[Chem. Pharm. Bull. 33(8)3096—3100(1985)]

^{1}H-Nuclear Magnetic Resonance Study of the Interaction of Zinc(II) Ion with a Histidine-Containing Peptide, L-Histidylglycylglycine

JUN-ICHI UEDA,*[a] AKIRA HANAKI,[a] NAOKO YOSHIDA,[b] and TERUMI NAKAJIMA[c]

National Institute of Radiological Sciences,[a] 4–9–1, Anagawa, Chiba 260, Institute for Medical and Dental Engineering, Tokyo Medical and Dental University,[b] 2–3–10 Surugadai, Kanda, Chiyoda-ku, Tokyo 101, and Faculty of Pharmaceutical Sciences, University of Tokyo,[c] Hongo, Bunkyo-ku, Tokyo 113, Japan

(Received November 6, 1984)

A proton nuclear magnetic resonance (^{1}H-NMR) study of the interaction of Zn(II) ion with histidine-containing peptide, L-histidylglycylglycine (his-gly-gly), was carried out. The 1 : 2 complex of Zn(II)–(his-gly-gly) takes different coordination structures depending on the pH. In the region of pH 4.5—6.5, the complex is a tautomer in which the Zn(II) ion is exchanged dynamically among amide carbonyl oxygen, imidazole nitrogen, and amino nitrogens. The Zn(II) ion coordinates with amino and imidazole nitrogens above pH 6.5.

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

In many zinc-containing enzymes, the Zn(II) ion is situated in a cluster composed of histidyl residues of the polypeptide chain and forms the active site of the enzymes. Therefore, the interaction of the Zn(II) ion with imidazole of the histidyl residues is of interest from the viewpoint of bioinorganic chemistry. However, since the Zn(II) ion has neither intrinsic color nor unpaired electrons, the range of spectroscopic techniques available for investigation of its binding properties is limited as compared with those available for other metalloenzymes. Nuclear magnetic resonance (NMR) is a useful technique for elucidating the coordination structure of zinc site in the proteins,[1)] but since proteins commonly possess many metal-binding groups including histidyl, cysteinyl, tyrosyl, and other functional groups, studies on the metal-binding of protein are likely to afford ambiguous results. Thus, we decided to use oligopeptide as a model compound of protein and to examine the zinc interaction of the peptide by NMR spectroscopy.

Many NMR studies on the complexation of metal ions with L-histidine have been reported,[2)] though few studies have been done on histidine-containing peptides.[3)] The complexation of Zn(II) ion with histidine-containing peptides has been investigated by the potentiometric titration method,[4)] which is valuable for the study of the stoichiometry and equilibrium of the metal–peptide interaction. On the other hand, the NMR technique should provide structural information about the metal-binding sites. In the present paper, we describe the mode of interaction of Zn(II) ion with a peptide containing a histidyl residue at the amino terminal, namely, L-histidylglycylglycine (his-gly-gly), and with L-histidine derivatives, as determined by ^{1}H-NMR spectroscopy.

Experimental

Material——L-Histidylglycylglycine (Bachem. Feinkemikalien Co.), L-histidine monohydrochloride (Kokusan

NII-Electronic Library Service

Kagaku), and histamine dihydrochloride (Merck) were commercial products. L-Histidine-*N*-methylamide dihydrochloride was synthesized by the conventional solution method. These peptides were chromatographically pure. Reagent grade $ZnSO_4 \cdot 7H_2O$ was used without further purification. To obtain deuterated reagents, the Zn(II) salt and the ligands were separately dissolved in D_2O (Showa Denko) and then D_2O was removed from the solutions under a vacuum.

NMR Measurements——^{1}H-NMR spectra were measured using a JEOL JNM FX-270 FT NMR spectrometer. Chemical shift values are quoted in parts per million (ppm) downfield from sodium 2,2-dimethyl-2-silapentane-5-sulfonate (DSS), used as an internal standard. The peptide solution in D_2O was titrated with either DNO_3 or NaOD solution. Whenever the pH of the solution reached the desired values, aliquots were withdrawn and placed in sample tubes for NMR measurement. The titration was done in the presence and absence of Zn(II) ion. Because of the low coordinating ability of Zn(II) ion, the peptides examined function as bidentate ligands toward Zn(II) ion. Precipitates of Zn(II) hydroxide were formed from the 1 : 1 solution of Zn(II)–peptide above pH 6. Thus, we examined the interaction of Zn(II) ion with histidine-containing peptide in 1 : 2 (Zn(II) : peptide) solutions. The concentrations of peptide and Zn(II) ion were 4.4×10^{-3} and 2.1×10^{-3} M, respectively, at the start of titration. The pH values given in the text are the readings of the instrument.

Results and Discussion

The ^{1}H-NMR spectra of his-gly-gly at pH 5.4 and 7.5 in the absence and presence of Zn(II) ion are shown in Fig. 1. The signals due to the imidazole C-2 and C-4 protons of the histidyl residue appear to change significantly on addition of Zn(II) ion.

The NMR titration curves of the imidazole C-2 and C-4 protons, methine and methylene protons of the histidyl residue, and two methylene protons of glycine residues at the middle and at the carboxylate end (abbreviated as C2-H, C4-H, C_α-H, C_β-H, mid-CH_2, and C–CH_2, respectively, in Fig. 1) in the absence and presence of Zn(II) ion are shown in Fig. 2. The titration curves in the absence of Zn(II) ion for imidazole C-2 and C-4 protons and His-C_α and -C_β protons display double sigmoid curves indicating deprotonation of the imidazolium ($pK_a = 5.5$[5]) and ammonium groups ($pK_a = 6.7$[5]). The addition of Zn(II) ion induces an upfield shift and line broadening of the signals of the imidazole C-2 and C-4 protons in the pH 4.5—6.5 region. Line broadening of the signals due to His-C_α and -C_β protons was also observed under the same conditions. The line broadening of signals of imidazole protons reached a maximum at pH 5.5, which corresponds to the pK_a of the imidazolium group. As the pH increased over 6.5, the addition of Zn(II) ion began to cause downfield shifts of the

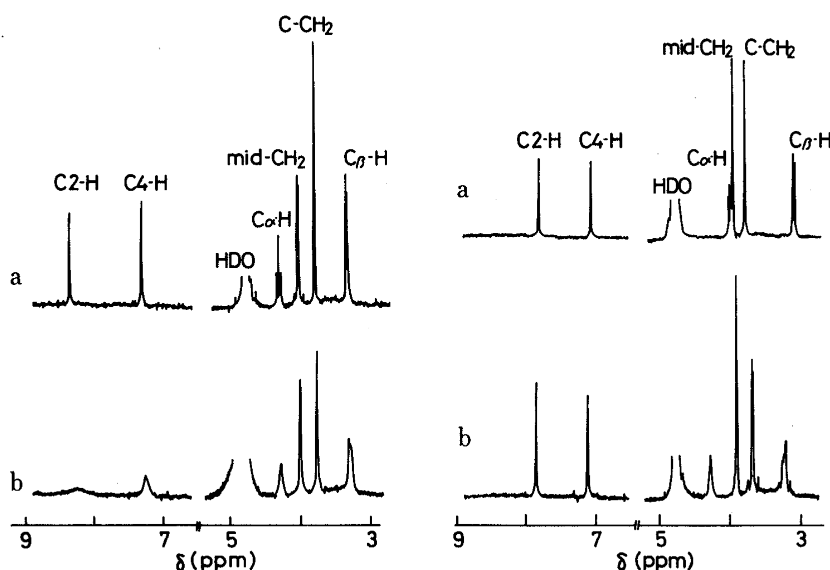

Fig. 1. The ^{1}H-NMR Spectra of (a) 4.4 mM his-gly-gly in D_2O and (b) 4.4 mM his-gly-gly in the Presence of 2.1 mM $ZnSO_4$ in D_2O Solution at pH 5.4 (Left) and pH 7.5 (Right)

NII-Electronic Library Service

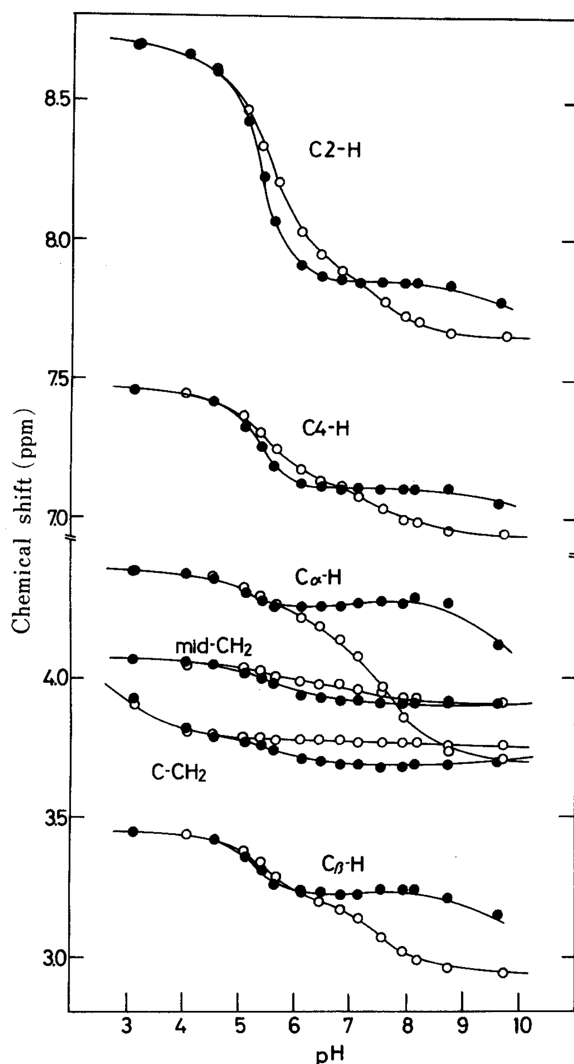

Fig. 2. The NMR Titration Curve of Imidazole C-2 and C-4 Protons, His-C_α Proton, His-C_β Proton, and Two Methylene Protons of Glycyl Residues of his-gly-gly in the Absence (○) and Presence (●) of Zn(II) Ion

Values of pH are uncorrected for D_2O.

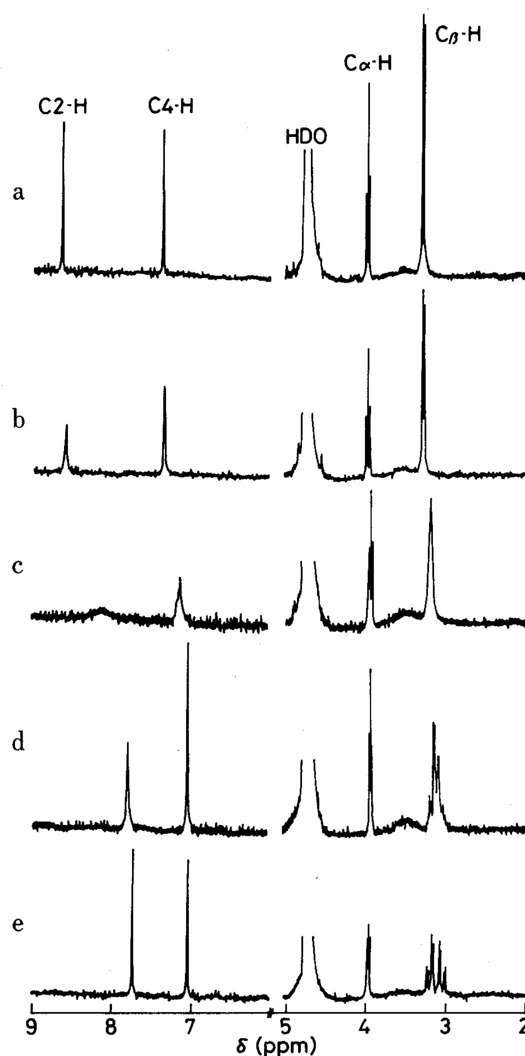

Fig. 3. The ^{1}H-NMR Spectra of 4.4 mM L-Histidine in the Presence of 2.1 mM $ZnSO_4$ in D_2O Solution at pH 4.0 (a), 4.9 (b), 5.8 (c), 6.7 (d), and 8.5 (e)

signals of the imidazole C-2 and C-4 protons, His-C_α and -C_β protons.

his-gly-gly is considered to have at least four ligating groups, namely, the nitrogen atoms of the amino and imidazole groups, and the oxygen atoms of the amide group at the amino terminal and the carboxylate group. Since the stoichiometry of the Zn(II) complex is 1:2 (Zn(II) : ligand), Zn(II) ion should be preferentially associated with two ligating groups from the four. The formation of molecular species in which Zn(II) chelates to the peptide *via* carboxylate and other ligating groups is unlikely because the 13-membered chelate ring involving the Zn(II)–O(carboxylate) would be rather unstable. It has been suggested that the stability of chelate complexes generally decreases as the size of the chelate ring increases over a 7-membered ring.[6] Thus, in the pH 4.5—6.5 region, three Zn(II) complex species, (i), (ii), and (iii), as shown in Chart 1 may exist in equilibrium.

The dynamic exchange of Zn(II) ion among those three species would result in the occurrence of line broadening of the signals due to the imidazole protons. Furthermore, upfield shifts of the signals of the imidazole protons may be explained by the proximal effect

NII-Electronic Library Service

(R = NH-CH2-CONH-CH2-CO2⁻)

Chart 1

Fig. 4. The ^{1}H-NMR Spectra of 4.4 mM L-Histidine-*N*-methylamide in the Presence of 2.1 mM $ZnSO_4$ in D_2O Solution at pH 4.1 (a), 4.9 (b), 5.5 (c), 5.8 (d), and 8.6 (e)

Fig. 5. The ^{1}H-NMR Spectra of 4.4 mM Histamine in the Presence of 2.1 mM $ZnSO_4$ in D_2O Solution at pH 4.0 (a), 5.8 (b), 6.6 (c), and 8.6 (d)

of the positively charged Zn(II) ion, which causes a decrease of the pK_a of the imidazolium group. As the pH increases, the Zn(II)–O(amide) bond gradually becomes unstable. We have observed that the 1 : 2 complex of Zn(II)–(gly-gly-gly) is hydrolyzed above pH 8 and Zn(II) hydroxide is precipitated. Since complexes (i) and (ii) become unstable with an increase of pH, the Zn(II) complex (iii) may exist as the predominant species. The signals due to the imidazole protons, His-C_α and -C_β protons, may move downfield as a result of electron with-

NII-Electronic Library Service

drawal from the amino and imidazole nitrogens caused by the binding with Zn(II) ion.[1d,7)]

In order to verify these assumptions, we examined the Zn(II) interactions with other ligands having three ligating groups, namely, amino, imidazole, and oxygen-containing groups, and with a ligand having two ligating groups, namely, amino and imidazole groups. L-Histidine and L-histidine-*N*-methylamide were used as examples of the former category, and histamine as an example of the latter.

The ^{1}H-NMR spectra of L-histidine at various pHs in the presence of Zn(II) ion are shown in Fig. 3; line broadening of the signals of imidazole protons can be seen, as observed in the case of his-gly-gly. The signals of imidazole C-2 and C-4 protons and His-C_β proton were shifted upfield by the addition of Zn(II) ion in the range of pH 5—7.5. In the pH range over 7.5, the addition of Zn(II) ion caused all the signals to shift to lower field. This indicates that Zn(II) ion coordinates with the amino- and imidazole-nitrogens as the pH is increased above 7.5. This result is consistent with the X-ray crystal structure analysis of di-(L-histidino)zinc-(II) dihydrate[8)] which showed that the zinc atom is coordinated by two amino nitrogen atoms and by two imidazole nitrogen atoms arranged in a somewhat distorted tetrahedral array, and the two carboxyl oxygen atoms approach the Zn(II) ion closely. A similar result was also obtained in the case of L-histidine-*N*-methylamide, as shown in Fig. 4.

The ^{1}H-NMR spectra of histamine do not show line broadening in the presence of Zn(II) ion as shown in Fig. 5. The signals of the imidazole protons did not broaden but were shifted a little to lower field by the addition of Zn(II) ion at pH above 7. These results support the assumption stated above.

Hence, the complexation of Zn(II) ion with his-gly-gly may be interpreted as follows on the basis of the NMR data. In the pH region between 4.5 and 6.5, the Zn(II) ion associated with his-gly-gly is exchanged rapidly on the NMR time scale among three ligating groups (*i.e.*, carbonyl oxygen of the N-terminal amide linkage, and amino and imidazole nitrogens). As a result, the signals of the imidazole protons are broadened. As the pH increases over 6.5, the Zn(II) ion may be chelated by amino and imidazole nitrogens of the peptide, and the rate of ligand exchange becomes fairly slow. This is consistent with the result obtained from a potentiometric study of the complexation of Zn(II) with L-histidylglycine.[4b)]

References

1) *a*) G. S. Baldwin, A. Galdes, H. A. O. Hill, B. E. Smith, S. G. Waley, and E. P. Abraham, *Biochem. J.*, **175**, 441 (1978); *b*) I. D. Campbell, S. Lindskog, and A. I. White, *J. Mol. Biol.*, **90**, 469 (1974); *c*) S. J. Lippard, A. R. Burger, K. Ugurbil, M. W. Pantoliano, and J. S. Valentine, *Biochemistry*, **16**, 1136 (1977); *d*) J. H. Bradbury, V. Ramesh, and G. Dodson, *J. Mol. Biol.*, **150**, 609 (1981).
2) *a*) R. H. Carlson and T. L. Brown, *Inorg. Chem.*, **5**, 268 (1966); *b*) G. C. K. Roberts and O. Jardetzky, *Adv. Protein Chem.*, **24**, 447 (1970).
3) H. Lakusta, C. M. Deber, and B. Sarkar, *Can. J. Chem.*, **58**, 757 (1980).
4) *a*) H. Lakusta and B. Sarkar, *J. Inorg. Biochem.*, **11**, 303 (1979); *b*) R. P. Agarwal and D. D. Perrin, *J. Chem. Soc., Dalton Trans.*, **1975**, 1045; *c*) R. P. Agarwal and D. D. Perrin, *J. Chem. Soc., Dalton Trans.*, **1976**, 89.
5) A. Yokoyama, H. Aiba, and H. Tanaka, *Bull. Chem. Soc. Jpn.*, **47**, 112 (1974).
6) F. J. C. Rossoti, "Modern Coordination Chemistry," ed. by J. Lewis and R. G. Wilkins, Interscience Publishers, Inc., New York, 1960.
7) N. J. Oppenheimer, L. O. Rodriguez, and S. M. Hecht, *Biochemistry*, **18**, 3439 (1979).
8) H. C. Freeman, *Adv. Protein Chem.*, **22**, 257 (1967).

NII-Electronic Library Service