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METAL COMPLEXES OF SULFHYDRYL-CONTAINING PEPTIDES : NICKEL(II) COMPLEX OF 2-MERCAPTOPROPIONYLGLYCINAMIDE IN COMPARISON WITH 2-MERCAPTOPROPIONYLGLYCINE

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The pK_{c_1} (7.40) and pK_{c_2} (8.04) values for deprotonation of the peptide and terminal amide groups in the 1:1 2-mercaptopropionylglycinamide(2-MPGA)-Ni(II) complex were determined. In the ^{1}H NMR spectrum of 2-MPGA-Ni(II) complex, the methylene protons of the ligand were observed as two signals at 3.96 and 3.31 ppm. The result suggests that two protons of the methylene group are in different environment which is induced by the fused-chelate ring formation.

It is surprising that only a few studies have been reported concerning the interaction of the sulfhydryl and amide-containing peptides with metal ions, in spite of the importance of sulfhydryl and amide groups of cysteine and glutamine(or asparagine) residues in peptide and protein ligands. Recently, we have characterized the Ni(II) and Cu(II) complexes of 2-mercaptopropionylglycine(2-MPG), and proposed that of interest is its Cu(II) complex as a new model for the "blue" copper centers.1) Amide groups even in several peptides which involve no sulfhydryl groups, exhibit interesting reactivity toward Cu(II) and Ni(II) ions.2) Herein, 2-mercaptopropionylglycinamide(2-MPGA)-Ni(II) complex has been investigated by electronic spectra, ^{1}H NMR spectra, and potentiometric pH titration, in comparison with 2-MPG- and glycylglycinamide-Ni(II) complexes.

2-MPGA and 2-MPG were gift of Santen Seiyaku Co. Visible absorption spectra of the Ni(II) complexes were determined in an aqueous solution(pH 9.2) using a Shimadzu recording spectrophotometer, Model Double-40R. Exactly equimolar amounts(0.004 M) of the ligand and Ni(II) ion were mixed in 18 ml of water and to this was added 2 ml of 1 M KNO_3 to make the total volume of 20 ml. The mixture was titrated with 0.1 N KOH at 20° under a nitrogen atmosphere, and the pH measurements were carried out with a Radiometer titrator, Type TTT-1C. The K_1 and K_c values were calculated according to the modified method of Datta and Rabin.3) ^{1}H NMR spectra were recorded at 100 MHz on a Varian HA-100 NMR spectrometer. Sample concentration was 0.2 M in D_2O(pD 9.6) and chemical shifts were measured from internal TSP.

Visible Spectra : 2-MPGA reacts with Ni(II) ion to form stable orange colored 1:1 complex in an alkaline solution. The visible spectral data of 2-MPGA-Ni(II) complex are given in Table I, together with those of the related Ni(II) complexes. The spectral feature of 2-MPGA-Ni(II) complex resembles remarkably that of 2-MPG-Ni(II) complex, though the absorption maxima of 2-MPGA-Ni(II) complex shift to shorter wavelength than that of 2-MPG-Ni(II) complex. The fact strongly suggests that 2-MPGA forms the Ni(II) complex similar to that of 2-MPG which coordinates to Ni(II) through the sulfhydryl, deprotonated peptide nitrogen, and terminal carboxylate groups.[1a)]

Potentiometric pH Titration : In the pH titration curves of 2-MPGA only and 1:1 2-MPGA-Ni(II) systems, the pH inflections are clearly observed at a=1 and a=3(a:moles of base per ligand), respectively. The result indicates that one proton from the sulfhydryl and two protons from the peptide and terminal amide groups dissociate in the course of the complex formation, and that 2-MPGA behaves as tridentate ligand in the 1:1 Ni(II) complex. In addition, it is reasonable to assume from the result the following reaction sequences and species involved herein :

$$Ni^{2+} + H_2L^- \xrightleftharpoons{K_1} Ni(H_2L)^+$$

$$Ni(H_2L)^+ \xrightleftharpoons{K_{c_1}} Ni(HL) + H^+$$

$$Ni(HL) \xrightleftharpoons{K_{c_2}} Ni(L)^- + H^+$$

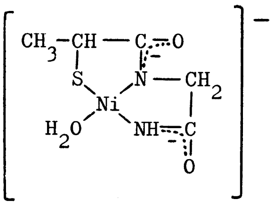
Table I Visible Spectral Data and Formation Constants of Ni(II) Complexes of 2-Mercaptopropionylglycinamide and Its Related Ligands

Ligand	Donor Set	Fused Ring Member	λ_{max} of Ni(II) Complex nm(ε)	Acid Dissociation Constant of Ligand pK_{COOH}	Acid Dissociation Constant of Ligand pK_{SH} or NH_3^+	Formation Constant of Ni(II) Complex log K_1	log K_2	log K_3	pK_{c_1}	pK_{c_2}	pK_{c_3}
2-Mercaptopropionylglycinamide	SNN	5-5	358(2790) 433(620)	——	8.20	4.59	——	——	7.40	8.04	——
2-Mercaptopropionylglycine[1a)]	SNO	5-5	375(2750) 475(410)	3.60	8.74	5.44	——	——	6.88	——	——
Glycylglycinamide(GGA)[2b)]	NNN	5-5	454(140)†	——	7.78	3.42	2.79	2.4	8.52	9.34	10.53
Glycylglycine(HGG)[4)]	NNO	5-5	605(10)††	3.14	8.09	4.07	3.34	2.5	not determined	not determined	——

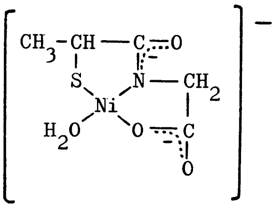
†Species is square-planar $Ni(H_{-2}GGA)(OH)^-$. ††Species is octahedral $Ni(H_{-1}GG)_2^{2-}$.

where H_3L refers to free amide ligand, and Ni(HL) and $Ni(L)^-$ to the complexes formed upon removal of one and two protons, respectively, from the peptide and terminal amide linkages in the positive complex $Ni(H_2L)^+$. The dissociation constant of 2-MPGA and formation constant of 2-MPGA-Ni(II) complex are listed in Table I, compared with those of the related ligands. The fact that the log K_1(4.59) of 2-MPGA-Ni(II) complex is smaller than that of 2-MPG-Ni(II) complex, is attributed to weaker basicity of the sulfhydryl group in 2-MPGA ligand. The pK_{c_1} (7.40) of 2-MPGA-Ni(II) complex for deprotonation of the peptide group is somewhat higher than that of 2-MPG-Ni(II) complex, whereas the pK_{c_2} (8.04) for the terminal amide group is much lower than that for the glycylglycinamide-Ni(II) complex(see Table I).

^{1}H NMR Spectra : Fig. 1 shows typical ^{1}H NMR spectra of 2-MPGA, 2-MPG, and their 1:1 Ni(II) complexes at pD 9.6. Both the Ni(II) complexes have similar spectral feature which indicates the presence of diamagnetic and square-planar species. The methylene protons of 2-MPGA(or 2-MPG) are observed as a single peak at 3.91(or 3.77) ppm. On the other hand, the methylene protons of 2-MPGA-Ni(II)(or 2-MPG-Ni(II)) complex are observed as two signals at 3.96 and 3.31(or 3.79 and 3.36) ppm. The result provides the fact that two protons of the methylene group are in different environment which is induced by the formation of complex with a rigid configuration, fused-chelate ring formation. Of two methylene protons of the Ni(II) complexes, the proton at lower field(3.96 or 3.79 ppm) is considered to be in a pseudo-equatorial position nearly trans to the Ni(II). The proton at higher field(3.31 or 3.36 ppm) would possess more axial character. Considerable evidence supports the view that the shifts of the methylene protons reflect the extent of the distortion from planarity as measured by the dihedral angle between Ni and H in Ni-N-C-H fragment.$^{5)}$ The determination of the dihedral angle between the H-C-N and C-N-Ni planes in 2-MPGA-Ni(II) and 2-MPG-Ni(II) complexes, is now under way. On the basis of these experimental results, the structure considered for 2-MPGA-Ni(II) and 2-MPG-Ni(II) complexes is as follows.



MPGA-Ni(II) complex



MPG-Ni(II) complex

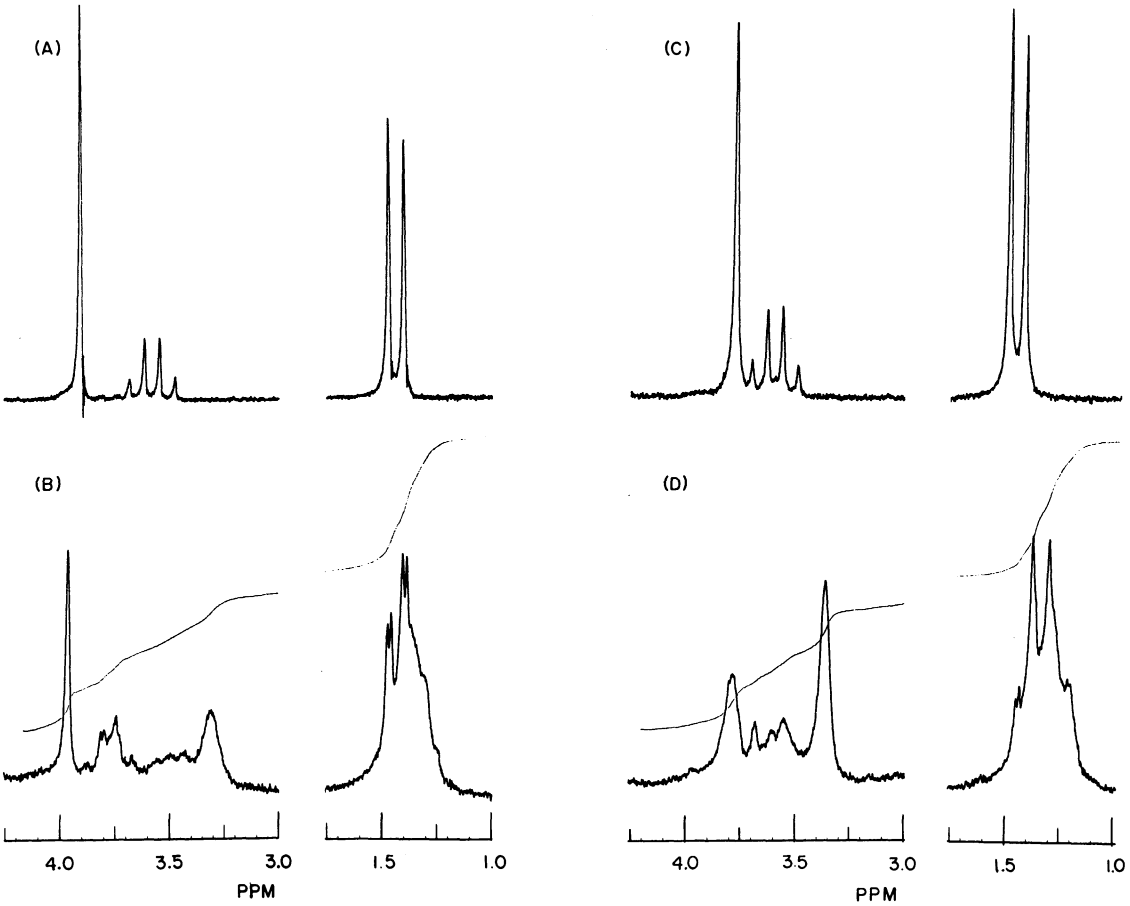


Fig. 1 100 MHz-^{1}H NMR spectra of 2-mercaptopropionylglycinamide(2-MPGA)(A), 2-MPGA-Ni(II) complex(B), 2-mercaptopropionylglycine(2-MPG)(C), and 2-MPG-Ni(II) complex(D) The spectra of the Ni(II) complexes were obtained by mixing ligand(0.2 M) and $NiCl_2$ (0.2 M) in borate buffer solution of pD 9.6.

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