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Complex Formation of Zinc(II) Ion with Glycylglycyl-L-histidine: An Investigation by Proton Nuclear Magnetic Resonance Spectroscopy

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A proton nuclear magnetic resonance (^{1}H-NMR) study of the interaction of zinc(II) ion with glycylglycyl-L-histidine (Gly–Gly–His) was carried out. The addition of zinc(II) ion affected the NMR spectrum of Gly–Gly–His above pH 6; the signal of the imidazole C-2 proton underwent line broadening and upfield shift, and that of the methylene proton of glycine at the amino terminal was also shifted upfield. It is suggested that Gly–Gly–His functions as a bidentate ligand, coordinating with the zinc(II) ion through the amino nitrogen and the neighboring peptide oxygen, and that the imidazole nitrogen of the complex is linked intermolecularly to the zinc(II) ion of another peptide complex.

Keywords——^{1}H-NMR; L-histidine; glycylglycyl-L-histidine; metal–ligand interaction

Serum albumin has been considered to function in the transport of trace metals between tissues and blood.[1] The peptide segment, L-aspartyl-L-alanyl-L-histidine (Asp–Ala–His), at the N-terminal of the polypeptide chain in albumin coordinates strongly to copper (II) ion, forming a square planar complex which involves amino nitrogen of the aspartic acid residue, imidazole nitrogen of the histidine, and two intervening deprotonated peptide nitrogens as the ligands.[2–4] Glycylglycyl-L-histidine (Gly–Gly–His), containing similar peptide backbone and metal-ligating sites, has been extensively studied as regards its complexing affinity for metal ions, principally copper (II) and nickel (II) ions.[5] However, a little work has been done on its interaction with the zinc (II) ion, which is of biologically importance. Since the zinc (II) ion has neither intrinsic color nor unpaired electrons, the range of spectroscopic techniques available for investigation of its coordination properties is limited. The complexation of zinc (II) ion with histidine-containing peptides has been investigated by the potentiometric method, which can provide valuable information about the stoichiometry and equilibrium of the metal–peptide interaction.[6] The nuclear magnetic resonance (NMR) technique would provide structural information about the environment of the metal-binding site. In the present paper, we describe a study of the mode of interaction of zinc (II) ion with Gly–Gly–His by ^{1}H-NMR spectroscopy.

Experimental

Materials——Gly–Gly–His (Protein Research Foundation) and $ZnSO_4 \cdot 7H_2O$ (Merck) were used without

further purification. For the measurements of NMR spectra, the zinc (II) salt and peptide deuterated thoroughly with D_2O as reported previously were used.[7]

Method——The ^{1}H-NMR measurements were carried out at room temperature with a JEOL JNM FX-270 FT NMR spectrometer as described previously.[7]

Results and Discussion

Tripeptides generally function as bidentate, tridentate, or quadridentate ligands.[5] In the formation of the zinc(II) complex, because of its low coordinating ability, the peptide functions as a bidentate ligand. Actually, precipitates are formed from a 1:1 solution of zinc(II)–peptide at pH 7.5.[8] Therefore, we decided to examine the interaction of zinc(II) ion with Gly–Gly–His in 1:2 (zinc(II): peptide) solution.

The ^{1}H-NMR spectra of Gly–Gly–His in the presence of a half equivalent of zinc(II) ion at various pHs are shown in Fig. 1. Assignments of signals were made on the basis of chemical shift changes with pH and by reference to reported values for peptides and peptide complexes.[9-11] None of the signals of the NMR spectra were affected by the addition of zinc(II) ion below pH 6. This suggests that the peptide does not coordinate with zinc(II) ion below pH 6. However, above pH 6, the peptide undergoes significant structural alteration. The signal due to the imidazole C-2 proton underwent line broadening at pH 7.2, probably due to a rapid equilibrium between free and complexed Gly–Gly–His species on the NMR

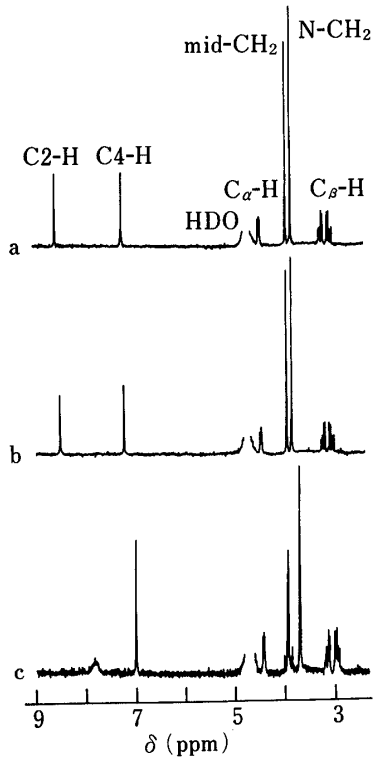

Fig. 1. ^{1}H-NMR Spectra of Gly–Gly–His in the Presence of $ZnSO_4$ in D_2O Solution

[Gly–Gly–His] = 4.4×10^{-3} M, [$ZnSO_4$] = 2.2×10^{-3} M, a) pH 5.0, b) pH 6.0, c) pH 7.2. Imidazole C-2 and C-4 protons, His-C_α and -C_β protons, and methylene protons of glycyl residues at the N-terminal and middle are abbreviated as C2-H, C4-H, C_α-H, C_β-H, N-CH_2, and mid-CH_2, respectively.

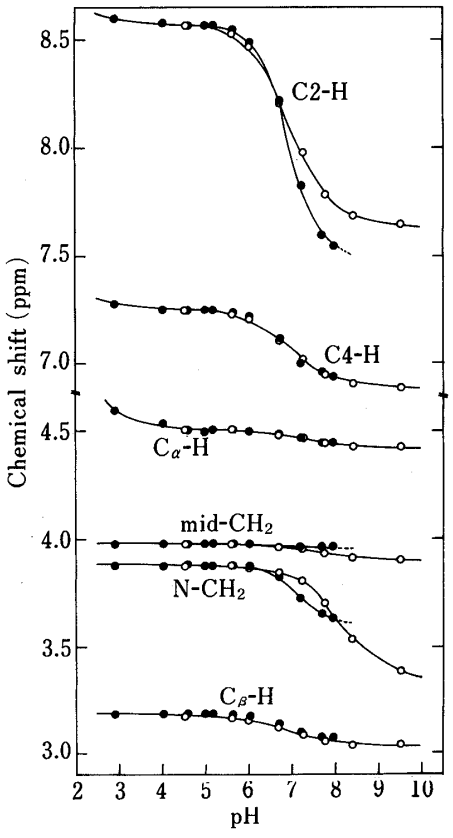

Fig. 2. NMR Titration Curves of Gly–Gly–His in the Absence (○) and Presence (●) of Zinc(II) Ion

Concentrations of the peptide and zinc(II) ion are the same as used for Fig. 1.

time scale. The measurement was carried out below pH 8.5, because the solution became turbid above pH 8.5 and precipitates were observed. In the complexation of zinc (II) ion with other histidine-containing tripeptides (*e.g.*, His–Gly–Gly[7] and Gly–His–Gly[12]), precipitates are not produced.

In order to analyze further the spectral changes that resulted from the metal interaction, NMR titration of the imidazole C-2 and C-4 protons, C_α and C_β protons of the histidine side chain, and methylene protons of glycyl residues at the amino terminal and in the middle position was carried out in the presence of zinc (II) ion and in the absence of zinc (II) ion. The titration curves are shown in Fig. 2. The effect of zinc (II) ion on the NMR spectrum began to appear at pH 7.2. The signals of the imidazole C-2 proton and methylene proton of glycine at the amino terminal were shifted upfield ($\Delta\delta$[13] $= -0.17$ and -0.08 ppm, respectively). This is suggestive of the interaction of zinc (II) ion with imidazole and amino nitrogens. A singlet due to the methylene proton in the middle position was split into a quadruplet and shifted a little downfield. This is suggestive of the interaction of zinc (II) ion with the carbonyl oxygen. It has been shown that a metal ion possessing weak coordination affinity, *e.g.*, zinc (II) ion, binds to the oxygen of the peptide bond adjacent to the amino terminal and a metal ion possessing strong affinity, *e.g.*, copper (II) ion, binds to the nitrogen of the deprotonated peptide bond.[5]

It is of interest that the imidazole C-4, His-C_α, and -C_β protons were not affected by the addition of zinc (II) ion. If the zinc (II) ion coordinates to both the amino and imidazole nitrogens, giving a six-membered chelate ring such as that formed in the L-histidine complexes, the signals of both imidazole C-2 and C-4 protons should undergo line broadening with an upfield or downfield shift. Though the imidazole group of Gly–Gly–His coordinates to the zinc (II) ion as stated above, it is probably not involved in the chelate ring of the complex. Nevertheless, it may bind weakly to the metal ion. A similar mode of coordination was found in zinc-bis[cyclo(L-histidyl-L-histidyl)]. A ^{1}H-NMR study of this complex indicated that only one of the two imidazole groups coordinates to zinc (II) ion and that the signal of the imidazole C-4 proton is not affected by the addition of zinc (II) ion.[14]

As for the modes of coordination between the zinc (II) ion and imidazole group in the zinc (II)–(Gly–Gly–His) complex, two possible ways can be considered, as shown in Chart 1.

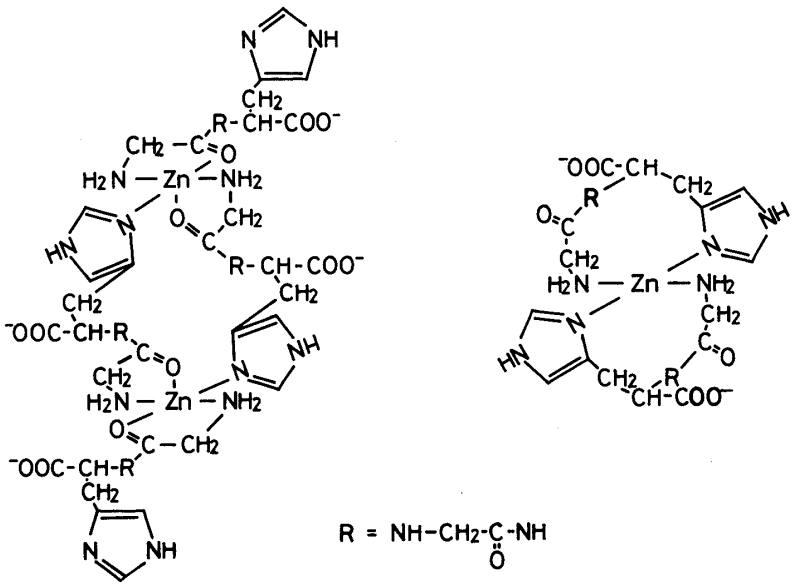

Chart 1

One is that two zinc (II) complexes are linked to each other through the imidazole group to form a dimer or an oligomer. The other is that the peptide coordinates with zinc (II) ion

through amino and imidazole groups as a bidentate ligand, forming a 12-membered chelate ring. A potentiometric study suggested that a binuclear complex, in which two zinc(II) ions are linked by cross-coordination through imidazole nitrogens, is formed.[8] On the other hand, the zinc(II) complex of Gly–Gly–His–*N*-methylamide was shown to form a large chelate ring by ^{13}C-NMR spectroscopy.[15] A mode of coordination in which all of the amino nitrogen, the vicinal carbonyl oxygen, and the imidazole nitrogen of Gly–Gly–His coordinate simultaneously to the zinc(II) ion seems to be sterically impossible.

The present study by ^{1}H-NMR spectroscopy has shown that zinc(II) ion coordinates to Gly–Gly–His through the amino nitrogen and the neighboring peptide oxygen to form a five-membered chelate ring, and that zinc(II)–(Gly–Gly–His) complexes may be bridged weakly by the imidazole group to form a dimer or an oligomer above pH 6. Since the stability constant for the zinc complex of Gly–Gly–His ($\log K_1 = 3.31$) is small,[8] the zinc complex may be easily hydrolyzed in an alkaline medium. In fact, as the pH was increased (8.5), precipitates of zinc(II) hydroxide, which did not include the peptide, were produced.

References and Notes

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