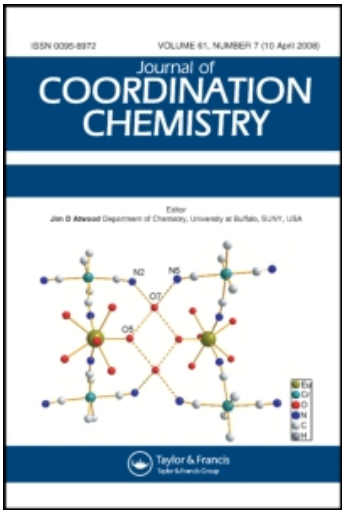

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INFRARED SPECTRA AND COORDINATE BONDING OF COPPER(II) AND NICKEL(II) POLYGLYCINE CHELATES IN AQUEOUS SOLUTION

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INFRARED SPECTRA AND COORDINATE BONDING OF COPPER(II) AND NICKEL(II) POLYGLYCINE CHELATES IN AQUEOUS SOLUTION

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Infrared absorption spectra in aqueous (D_2O) solution are employed to elucidate the nature of coordinate bonding in copper(II)- and nickel(II)-polyglycine chelates formed over the full pH range from strongly acid to strongly alkaline solution. These assignments provide the first microscopic evidence for metal-polyglycine coordinate sites in acid solution. Shifts in peptide carbonyl and terminal carboxylate frequencies are interpreted as indications of the conformational changes in both copper(II) and nickel(II) chelates as dissociation of hydrogen ion takes place to convert the initial complexes to the ultimate high pH forms, $MH_{-n}L^{1-n}$ and $MH_{-n}LOH^{-n}$, where n is the number of peptide linkages in the ligand.

INTRODUCTION

X-ray crystal structure studies of Freeman and coworkers[1] have elucidated the structures of many metal peptidechelates in the solid state. Because of the different conditions that exist in dilute solution it is probable that solution structures will be different in many cases. During the past few years considerable microscopic information has become available on aqueous peptide complexes. It is the purpose of this paper to recheck and analyze the available infrared data in order to determine the nature of the coordinate bonding in the metal chelate species formed in aqueous solution.

A series of studies of the stabilities of copper (II) complexes of di-, tri, and tetraglycine have been reported by Koltun et al.[2-4], who suggested some have new species to explain their potentiomentric measurements. Stability constants and coordinate bonding sites were reported by Martin et al.[5] for nickel(II) chelates of glycine peptides. Kim and Martell reported equilibrium constants for the interaction of glycine peptides with copper (I)[6,7] and nickel(II)[8], and described the aqueous infrared spectra of the glycine peptides[9], and of their metal chelates[6-8]. Additional microscopic data on these metal peptide chelates have been provided by Kim and Martell[10] in the form of aqueous proton nmr spectra. Mathur and Martin[11] have also reported the aqueous nmr spectra of glycine peptides and of the nickel(II) tetraglycine chelate. Further microscopic information on optically active di- and tripeptide chelates of copper(II) has recently been provided in the form of optical rotatory dispersion studies by Bryce *et al*[12-14] and by Kim and Martell[15].

Kaneda and Martell[16] have reinvestigated the copper(II) and nickel(II)-polyglycine equilibrium and have provided further evidence on the nature of the metal complex species formed under varying solution conditions. Motekaitis and Martell[17] have completed an aqueous solution study of the interaction of the N,N-diacetic acid derivatives of di, tri, and tetraglycine with copper(II), nickel(II), and cobalt (II), and, in addition to obtaining quantiative equilibrium data, have made a detailed aqueous infrared carbonyl band assignment for each carbonyl group present. Probable structures of the complexes consistent with the data were proposed.

EXPERIMENTAL

Spectral Measurements

Infrared spectra were measured with a Perkin Elmer Model 21 spectrophotometer using silver chloride absorption cells of 0.0165 mm thickness. The concentrations of the solutions were 0.020 M ligand in 99.8% D_2O and the ionic strength was adjusted to

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1.0 M with potassium chloride. Deuterium ion concentrations were measured with a Beckman model GS pH meter fitted with the special "one-drop" type electrodes and calibrated at 20° using NaOD, DCl, and $DC_2H_3O_2$.

Reagents

Glycylglycine was obtained from Calbiochem, La Jolla, California; triglycine was purchased from Mann Research Laboratory, New York; and tetraglycine was obtained from the Nutritional Biochemicals Corporation, Cleveland, Ohio. D_2O was purchased from Bio Rad Laboratory, Richmond, California. The peptides were recrystallized from aqueous alcohol solutions and were found to be chromatographically pure.

RESULTS

The ir spectra of diglycine, and of its copper(II) and nickel(II) chelates in aqueous solution, were measured as a function of pH. Similar spectra were obtained for triglycine and tetraglycine systems in the absence and in the presence of copper(II) and nickel(II) ions, and corresponding infrared assignments were made. The spectral data obtained were essentially the same as previously reported[6-9], and are therefore not presented here, however the present assignments are more complete and differ in several respects from those reported previously[6-9]. These new assignments, when compared to the reactions occurring in solution at various pH values may be used to infer the nature and coordinate bonding sites of specific metal chelate species in solution. These

TABLE I

Aqueous IR frequencies of diglycine and its Cu(II) and Ni(II) chelates

	Infrared Frequencies, cm^{-1}							
Species	-COOH	$\overset{\oplus}{\text{-CONH-}}$	-CONH-	$\overset{O---M^{2+}}{\underset{\|}{\text{-C-NH-}}}$	$\overset{M^{2+}}{\underset{\|}{\text{-CO-N-}}}$	-COO^-	$\overset{\ominus}{\text{-COO}}\overset{M^{2+}}{\underset{\|}{}}\text{-}$	$\overset{\ominus}{\text{-COO}}^-$
H_2L^+	1720	1675						
$HL^{\pm}$		1665				1595		
L^-			1632			1595		
CuL^+				1625		1598		
$CuH_{-1}L$					1610		∿1585 (sh)	
$NiHL^{2+}$				1630			1597[a]	
NiL^+				1633		1600		
$Ni(H_{-1}L)_2^{2-}$					1620			1565
$Ni(H_{-1}L)_2(OH)_2^{4-}$					1618			∿1564

[a] $-COO^-...M^{2+}$

assignments to specific solution species are listed in Tables I, II and III.

DISCUSSION

Reaction Stoichiometry

For the interpretation of the infrared spectra, it was imperative to consider the stoichiometry of reactions in solution as a function of pH. The determination of these reactions with various metal ions had been carried out during previous potentiometric and spectrophotometric investigations[2-11,16]. All of the mononuclear 1:1 complexes determined in previous investigations were taken into account in this interpretation and most of these are explicitly set forth as formulas I–XIII.

Infrared Spectra

Diglycine and Its Chelates

a. *The Free Ligand.* The cationic form of diglycine has a strong peptide –CONH– absorption at 1675 cm^{-1} and a weaker –COOH absorption at 1720 cm^{-1}. In the neutral (dipolar) form, the neutral carboxyl band is replaced by the negative carboxylate band at 1595 cm^{-1}, while the position of the peptide carbonyl band, which is mainly influenced by the

TABLE II

Aqueous IR frequencies of triglycine and its Cu(II) and Ni(II) chelates

	Infrared Frequencies, cm^{-1}							
Species	-COOH	$\overset{\oplus}{-}$CONH-	-CONH-	$\overset{O---M^{2+}}{\|}$ -C-NH-	-CO-$\overset{M^{2+}}{N}$-	$-COO^-$	$\overset{M^{2+}}{\ominus}$ $-COO^-$	$\ominus$ $-COO^-$
H_2L^+	1723	1678	1656					
$HL^{\pm}$		1678	1648			1597		
L^-			1644			1597		
CuL^+			1645	1630		1595		
$CuH_{-1}L$					1620	1595		
$CuH_{-1}L(OH)^-$					∿1615		1595	
$CuH_{-2}LOH^{2-}$					1610			1563
NiL^+			∿1640	1630		1595		
$NiH_{-2}L^-$					∿1605		1598	
$NiH_{-2}LOH^{2-}$					∿1605			1565
$NiHL^{2+}$		1680		1640		1595		

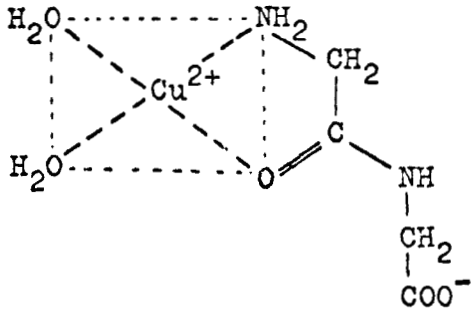
adjacent positive ammonium group, moves slightly ($10\ cm^{-1}$) to longer wave lengths. The strong influence of the terminal positive ammonium group is seen in the further shift of the peptide carbonyl by $33\ cm^{-1}$ to $1632\ cm^{-1}$ when it is converted to the neutral amino group in the negative form of the ligand.

b. *Cu(II) Chelates.* The infrared spectrum of the Cu(II) chelate, CuL^{+}, clearly indicates coordination of the peptide carbonyl with Cu(II) ion through the peptide oxygen, in view of the shift of the carbonyl band to longer wave lengths (from 1632 to 1625 cm^{-1}). The observed decrease in frequency ($7\ cm^{-1}$) is much less than it would be if the peptide carbonyl were the only group affected by coordination, since coordination of the terminal amino group to the Cu(II) ion shifts the peptide carbonyl in the reverse direction. If it is estimated that the influence of terminal amino-Cu(II) coordination has about half the electronic effect on the peptide carbonyl as that observed for proton coordination, it is seen that the peptide carbonyl frequency is lowered 20–25 cm^{-1} by coordination of the carbonyl oxygen to the metal ion. Coordination of the Cu(II) ion to the terminal amino group is indicated by the potentiometric data,[6] in which the metal ion is seen to displace a proton from $HL^{\pm}$ at relatively low pH. The infrared and potentiometric data, taken together, thus provide conclusive evidence for the metal coordination indicated in formula I. Molecular models indicate that in such a structure, the carboxylate group cannot coordinate with the copper(II) ion.

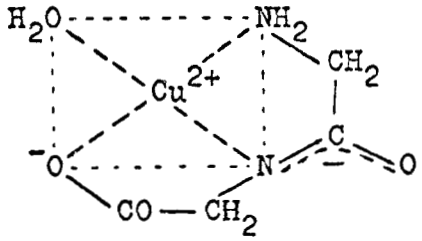
TABLE III

Aqueous IR frequencies of tetraglycine and its Cu(II) and Ni(II) chelates

	Infrared Frequencies, cm^{-1}						
Species	-COOH	$\overset{\oplus}{\text{-CONH-}}$	-CONH-	$\overset{O\text{--}M^{2+}}{\underset{\|}{\text{-C-NH-}}}$	$\overset{M^{2+}}{\underset{\|}{\text{-CO-N-}}}^{-}$	-COO^{-}	$\overset{\ominus}{\text{-COO}^{-}}$
H_2L^{+}	1722	~1675	1658				
$HL^{\pm}$		1673	1648			1596	
L^{-}			1643			1597	
CuL^{+}			1645	1620		1595	
$CuH_{-1}L$ } $CuH_{-2}L^{-}$				~1610	1600		~1570
$CuH_{-3}L^{2-}$					1610 sh 1592		1557
NiL^{+}			1650	1640		1598	
$NiH_{-3}L^{2-}$					1610 1595		1560
$NiHL^{2+}$						1596	



I CuL^+

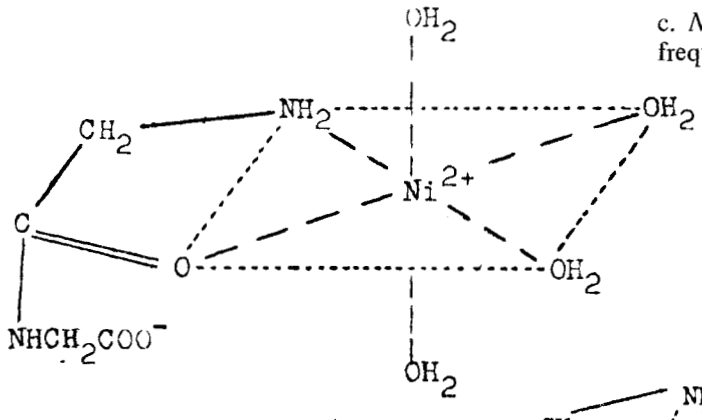


II $CuH_{-1}L$

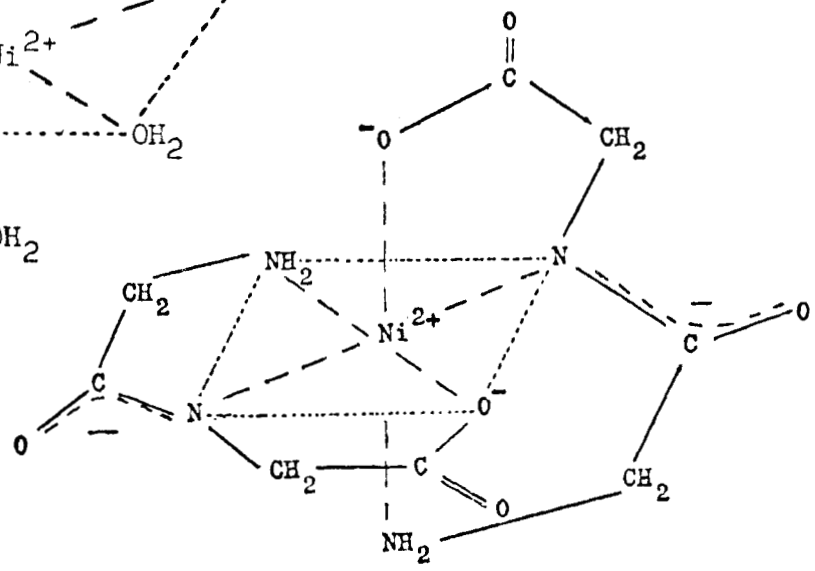
Copper(II)-diglycine complexes in solution

At higher pH, where the complex $CuH_{-1}L$ is formed, replacement of the peptide proton by a Cu(II) ion results in a further shift of the peptide carbonyl to 1610 cm^{-1}. The lower frequency is to be expected even though the peptide nitrogen is coordinated to the metal ion (II), since there is considerable negative charge on the coordinated peptide group. The shift of frequency observed in going from peptide proton to peptide nitrogen coordination, 22 cm^{-1}, may be considered the result of two opposing effects: the release of a proton would lower the frequency considerably, while coordination to the Cu(II) ion would partially reverse the effect of the proton dissociation and increase the frequency. Therefore it is seen that the stronger the binding of the metal ion to the ligand, the more closely will the carbonyl frequency approach that of the protonated peptide group in the free ligand, L^-. The carboxylate band does not change much when the peptide proton is displaced. The influence of the negative charge of the adjacent peptide linkage, which lowers the carboxylate frequency, is counteracted by coordination of the previously-uncoordinated carboxylate group (formula II), which tends to increase the carboxylate frequency.

c. *Ni(II) Chelates*. The neutral peptide carbonyl frequency of the Ni(II) chelate, NiL^+, III, has an



III NiL^+



IV $Ni(H_{-1}L)_2^{2-}$

Nickel(II)-diglycine complexes in solution

infrared carbonyl band at 1633 cm^{-1}, significantly higher than that (1625 cm^{-1}) of the corresponding copper(II) chelate, and indicating weaker bonding of the carbonyl oxygen to the metal ion. The carboxylate group is not in a sterically favorable position to coordinate strongly with the metal ion in this octahedral complex, and there is no significant effect observable in the infrared frequency of the carboxylate carbonyl. Potentiometric evidence for the $NiHL^{2+}$ chelate is borne out by the absorptions at pH 5.0 at 1630 cm^{-1} (amide O-bound) and at 1597 cm^{-1} ($Ni^{2+} - {}^{-}OOC$).

At high pH the 1:1 nickel(II) chelate, $NiH_{-1}L$, seems not to be sufficiently stable in solution to resist hydrolysis, but at higher ratios of ligand to metal ion, there is definite infrared evidence for a coordinated negative peptide carbonyl absorption at 1620 cm^{-1}, corresponding to the formation of a chelate compound such as that indicated by formula IV. It was noted that solutions of this complex at high pH are bluish green, and it is therefore tentatively assigned an octahedral structure in which the peptide nitrogen is deprotonated.

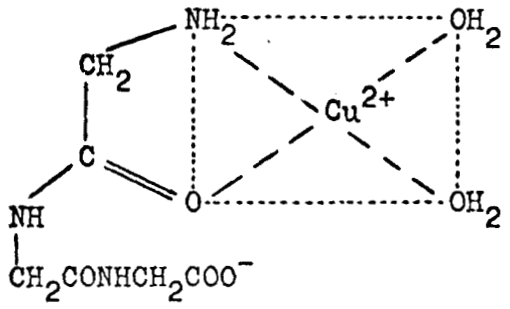
The infrared spectra of nickel(II)-glycylglycine indicate the growth of a strong carboxylate carbonyl band at the unusually low frequency of 1564 cm^{-1} The only reasonable explanation for the observed frequency is the displacement of coordinated carboxylate groups by hydroxide ion, to give a chelate compound, in which both carboxylate groups are uncoordinated. The low frequency is due to the influence of the negative charge on the adjacent peptide linkage. A similar effect can be seen at the high pH values for the corresponding copper(II) complexes in the growth of a shoulder at 1575 cm^{-1} The lower frequency of the nickel(II) carboxylate carbonyl group is in accord with the greater negative charge on the adjacent coordinated peptide linkage. That build-up of negative charge lowers these frequencies is now corroborated by the NGDA studies[17]

Triglycine and Its Chelates

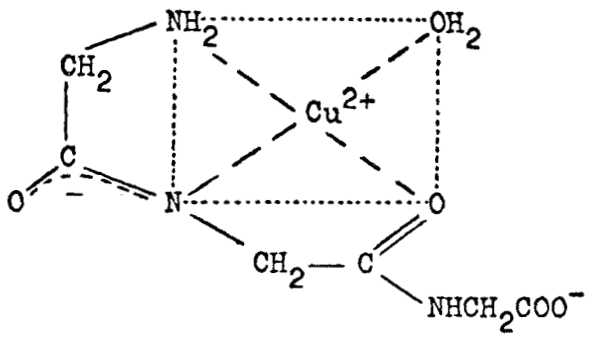
a. *The Free Ligand.* Whereas the infrared frequencies of the carboxyl and carboxylate groups of triglycine (Table II) are very similar to those of diglycine (Table I), the peptide carbonyl frequencies are quite different. In the ligand cation, as well as in the dipolar ion, two separate carbonyl frequencies are observed, and both shift to longer wave length with increasing negative charge on the ligand. The higher frequency band at 1678 cm^{-1} is assigned to the peptide carbonyl adjacent to the terminal amino group, since it is more strongly influenced by the terminal positive nitrogen atom, and is not influenced by ionization of the carboxyl group. The other peptide carbonyl band at 1656 cm^{-1} in the cation shifts to longer wave length (1648 cm^{-1}) in the dipolar ion. Both carbonyl bands have the same frequency, 1644 cm^{-1}, in the ligand anion, L^{-}.

b. *Cu(II) Chelates.* The copper(II) chelate, CuL^{+}, has two peptide carbonyl absorptions, one corresponding to an uncoordinated peptide group, and the other at the same frequency as that observed for the oxygen-coordinated peptide carbonyl of the corresponding diglycine chelate. These data therefore provide definite evidence for a bidentate structure corresponding to formula V. Although there is potentiometric evidence for $CuHL^{2+}$, its concentration was too dilute to assign the infrared spectrum. At higher pH the coordinated peptide groups undergo successive proton dissociation steps, beginning with the group nearest the amino group, giving rise to chelate species VI and VII which have negative peptide carbonyl absorptions about 1610–1620 cm^{-1}. It is noted that whereas simultaneous peptide dissociation and carboxylate coordination does not appreciably influence the carboxylate carbonyl frequency, a large frequency shift to longer wave lengths is again seen when the carboxylate group is displaced from the metal by a hydroxide ion to give a complex similar to VIII. The fact that the free carboxylate band now appears at lower frequency than was observed for the corresponding glycylglycine chelate, $CuH_{-1}L$, is in accord with the greater negative charge on the triglycinate ligand in the chelate compound $CuH_{-1}L^{-}(OH)^{2-}$.

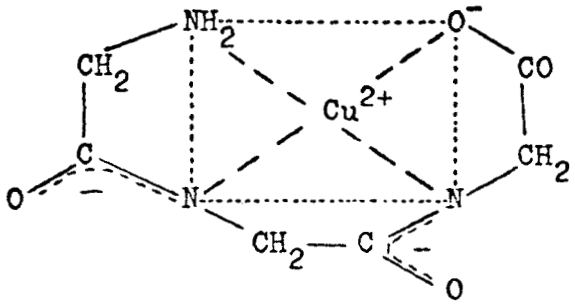
c. *Ni(II) Chelates.* The nickel(II) chelates of triglycine in acid solution, NiL^{+} and $NiHL^{2+}$ are similar to the analogous Cu(II) chelates. NiL^{+} exhibits two peptide carbonyl frequencies, one of which (at 1630 cm^{-1}) corresponds to a neutral peptide linkage coordinated to the metal ion through the carbonyl oxygen. The chelate in which both peptide groups are ionized and coordinated to the nickel(II) ion has a characteristic peptide absorption band at 1605 cm^{-1}. The frequency of the carboxylate carbonyl band of $NiH_{-2}L^{-}$ (1598 cm^{-1}) is slightly higher than expected, in view of the 1595 cm^{-1} value found for the corresponding copper(II) chelate. A shift to higher frequency compared to the spectrum of the octahedral nickel(II)-triglycine chelate ($Ni(H_{-1}L)_2^{2-}$), would not be unexpected because of the tighter



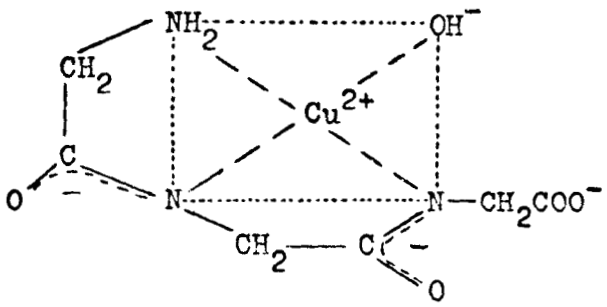
V CuL^{+}



VI $CuH_{-1}L$

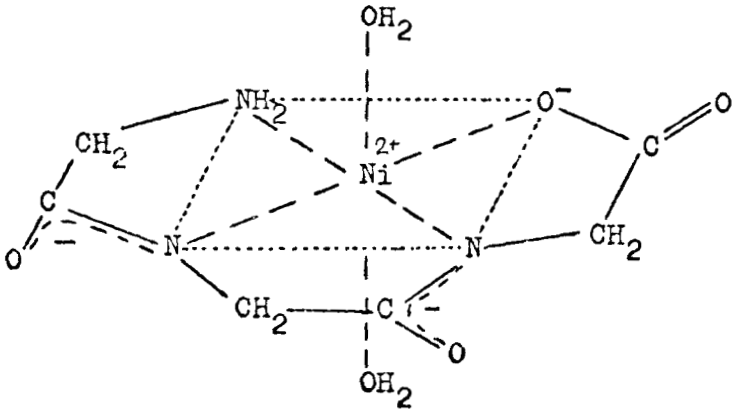


VII $CuH_{-2}L^{-}$



VIII $CuH_{-2}LOH^{2-}$

Copper(II)-triglycine complexes in solution



IX $NiH_{-2}L^{-}$

Nickel(II)-triglycine complexes in solution

binding of the carboxylate group in the square planar Ni(II) chelate illustrated by formula IX. When the carboxylate group is displaced from the coordination sphere of the metal ion by a hydroxide ion, its frequency shifts by 33 cm^{-1} to longer wave length, which is about the same, as the corresponding shift observed for the diglycine chelate.

It is noted that the free and coordinated carboxylate bands appear at about the same frequencies for both the Ni(II) and Cu(II)-triglycine chelates. The lack of appreciable shifts is due to two opposite effects. Deprotonation of the adjacent peptide linkage lowers the frequency of the carboxylate band, while coordination to the metal

ion tends to increase the frequency, resulting in little or no change in the observed value.

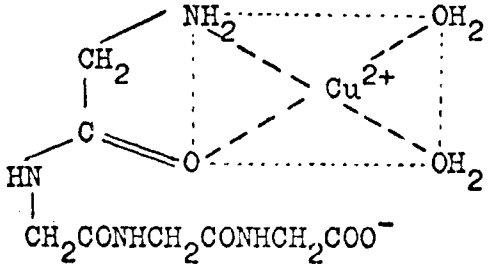
Tetraglycine and Its Chelates

a. *The Free Ligand.* The frequencies of the carbonyl absorption bands of the various ionic species of tetraglycine are almost identical to those of the corresponding forms of triglycine, and are given the same assignments.

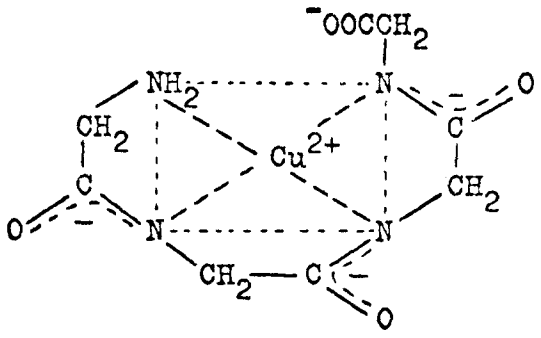
b. *Cu(II) Chelates.* As in the case of triglycine, the Cu(II)-tetraglycine chelate, CuL^+, has two peptide carbonyl absorption bands, one at 1620 cm^{-1} corresponding to an oxygen coordinated neutral peptide group, and the other at 1645 cm^{-1}, essentially unchanged from that of the free ligand. Thus the infrared data provide evidence for metal ion coordination of the terminal amino group, and the adjacent peptide carbonyl group, as indicated in formula X.

At higher pH the mixture of protonated and deprotonated, metal-coordinated, peptide groups give rise to two broad peptide carbonyl absorption bands, and a free carboxylate absorption band at longer wave length. The completely deprotonated chelate, $CuH_{-3}L^{2-}$, has only one peptide carbonyl band, in accordance with formula XI. The frequency of this band, 1595 cm^{-1}, is the lowest of any Cu(II) chelate studied in this research, reflecting the strong electronic effects of the three adjacent negative peptide groups on each other. The free carboxylate group of XI has a carbonyl band at 1557 cm^{-1}, the lowest frequency observed for any carbonyl group in the compounds investigated. The unusually low frequency of this band is due to the influence of the three negative charges of the ligand donor groups on the electron density in the uncoordinated carboxylate group.

c. *Ni(II) Chelates.* The frequencies of the neutral uncoordinated and the neutral coordinated peptide linkages in the low pH nickel(II) chelate, NiL^+, indicate a structure, XII, analogous to that of the

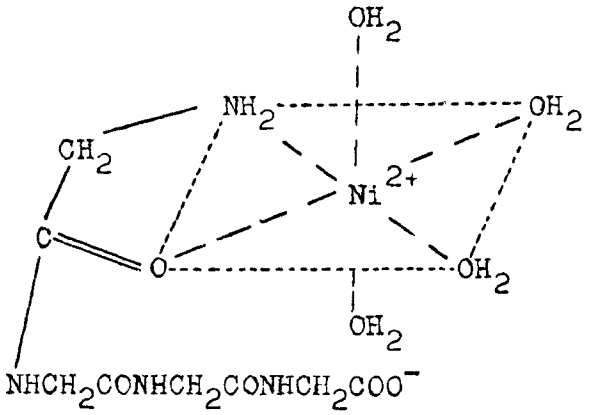


X CuL^+

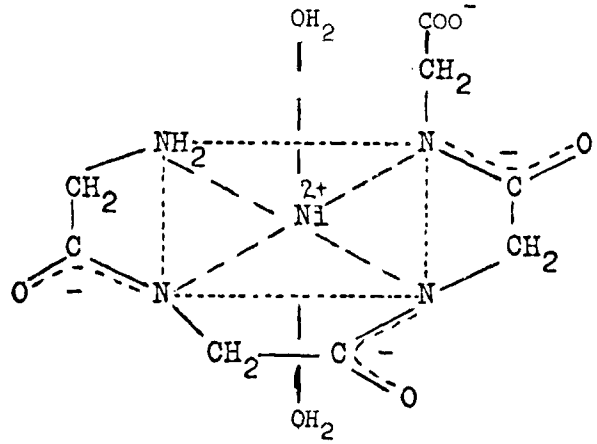


XI $CuH_{-3}L^{2-}$

Copper(II)-tetraglycine complexes in solution



XII NiL^+



XIII $NiH_{-3}L^{2-}$

Nickel(II)-tetraglycine complexes in solution

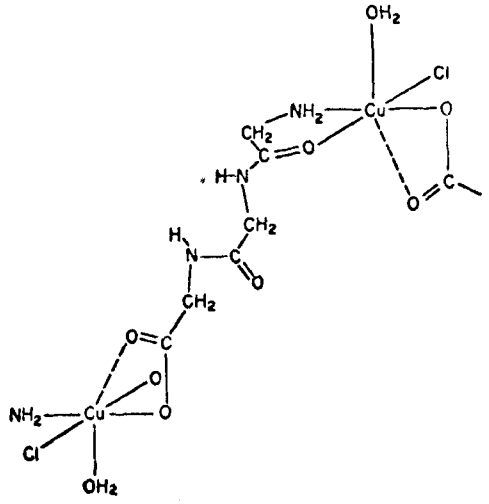
corresponding copper(II) chelate, X. The frequency of the peptide carbonyl coordinated at Ni(II) through oxygen is higher than that of the same group coordinated to Cu(II), as would be expected if the Ni(II)-carbonyl interaction is weaker than that of Cu(II). The fact that the shift of the carbonyl band from uncoordinated to coordinated is less for Ni(II) than it is for Cu(II) is in accord with this conclusion. It is also noted that the magnitude of this shift (10 cm^{-1}) to lower frequencies is the same for Ni(II) chelates of both tri- and tetraglycine.

For the fully deprotonated chelate, the peptide carbonyl frequencies at 1595–1610 cm^{-1}, and the free carboxylate carbonyl frequency at 1560 cm^{-1} are fully in accord with formula XIII. It is interesting that in this case two peptide carbonyl bands have been resolved; of these, the higher frequency band is considered to be due to the carbonyl group adjacent to the terminal amino group. The lower frequency of the carboxylate carbonyl compared to that of the corresponding triglycine chelate is due to the influence of the negative charge in the ionized ligand in the tetraglycine chelate upon the uncoordinated terminal carboxylate.

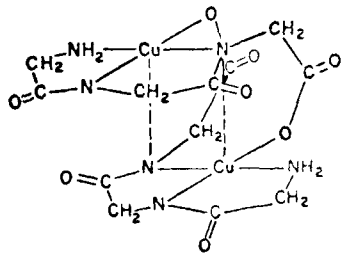
Comparison with X-Ray Crystal Studies

The accumulation of a large body of X-ray data on structure and bond distances in solid metal-peptide chelates[1,18] now makes possible many comparisons with analogous chelates in solution. Formulas XIV–XVII are examples of crystal structures of copper(II)-peptide chelates reported[18] that illustrate the principles involved. In many cases, the x-ray data suggest probable structures of the chelates in solution.

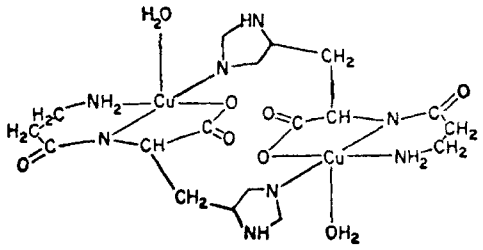
While metal chelates and complexes frequently crystallize without undergoing significant conformational changes, care of course must be taken in extrapolating solid state data to the elucidation of chelates in solution. In polar solutions, and especially in dilute solution, there is much greater tendency for intramolecular coordination than is true in the solid state. In fact there are no driving forces of the type classified under the term "chelate effect" governing the formation and structure of solid compounds, and the characteristic "stability" accorded to metal chelate ring formation is a reality only for dilute solutions. Thus it is seen that intermolecular coordination of the type illustrated by formulas XIV, XV, and XVI would not be expected in solution at moderate or dilute concentrations of solute. For the solution species of XIV, one would expect metal



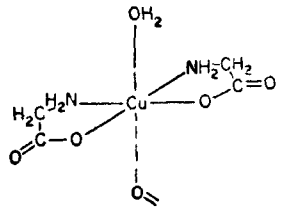
XIV



XV



XVI



XVII

binding only by the terminal amino and adjacent peptide carbonyl oxygen, with the remaining peptide carboxylate groups in an extended solvated form in solution. In other words, unidentate carboxylate bonding would be insufficient to hold two complexes together in dilute aqueous solution. The same conclusion applies to XV, which is known from potentiometric data to form only mononuclear species (VII) in solution. In the case of XVI, one would also expect the aqueous species to be mononuclear in dilute solution, perhaps with coordination of the imidazole nitrogen rather than the carboxylate group. Another example of a crystal structure that does not apply to solutions is the *cis* form of bisglycinato-copper(II) hydrate[18], XVII, which must be held in this energetically unfavorable conformation by crystal lattice forces (i.e., weak intermolecular forces, including hydrogen bonding). These forces would not exist in dilute aqueous solution, since water of solvation is uniformly available in all directions about a metal chelate compound. Thus a chelate compound of a labile metal ion of this type would be immediately converted to the more stable *trans* form in solution.

There are many examples of X-ray determinations of solid state structures that provide valuable information for the elucidation of the probable structures of solution complexes. The data on coordinate bond distances obtained from crystal structures is invaluable in determining relative coordinate bond strengths. The planar structures of the Cu(II) and Ni(II) chelates of deprotonated ligands $H_{-2}L^{3-}$ and $H_{-3}L^{4-}$, derived from tri- and tetraglycine, suggested for solution chelates, have been confirmed for the crystalline state[1,18,19]

Bisglycylglycinatonickel, $Ni(H_{-1}L)^{2-}$, formula IV, is another interesting example of how x-ray analysis can suggest a structure for an aqueous complex that would be difficult to deduce from evidence available for the ligand in solution. This complex cannot be accurately evaluated potentiometrically because precipitation occurs as base is added in the course of its formation in solution. Although the peptide group seems to be protonated, the 1:1 ligand: metal complex is not stable. The color of the 2:1 chelate in solution is greenish blue, rather than the yellow color observed for the planar nickel chelates of tri- and tetraglycine formed at high pH. The 2:1 crystalline chelate, $Na_2Ni(H_{-1}L)_2 \cdot 8H_2O$, which is reported[19] to have a structure similar to IV, strongly supports this conformation for the analogous chelate in solution.

Recently a more detailed study of equilibria over a wide range of solution conditions has revealed the existence of polynuclear species in solution at high concentrations[20-22]. This finding is of interest since it indicates the nature of the species present in saturated solutions, from which the crystalline materials described above are derived. It seems therefore that some of the structures described by Freeman[1] for the solid state may exist in solution under these special conditions of relatively high concentration.

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