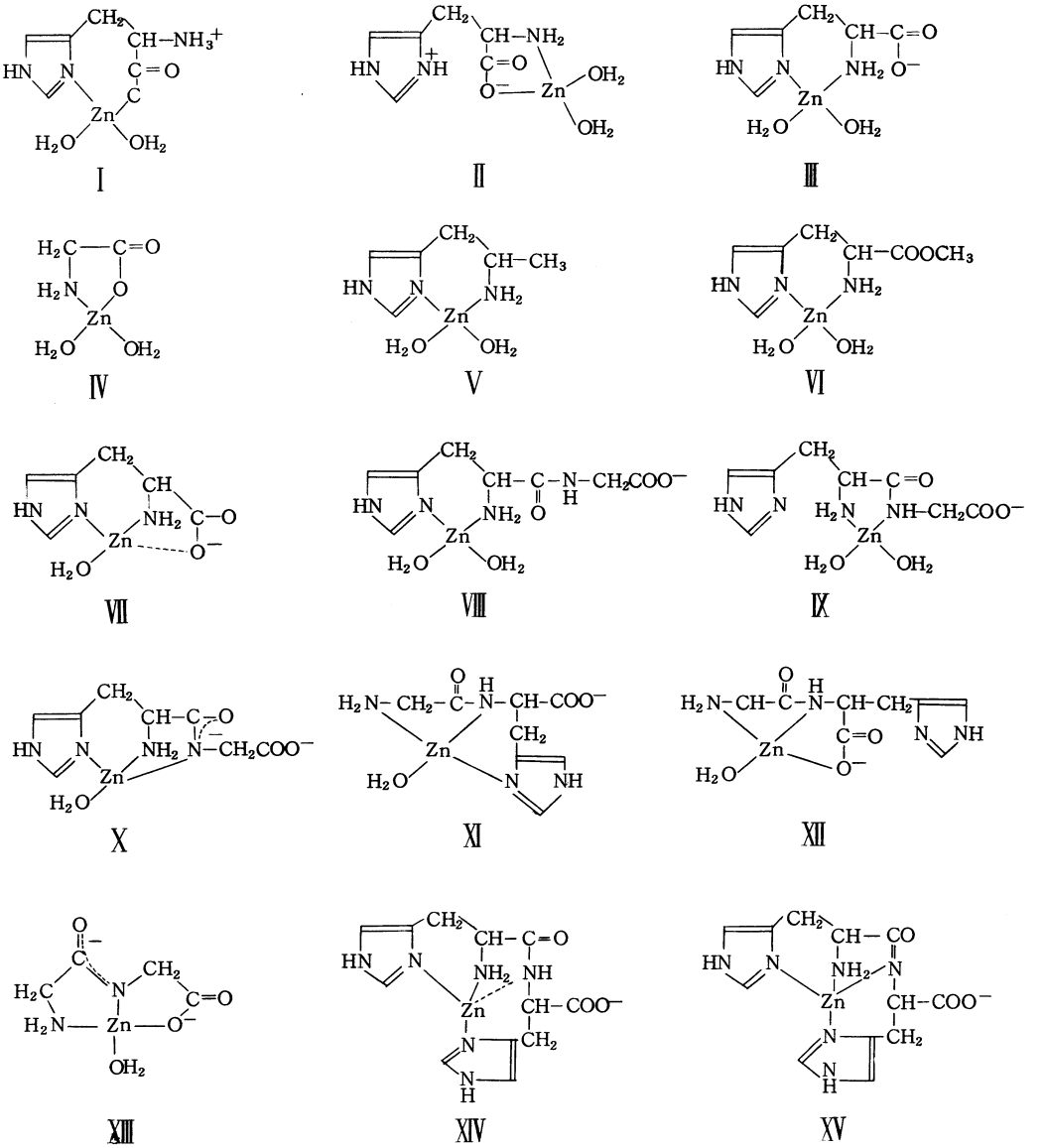

Fig. 2. Assumed chemical structures for Zn-complexes of His and the related compounds.

Fig. 1–4, 1–5, and 1–6, respectively. The reason for selecting Gly as the other amino acid residue is that Gly has no side chain.

In Fig. 1–4, the titration curves in the region of $a = 1 \sim 3$ resemble those of the Zn-His system. The results indicate that two donor groups of HisGly coordinate to the Zn ion. HisGly has four donor groups, i.e., the α-NH_2 and the imidazole groups of His residue, the α-COOH group of Gly residue and the NH group of the peptide linkage. Accordingly, it is necessary to make clear which two groups of the four would participate in the Zn-complex formation. The possibility of the participation of the NH group of the peptide linakge is probably excluded because of the difficulty of dissociating the proton at pH values near neutrality. If the COOH group participates, the resulting complex would have an 8-membered chelate ring in the structure. Such a structure seems improbable because of its instability. The residual two groups are the imidazole and the α-NH_2 groups of His residue. Therefore, the structure of the Zn-HisGly complex is assumed to be illustrated by Formula VIII in Fig. 2.

In the region of $a = 3 \sim 4$, the titration curves of the Zn-HisGly (1 : 1) system show the second lowering of pH, namely, the second protonation which indicates the formation of another complex. The protonation is considered to be due to the dissociation of the NH group of the peptide linkage, because the other three groups of HisGly should have already been dissociated below $a = 3$. For the structure of the Zn-complex, either IX or X is assumed. The chelate ring of Formula IX resembles that of Formula IV for the Zn-Gly complex. The stability of metal chelate complexes in general increases with number of the chelate ring of the structures. Formula X, accordingly, is given as a more possible structure than IX.

GlyHis is the isomer of HisGly in regard to the terminal NH_2 and COOH groups. The titration curves of the Zn-GlyHis systems are illustrated in Fig. 1–5. The results indicate that the liberation of two or three protons per mole of GlyHis occurred in the Zn-complex formation. When the number of the proton released is three, Formula XI or XII is probably given for the complex. Both formulae have two chelate rings.

Fig. 1–6 shows the titration curves of the Zn-GlyGly systems. It should be mentioned that the precipitation of $Zn(OH)_2$ occurred in the earlier stages of the titrations, especially in the case of the (1 : 2) system. Similar results were obtained also with Zn-Gly systems, although the titration curves were not revealed (see Table 1). A feature common to the Zn-Gly and the Zn-GlyGly systems is that only Gly coordinates to the Zn ion. In view of these facts, it may generally be said that the participation of the imidazole group of His is important in the stabilization of the Zn-complexes. For the structure of the Zn-GlyGly complex, Formula XIII may be postulated by reference to the structure proposed for the Cu-GlyGly complex by DORAN *et al.*[9]

In this investigation, none of the Zn-HisHis systems were examined. The structure of the Zn-complex, however, can be illustrated as Formula XIV or XV by reference to the DORAN's paper on the Cu-HisHis complex.[9]

SUMMARY

Formations of Zn-histidine, -histamine, -histidine methylester, -histidylglycine, -glycylhistidine and -glycylglycine complexes in aqueous solution were examined by means of a potentiometric titration method. On the basis of the results obtained the possible chemical structures were given to the individual complexes. The main object of this investigation is to know what donor groups of histidine participate in the Zn-complex formation at a given pH value.

In neutral and alkaline solutions above pH 6, the imidazole and the α-NH_2 groups of histidine coordinate to the Zn ion and form the Zn-histidine (1 : 2) complex. In acidic solutions, the α-COOH group also coordinates and forms the Zn-histidine (1 : 1) complex together with the above two groups. The complexes formed by the participation of the imidazole group of histidine are considered to be more stable than the Zn-glycine or -glycylglycine complex by reason of the difficulty in the precipitation of $Zn(OH)_2$ during the titrations. This was also the case for the Zn-histidine-containing dipeptide complexes.

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亜鉛とヒスチジンならびにその関連化合物との錯体生成について

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食品やその原料となる生物体には各種の金属元素とともに，多くの酸素－および窒素－配位子が含まれる。そこで金属元素の挙動を知るためには，これら金属－配位子の錯体形成に関する知識が必要となる。本報では，pHの異なる各種溶液中で生成するZn–Hisおよび Zn– 含 His ジペプチド錯体について，電位差滴定曲線に基づいて考察した。考察の中心は，Zn 錯体形成に関与する配位基と溶液の pH の関係であって，遊離

His ならびにペプチドの構成単位となったHis残基に分けて検討した。

まず，pH6以上の中性およびアルカリ性溶液中ではイミダゾール -NH と α-NH_2 が配位することを推測し，Fig. 2 の式Ⅰを示した。またHistaおよびHisMeと比較することによって，α-COOH 基の影響もあり得ると考え，式Ⅶを示した。

次に，全く側鎖をもたないGlyとHisから成るジペプチドすなわちHisGlyおよびGlyHisを比較し，さらにGlyGlyと比べることによって，イミダゾール核がZn錯体を安定化させ，$Zn(OH)_2$ の沈澱形成をかなり抑制することについて考察を加えた。

この種の報文は少なく，とくにHisを含むペプチドとZnの錯体生成に関する報文は始めてのものである。