Infrared Spectra and Structures of Copper(II) and Zinc(II) Complexes of Glycylglycine in Aqueous Solution

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(Received January 21, 1975)

The infrared spectra of the Cu(II) and Zn(II) complexes of glycylglycine were observed in D_2O at various pD values. Large spectral changes in going from acidic to neutral solution were interpreted in terms of the equilibria among free and complexed forms of glycylglycine. In the Cu(II) complex formed for $3<pD<5$ the metal-ligand bonding occurs through the amino nitrogen and peptide oxygen. As pD is increased further, the structure of the Cu(II) complex changes into a form in which the metal-ligand bonding occurs through the amino nitrogen, peptide nitrogen (deprotonated), and carboxyl oxygen. In contrast to the Cu(II) complex, no change of structure is found for the Zn(II) complex. The Zn(II) complex formed for $pD>5$ is similar to the Cu(II) complex present for $3<pD<5$.

The interaction between metal ions and peptides is an important subject not only in the chemistry of coordination compounds but also from biological viewpoint. In this and subsequent papers we study the structures of metal(II) complexes of some dipeptides in aqueous (or deuterium oxide) solution of various pH (or pD) and discuss, on this basis, the role of metal ions in the hydrolysis of dipeptides.

The structure of Cu(II) complex of glycylglycine, the simplest dipeptide, has been studied by many authors in crystal[1-3] and in aqueous solution.[4-8] The most interesting feature derived from the previous studies is the change of complex structure with the increase in pH (or pD). All the authors seem to agree that the peptide group is deprotonated in going from acidic to neutral solution and that in neutral solution the metal-ligand bonding occurs through the amino nitrogen, peptide nitrogen (deprotonated), and carboxyl oxygen. As for the structure in acidic solution, two different forms have been proposed with respect to the metal-ligand bonding. We discuss this matter through the analysis of infrared spectra of this complex in deuterium oxide solution. The structure of the Zn(II) complex in aqueous solution is also examined by referring to the Cu(II) complex case.

Experimental

Materials. Glycylglycinatocopper(II) trihydrate[Cu-GG·$3H_2O$[9]], glycylglycinatocopper(II) chloride monohydrate[Cu(HGG)Cl·H_2O], bis(glycylglycinato)zinc(II) dihydrate[$Zn(HGG)_2\cdot 2H_2O$], and glycylglycinatozinc(II) hemihydrate[Zn(HGG)Cl·$0.5H_2O$] were prepared from glycylglycine and corresponding metal(II) chloride or nitrate according to the methods described in references 10—12.

Infrared Absorption Measurements. The infrared spectra were recorded for *ca.* 0.2 M D_2O solutions using a Hitachi EPI-G3 spectrophotometer and a pair of cells made of As_2Se_3 (sample layer thickness 0.05 mm). The amino and peptide protons were deuterated by conventional method before preparing the D_2O solution. The pD value was adjusted by adding suitable amount of the 1 M D_2O solution of DCl or NaOD. The pD value was determined using a Hitachi-Horiba pH-meter F-7 with a combination electrode and a set of pH test paper, and no correction for the obtained values was made.

Results and Discussion

Infrared Spectra and Structures of the Cu(II) Complexes. The spectrum of the D_2O solution of CuGG·$3D_2O$ was essentially the same as that of Cu(DGG)Cl·D_2O under common pD values. So, the structure of complexed glycylglycine in both solutions must be identical. The infrared spectra in the region between 1800 and 1200 cm^{-1} of Cu(DGG)Cl·D_2O are shown in Fig. 1. The results shown in this figure agree well with those obtained previously by Kim and Martell[5] for the mixture of the Cu(II) ion and glycylglycine in D_2O.

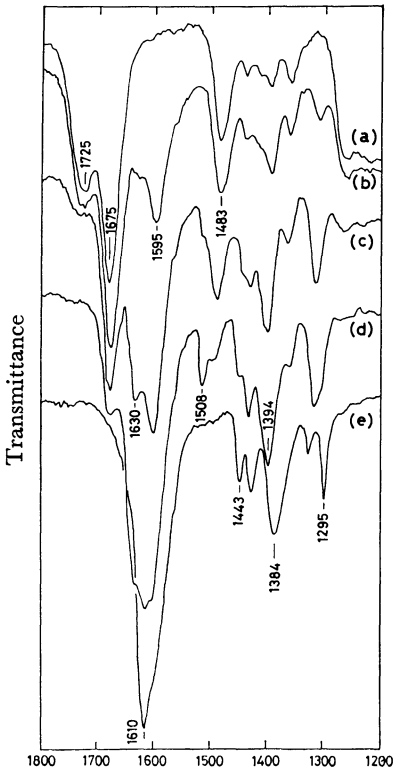


Fig. 1. Infrared spectra of the D_2O solution of the Cu(II) complex at various pD values. Concentration, *ca.* 0.2 M; sample layer thickness, 0.05 mm.
(a), pD 0.6; (b), pD 2.0; (c), pD 3.6; (d), pD 4.2; (e), pD 5.6.

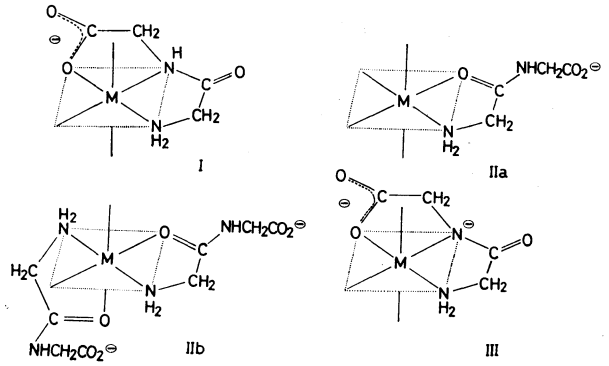


Fig. 2. Structures of the metal-glycylglycine complexes.[15]

Although Kim and Martell observed only the 1800—1500 cm^{-1} region, the region below 1500 cm^{-1} also gives useful information as will be described shortly. By comparing the spectra in Fig. 1 with pD variation of the spectra of glycylglycine itself,[13,14] it is clear that no complex is formed in the pD range below *ca.* 3. The band at 1630 cm^{-1} which appears only in the pD range between 3 and 5 was assigned by Kim and Martell[5] to the amide I vibration of Complex I shown in Fig. 2.[15] The formation of Complex I in acidic solution was later supported by a potentiometric analysis.[7] However, the present infrared results are better accounted for by assuming Complex IIa of Fig. 2 on the following grounds.

The amide II band which is located at about 1560 cm^{-1} in the spectra of simple peptides is known to shift, on deuteration, to about 1480 cm^{-1} (amide II').[16] The amide II' band is clearly seen at 1502 cm^{-1} in the spectrum of deuterated solid complex Cu(DGG)Cl·D_2O[17] (Fig. 3), whereas no corresponding band is found in the spectrum of deuterated solid complex CuGG·$3D_2O$ (Fig. 4). Since the peptide group in the latter complex is deprotonated, the normal modes would be different from those of ordinary peptide bond. So, it is reasonable that no band is found around 1500 cm^{-1} for CuGG·$3D_2O$. In D_2O the amide II' band of free glycylglycine is found at 1483 cm^{-1}. In the spectra of Cu(II) complexes in D_2O at pD 3, the presence of this band indicates, together with

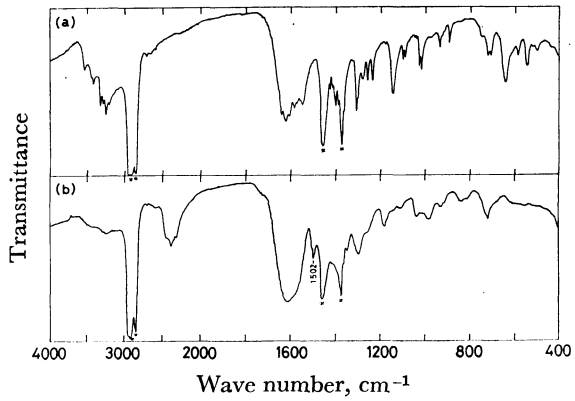


Fig. 3. Infrared spectra of solid (a) Cu(HGG)Cl·H_2O and (b) Cu(DGG)Cl·D_2O (Nujol mull).
The absorptions due to Nujol are indicated by asterisk.

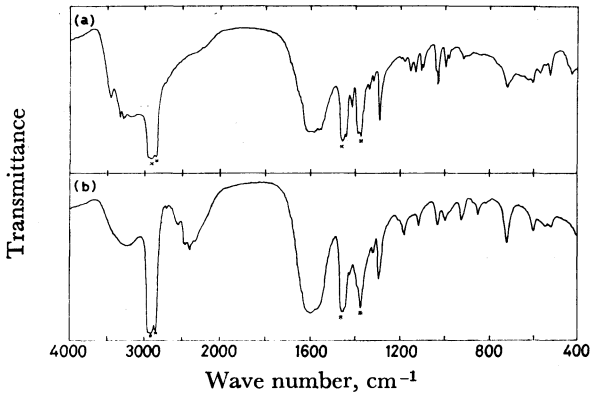
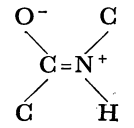


Fig. 4. Infrared spectra of solid (a) CuGG·$3H_2O$ and (b) CuGG·$3D_2O$ (Nujol mull).
The absorptions due to Nujol are indicated by asterisk.

other evidence, that the complexes are dissociated in this low pD range. In the pD range between 3 and 5 where the 1630 cm^{-1} band is observed, another band appears at 1508 cm^{-1} in addition to the 1483 cm^{-1} band. The 1508 cm^{-1} band can be assigned to the amide II' vibration of complexed glycylglycine. The shift of amide II' band to higher frequency is related to the increase in bond order of the peptide C-N linkage; in other words, the contribution of the resonance form



increases with the complex formation. This type of change can be expected for Complex IIa but not for Complex I (Fig. 2). In the latter the bond order of the peptide linkage would be reduced through the increased contribution of sp^3 configuration of the nitrogen atom.

The infrared analysis given above indicates that complex IIa and free glycylglycine coexist in the range $3<pD<5$. The presence of Complex IIa in acidic solution was first proposed by Rabin[4] from potentiometric studies and later supported by calorimetric[6] and kinetic[8] analyses. The X-ray diffraction analysis of Cu(HGG)Cl·H_2O crystallized from acidic solution gave a structure analogous to Complex IIa.[3]

In neutral solution with $pD>5$ new bands are observed at 1610, 1443, and 1295 cm^{-1}, whereas the bands at 1630 and 1508 cm^{-1} disappear. The spectra measured at pD values between 5 and 11 were essentially the same as that of Fig. 1(e). The new bands are undoubtedly due to Complex III shown in Fig. 2. The 1610 cm^{-1} band may be assigned to the C=O stretching and the latter two bands are probably associated with the $C-N^-$ stretching vibration. The conversion of complex structure in going from acidic to neutral solution and the presence of Complex III in neutral solution are unanimously accepted.[4-8] In this complex the terminal CO_2^- group is also coordinated to the metal. In fact, the CO_2 symmetric stretching band is shifted to 1384 cm^{-1} [Fig. 1(e)] from 1394 cm^{-1} of free CO_2^- [Fig. 1(b), (c), and (d)].

The assignments of bands observed in the region

Table 1. Assignments of the infrared bands of the D_2O solution of the Cu (II) complex in the region between 1750 and 1440 cm^{-1}.[a]

Obs. freq., cm^{-1} [a]	Assignment
1725 (1725)	C=O str. of terminal COOH
1675 (1675)	Amide I of free $D_3N^+CH_2CONDCH_2CO_2^-$
1630 (1640)	(1) Amide I of free $D_2NCH_2CONDCH_2CO_2^-$ (2) Amide I of Complexes IIa and IIb
1610	C=O str. of Complex III
1595 (1595)	Antisym. str. of free and complexed CO_2^-
1508 (1500)	Amide II′ of Complexes IIa and IIb
1483 (1483)	Amide II′ of free $D_3N^+CH_2CONDCH_2CO_2^-$
1443	C–N^- str. of Complex III[b]
1394 (1394)	Sym. str. of free CO_2^-
1384	Sym. str. of complexed CO_2^-

a) This table is also applicable to the D_2O solution of the Zn (II) complex. The observed frequencies for the D_2O solution of the Zn(II) complex are given in parentheses. b) The 1295 cm^{-1} band also may be assigned to a mode which has a considerable contribution of the C–N^- stretching.

between 1750 and 1300 cm^{-1} are given collectively in Table 1.

Infrared Spectra and Structures of the Zn(II) Complexes. The infrared spectrum of the D_2O solution of Zn(DGG)$_2$·2D_2O was not much different from that of Zn(DGG)Cl·0.5D_2O under common pD values. The structure of complexed glycylglycine in both solutions must therefore be identical. The infrared spectra in the region between 1800 and 1200 cm^{-1} of Zn(DGG)Cl·0.5D_2O in D_2O are shown in Fig. 5. The examination of these spectra shows that no complex is formed for pD<4. In this pD range the amide II′ band at 1483 cm^{-1} of free glycylglycine is observed similarly as in the case of the D_2O solution of the Cu(II) complexes for pD<3. The bands at 1640 and 1500 cm^{-1} begin to appear at pD about 5 and become stronger for higher pD values until the precipitation of the complex begins from alkaline solution with pD about 9. At pD 7.6 the amide II′ band of free glycylglycine is observed as a very weak shoulder. This indicates that Complex IIa and /or Complex IIb are the predominant species in this pD range. In both of these complexes the metal-ligand bonding occurs through the amino nitrogen and peptide oxygen. Complex IIb is consistent with the structure determined for the crystal of Zn(HGG)$_2$·2H_2O by X-ray diffraction.[2] It is interesting to note that no conversion of complex structure takes place for the Zn(II) complex in going from acidic to neutral solution.

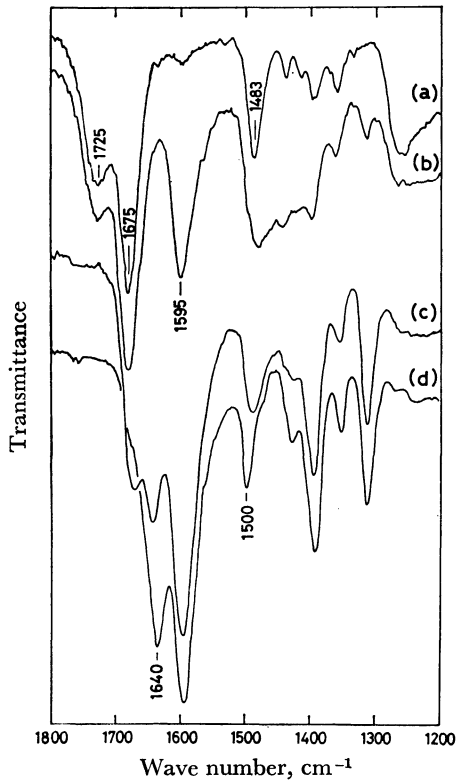


Fig. 5. Infrared spectra of the D_2O solution of the Zn(II) complex at various pD values.
Concentration, *ca.* 0.2 M; sample layer thickness, 0.05 mm. (a), pD 0.8; (b), pD 3.2; (c), pD 6.0; (d), pD 7.6.

Conclusion

The analysis of the infrared spectra of the Cu(II) complex of glycylglycine in D_2O shows that, for 3< pD<5, a complex is formed in which the metal-ligand bonding occurs through the amino nitrogen and peptide oxygen. As pD is increased, the structure of complex changes. In the complex which is present predominantly for pD>5 the metal-ligand bonding occurs through the amino nitrogen, peptide nitrogen (deprotonated), and carboxyl oxygen.

The behavior of the Zn(II) complex of glycylglycine is considerably different from that of the Cu(II) complex. No structure change of the complex is observed with increasing pD. In the complex formed for pD>5 the metal-ligand bonding is of the same type as found for the Cu(II) complex for 3<pD<5. Such structural information is useful in analysing the catalytic activities of these two metal ions in the hydrolysis of peptide bond. This point will be treated in a subsequent paper.

The authors wish to thank Miss Hideko Ito for the assistance throughout this work.

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9) The abbreviation GG stands for doubly deprotonated

glycylglycine. Free glycylglycine is therefore represented as H_2GG.

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14) The results of our measurements for glycylglycine itself were in agreement with those of Kim and Martell.[13]

15) In this figure only the metal-glycylglycine bonding is shown and other ligands such as OH^-, Cl^-, and H_2O are neglected, since no information about the latter ligands is available from the infrared spectra.

16) T. Miyazawa, T. Shimanouchi, and S. Mizushima, *J. Chem. Phys.*, **24**, 408 (1956).

17) In the spectra of solid complexes $Zn(DGG)_2 \cdot 2D_2O$ and $Zn(DGG)Cl \cdot 0.5D_2O$ the amide II′ bands are observed at 1490 and 1496 cm^{-1}, respectively.