the plane of the chelate oxygens and the lengthening of the copper–oxygen bond distance. However, the oxygen p orbital contribution to the singly occupied MO is highly sensitive to the choice of coordinates for for the adduct and may lead to a decrease in the d_{xy} contribution.

Conclusion

(1) Cu(*t*-Buoac)$_2$ forms well-defined 1:1 pyridine adducts in cyclohexane over the range of concentrations studied.

(2) The spectrophotometric technique employed is suitable for studying the thermodynamics of adduct formation for copper(II) β-diketonates.

(3) The order of donor ability is pyridine < 4-methylpyridine < 4-ethylpyridine < 4-*t*-butylpyridine and is maintained with Cu(*t*-Buoac)$_2$. The pyridine ligand steric requirements are constant throughout the series.

(4) The pyridine proton contact shifts predominantly occur by spin delocalization into the pyridine σ system and are free of steric effects. A small σ–π hyperconjugative effect is present, dominating at the γ position. Evidence is given that the contact shifts may follow the thermodynamics of adduct formation.

(5) The isotropic copper hyperfine coupling constant decreases dramatically upon adduct formation and does follow the thermodynamics of adduct formation. This decrease may be due to both decreasing d-orbital spin density and admixing of 4s character into the singly occupied MO.

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Proton Nuclear Magnetic Resonance Study of Metal–Glycine Peptide Complexes. Copper(II) and Nickel(II) Complexes[1,2]

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Abstract: Glycine peptides and their complexes with copper(II) and nickel(II) have been studied by aqueous (D_2O) proton nmr spectral measurements. For each dissociation step, as the pH is increased, the proton nmr peaks of the ligand methylene group nearest to the site of proton dissociation shift to higher field. In the presence of relatively small amounts of copper(II) or nickel(II) ion, these peaks disappear or broaden and the order of such change with increasing concentration of metal ion suggests the manner in which metal ion coordinates to the ligand and the sequence of coordination under changing solution conditions. Paramagnetic octahedral nickel(II) complexes undergo transition to diamagnetic planar forms with displacement of protons from the peptide linkages present in triglycine and tetraglycine. Direct evidence for the nature of this diamagnetic complex is obtained by proton nmr spectra. Contrary to the line broadening observed for paramagnetic metal complexes, three methylene peaks are observed for the nickel–triglycine complex and four methylene peaks, of which two peaks overlap, are observed for the nickel(II)–tetraglycine complex.

Copper(II) and nickel(II) complexes of glycine peptides have been studied by potentiometric pH measurements together with aqueous (D_2O) infrared absorption spectral techniques.[4–6] The purpose of the present aqueous (D_2O) proton nmr study is to obtain further information about the structures and the coordination sites of the metal peptide chelate species in solution.

Li and his coworkers[7–9] have already employed proton nmr measurements in studying metal complexes of diglycine and triglycine using heavy water as solvent, and proved this technique to be useful in obtaining

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(2) Abstracted in part from a thesis submitted by M. K. Kim to the faculty of Illinois Institute of Technology in partial fulfillment of the requirements of the degree of Doctor of Philosophy.

(3) (a) University of Illinois; (b) Texas A&M University.

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(8) N. C. Li, R. L. Scruggs, and E. D. Becker, *J. Am. Chem. Soc.*, **84**, 4650 (1962).

(9) P. Tang and N. C. Li, *ibid.*, **86**, 1293 (1964).

structural information. Mathur and Martin[10] investigated the proton nmr spectra of glycine peptides and the diagmagnetic nickel(II)–tetraglycine complex in aqueous D_2O solution.

Experimental Section

Reagents. Sources of the reagents used are the same as those reported earlier.[4,6,11]

Proton Nmr Spectral Measurements. Proton nmr spectra were obtained with a Varian A-60 spectrometer. About 5–10% (w/v) of ligand was dissolved in D_2O. To prepare acidic and basic solutions slightly more than equivalent amounts of DCl or NaOD were added. The methyl hydrogen peak of *t*-butyl alcohol obtained from Eastman Organic Chemicals, Rochester, N. Y., was used as an internal standard by adding a drop to about 0.5 ml of the sample solution. For paramagnetic copper(II) and nickel(II) complexes, small amounts of the metal chloride were added to the ligand solution. The metal ion concentration was varied between 10^{-2} and 10^{-4} *M*. For some nickel complexes of triglycine and tetraglycine, equivalent amounts of metal chloride and base were added to the ligand solution. If precipitation occurred, only the clear solution was transferred in the nmr tube. All spectra were measured at $37 \pm 2°$.

t-Butyl alcohol was chosen as an internal standard, because it is soluble in water, is chemically inert to the ligands, metal ions, or metal complexes under investigation, and has no absorption in the region where methylene hydrogens of the peptide absorbs. The τ value of methyl hydrogens of *t*-butyl alcohol is reported to be 8.80 by Jones, *et al.*[12] This corresponds to 72 cps at 60 Mc, at which all the spectra in the present investigation were measured. The magnetic field was first adjusted with TMS before the spectra of the experimental samples were taken. The methyl hydrogen peak of *t*-butyl alcohol appeared between 70 and 80 cps. This was corrected to 72 cps, and corresponding changes were applied to other peaks.

All hydrogens attached to nitrogen and oxygen atoms of glycine peptides exchange rapidly with deuterium when they are dissolved in D_2O, and absorption peaks are obtained only for methylene hydrogens. Because of this exchange and the small amount (less than 0.2%) of residual H_2O in the D_2O solvent, an HDO peak near 280 cps is always obtained. This peak varies in position, depending on the concentration of solute and on the acidity or basicity of the solution. It shifts its position and starts broadening when more than 10^{-3} *M* metal ion is added.

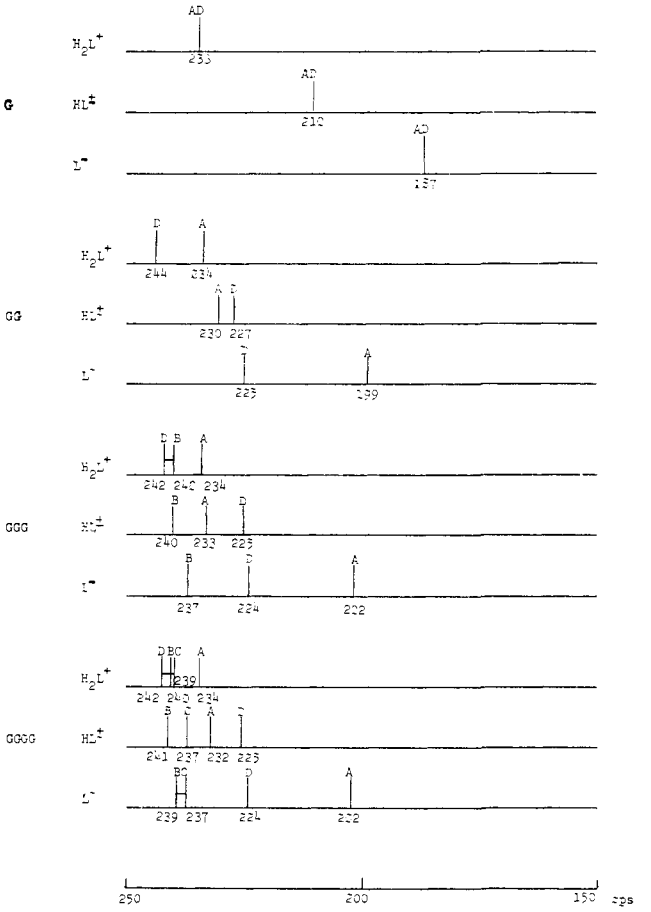

Figure 1. Proton nmr spectra of glycine peptides in aqueous (D_2O) solution: H_2L^+, cationic form; $HL^{\pm}$, dipolar form; L^-, anionic form. Positions of the peaks are given in cps; letters A, B, C, D indicate methylene groups in sequence from amine to carboxyl and of the peptide; G, GG, GGG, and GGGG represent glycine, diglycine, triglycine, and tetraglycine, respectively.

Results

Proton Nmr Spectra of Glycine Peptides. The change of peak position of the methylene hydrogens of glycine peptides as a function of solution basicity is shown schematically in Figure 1. The typical proton nmr spectra of tetraglycine are shown in Figure 2.

Glycine has one peak which shifts to higher field as the basicity increases. Diglycine has two peaks. The peak at lower field in acidic solution shifts to higher field in neutral and basic solution.

Three peaks are observed for triglycine. Among the three peaks found in acidic solution, 242, 240, and 234 cps, the first two are not completely separable. The 234-cps peak remains almost unchanged in neutral solution, but the doublet becomes a single peak and a new peak appears at higher field, 225 cps. As the solution becomes basic, the 234-cps peak shifts to high field and finally has the highest field position.

Tetraglycine has four peaks. Three of them are close together in acidic solution, but all four are distinct in neutral solution. In basic solution two peaks at low field again are not separable. It is interesting to note that for basic solutions the lowest field peak of triglycine and the two lowest field peaks of tetraglycine decrease in height after several hours and are eventually completely replaced by new peaks at 199 and 187 cps.

Proton Nmr Spectra of Metal Complexes. When relatively small amounts of copper(II) or nickel(II) ion are added gradually to solutions of the ligands, the ligand peaks broaden and finally some or all of them disappear. Table I shows the effect of the addition of metal ion on the ligand peaks when the concentration of added metal ion changes from 10^{-4} to 10^{-2} *M*. At metal ion concentrations larger than 10^{-2} *M*, all the peaks listed in Table I broaden or disappear. The observed peaks in basic solutions disappear at lower concentration of metal ion than is necessary to produce the same effect on the spectra of neutral solutions. The spectra of triglycine in the presence of varying concentrations of copper(II) ion in basic solution are shown in Figure 3 (A, B, C).

In the presence of copper(II) or nickel(II) ion, glycine has one peak, which broadens in basic solution as more metal ion is added.

In the presence of the copper(II) ion in neutral solution, diglycine has one peak at low concentration of metal ion, which broadens as more metal ion is added.

(10) R. Mathur and R. B. Martin, *J. Phys. Chem.*, **69**, 668 (1965).
(11) M. K. Kim and A. E. Martell, *J. Am. Chem. Soc.*, **85**, 3080 (1963).
(12) R. A. Y. Jones, A. R. Katritzky, J. N. Murrell, and N. Sheppard, *J. Chem. Soc.*, 2576 (1962).

Table I. The Effect of Cu(II) and Ni(II) Ions on the Proton Nmr Spectra of Glycine Peptides[a]

Metal ion	Ligand	Species present	Nmr peaks affected[b]	Order of disappearance[c]
Cu(II)	G (A)[e]	±	211	(A)[d]
		−	190^{I}	A
	GG (AB)	±	227^{I}	A → D
		−	226^{II}, 200^{I}	A → D
	GGG (ABD)	±	240^{II}, 234^{I}, 225^{II}	A → B,D
		−	238^{II}, 224^{II}, 203^{I}	A → B,D
	GGGG (ABCD)	±	242^{II}, 237^{III}, 225^{I}	A → D → B → C
		−	239^{II}, 238^{III}, 225^{IV}, 202^{I}	A → B → C → D
Ni(II)	G(A)	±	211	(A)[d]
		−	187^{I}	A
	GG (AD)	±	230, 227^{I}	D → (A)
		−	225^{II}, 199^{I}	A → D
	GGG (ABD)	±	240^{II}, 232, 225^{I}	D → B → (A)
		−	237^{II}, 224^{III}, 201^{I}	A → B → D
	GGGG (ABCD)	±	241^{II}, 237^{II}, 232, 225^{I}	D → B,C → (A)
		−	240^{II}, 238^{II}, 225^{III}, 202^{I}	A → B,C → D

[a] T_L = 5–8% (w/v), T_M = 1.0^{-4}–10^{-2} M. [b] Roman numerals indicate the order in which the peaks broaden as metal ion is added. [c] A, B, C, D represent terminal amino, one or more peptide, and carboxylate methylene groups, respectively, proceeding in order along the peptide chain from amino to carboxyl groups. [d] Disappears at higher than 10^{-2} M metal ion concentration.

For basic solutions there are two peaks, the one at high field disappearing first while the other broadens at higher concentrations of metal ion. In case of nickel(II) ion in neutral diglycine solution, two peaks appear close together. The high-field peak starts to broaden at large nickel(II) ion concentration, while the low-field peak remains unchanged.

When copper(II) ion is added to triglycine, three peaks are found. Of these, the 234-cps peak in neutral solution and 203-cps peak in basic solution (Figure 3) disappear first, while the remainder broaden and disappear at higher metal ion concentration. With the nickel(II) ion, the 225- and 240-cps peaks broaden in the order given, while the other peak remains unchanged in neutral solution.

A neutral solution of tetraglycine and copper(II) ion has three peaks at 225, 237, and 242 cps. The 225-cps peak disappears first, and the other two at 242 and 237 cps disappear later as more metal ion is added. Of the four peaks in basic solution, the 202-cps peak disappears first and the remainder follow in the order given. When nickel(II) ion is added, four peaks are found in both neutral and basic solution. For neutral solutions, the 225-cps peak broadens first, and the 241- and 237-cps peaks broaden later, with increasing nickel(II) ion concentration. For basic solutions, the addition of metal ion first causes the disappearance of the 202-cps peak and the other three follow at higher metal ion concentration.

The proton nmr spectra of yellow nickel(II) complexes of triglycine and tetraglycine were measured and are shown in Figures 4A and 4B. Three upfield-shifted peaks are observed for the solution made up of equimolar triglycine and nickel(II) ion and 3 equiv of base. Four peaks also shifted upfield, of which two overlap, are found when 4 equiv of base is added to a solution containing equimolar tetraglycine and nickel(II) ion. Attempts were made to measure spectra when 2 and 4 equiv of base are added to a 1:1 nickel(II)–triglycine solution. Two broad, obscure peaks are obtained in the former case whereas in the latter case two indistinct peaks are obtained. When only 3 equiv of base is added to nickel–tetraglycine solution, two broad peaks are obtained.

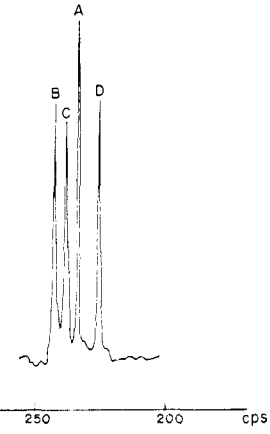

Figure 2. Proton nmr spectra of dipolar (neutral) form of tetraglycine in D_2O; concentration of peptide ≃ 0.13 M; letters refer to assignments in Figure 1.

Discussion

Glycine Peptides. The four different pairs of methylene hydrogens of tetraglycine are assigned letters A, B, C, and D, following the notation of Mathur and Martin.[10] If A is used to represent the pair of methylene hydrogens adjacent to the terminal amino group, D is used for those adjacent to the terminal carboxyl group and the others are labeled in alphabetical sequence. The designations of the methylene groups become: tetraglycine, ABCD; triglycine, ABD; diglycine, AD; and glycine, A (or D).

When the diprotonated form of glycine loses a proton to become a dipolar ion, the A (or D) peak is shifted upfield by 23 cps. The same extent of upfield shift results from the loss of the second proton from glycine.

In the case of diglycine, a larger shift is expected for the D peak than the A peak, in the change from cation

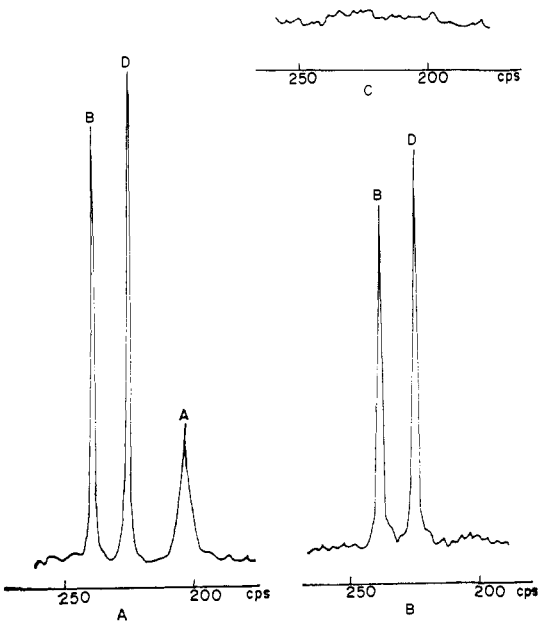

Figure 3. Proton nmr spectra of 0.37 M triglycine in basic D_2O solution in the presence of varying concentrations of copper(II) (A) $[Cu^{2+}] = 4.0 \times 10^{-4}$ M; (B) $[Cu^{2+}] = 2.0 \times 10^{-3}$ M; (C) $[Cu^{2+}] = 8.0 \times 10^{-3}$ M.

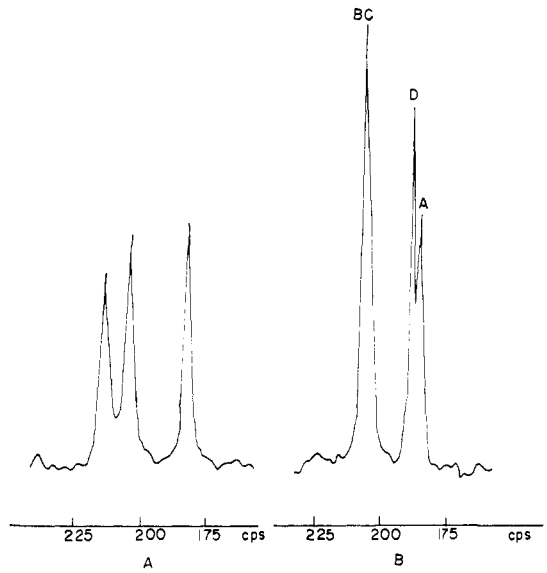

Figure 4. (A) Proton nmr spectrum of 0.41 M Ni(II)–triglycine chelate, $NiH_{-2}L^-$, in D_2O; (B) proton nmr spectrum of 0.16 M Ni(II)–tetraglycine chelate, $NiH_{-3}L^{2-}$, in D_2O.

to dipolar ion, since the D group is nearer to the site of proton dissociation than is A. The reverse is expected for the change from dipolar ion to the anionic form. Accordingly, of the two anion resonances, at 199 and 225 cps, the 199-cps absorption is assigned to the A group, and the 225-cps is assigned to the D group, since this assigns the greater shift from either of the dipolar resonances to the A group nearer the terminal amino group. In the same way, the shift from the 244-cps absorption of the cation to either the 230- or 227-cps absorption of the dipolar ion is larger than the shift from the 234-cps absorption, so that assignment of the 244-cps resonance to D and the 234-cps to A seems justified. The 230- and 227-cps peaks of the dipolar ion can be assigned to either A and D or D and A, but assigning the former to A and the latter to D is more reasonable, as indicated in Table II. The suggested assignment seems more logical, since it gives a shift more comparable with the analogous glycine shift on dissociation of the carboxyl group.

Table II. Diglycine Nmr Spectral Shifts as a Function of Ionic Charge

	Cation	Δ	Dipole	Δ	Anion
		Diglycine Assignments			
A	234	−4	230	−31	199
D	244	−17	227	−2	225
		Alternate Dipolar Assignments			
A	234	−7	227	−28	199
D	244	−14	230	−5	225

The three peaks of triglycine and four peaks of tetraglycine can be assigned in an analogous way. The assignments of all peaks are tabulated in Table III.

Table III. Proton Nmr Spectra of Glycine Peptides in Aqueous Solution at 60 Mc[a]

Peptide	Ionic form	Methylene hydrogen peaks, cps			
G	H_2L^+	233 (A)			
	$HL^\pm$	210 (A)			
	L^-	187 (A)			
GG	H_2L^+	244 (D)	234 (A)		
	$HL^\pm$	230 (A)	227 (D)		
	L^-	225 (D)	199 (A)		
GGG	H_2L^+	242 (D)	240 (B)	234 (A)	
	$HL^\pm$	240 (B)	233 (A)	225 (D)	
	L^-	237 (B)	224 (D)	202 (A)	
GGGG	H_2L^+	242 (D)	240 (B)	239 (C)	234 (A)
	$HL^\pm$	241 (B)	237 (C)	232 (A)	225 (D)
	L^-	239 (B)	237 (C)	224 (D)	202 (A)

[a] Internal standard, t-butyl alcohol.

It is interesting to note that in triglycine the B peak undergoes little change throughout all ionic forms, while the large shifts of the D peak from cation to dipolar ion, and of the A peak from dipolar ion to anion, correspond to the manner in which the peptide dissociates. The assignments of B and C peaks of tetraglycine may very well be reversed since the B and C methylene groups are away from the dissociation site of peptides, and there is very little difference between them.

The proton nmr spectra of the ligand species studied by Mathur and Martin[10] and by Morlino and Martin[13] are in good agreement with the results of the present investigation. When their values, reported in parts per million with respect to acetonitrile as internal standard, are converted to cps with the τ value of acetonitrile of 7.98,[2] the difference between their values and the present work was less than 3 cps for all peaks. However, the present assignments of the B and C peaks of the tetraglycine anion are reversed from those given by Mathur and Martin.[10] Probably because of its low solubility,

(13) V. J. Morlino and R. B. Martin, *J. Am. Chem. Soc.*, **89**, 3107 (1967).

the spectra of the tetraglycine dipolar ion was not studied by Mathur and Martin. In the present investigation, the spectra were obtained with the very low concentration of 3%. The gradual disappearance of 237-cps peak of triglycine anion and 239- and 237-cps peaks of tetraglycine anion was also observed by Mathur and Martin and was explained as exchange of B and C methylene hydrogens with deuterium. Since after complete disappearance of these peaks, new peaks appear at 199 and 187 cps, where glycine and diglycine have A-type resonance, it is likely that the exchange of B and C methylene hydrogens with deuterium is accompanied by hydrolysis of the adjacent peptide linkages. Further studies are needed to establish the mechanism of this reaction.

Metal–Glycine Peptide Complexes. Li and coworkers[7–9] indicated that the presence of a paramagnetic metal ion broadens and finally flattens the proton nmr peak of the group adjacent to the binding site, because its relaxation time is decreased by the magnetic field of metal ion, and exchange occurs between free and coordinated ligands. On the other hand, a diamagnetic metal ion produces downfield or upfield shifts, the extent of which is dependent upon the strength of binding. Their findings that for diglycine and triglycine the peaks of the methylene group adjacent to the terminal amino group (*i.e.*, "A" peaks) are affected to a greater extent than the other peak(s) by the presence of paramagnetic copper(II) or cobalt(II) ion was taken as evidence that the terminal amino and peptide groups, rather than carboxylate group, are the preferred binding sites for these metal ions.

In the present investigation, the order of broadening or disappearance of ligand peaks with increasing concentration of paramagnetic copper(II) or nickel(II) ion suggests the manner in which metal ion coordinates to the ligand and the sequence of coordination under changing conditions. As will be shown below for individual complexes, it is interesting that the presence of strong coordinate bonds between the metal ion and amino and/or peptide nitrogens is revealed by the fact that the A peaks are the first to disappear in the case of copper(II), both in neutral and in basic solutions, and in the case of nickel(II) in basic solutions. The nickel(II) ion in neutral solution gives somewhat different results from the other cases in that the influence of the metal ion on the D peak is always larger than on the A peak. This is not surprising if the difference in the behavior of nickel(II) and copper(II) ions toward the ligand is considered. The potentiometric titration curves of the 1:1 solutions at "*a*" values between 0 and 1 show that the coordination of the amino group to the nickel(II) ion is much weaker than to the copper(II) ion, prior to displacement of protons from the peptide linkages.[4–6] On the other hand, it is probable that the negatively charged carboxylate group of the dipolar ligand species can bind to the positively charged nickel(II) ion through a coordinate bond of predominantly ionic character. Carboxylate binding of peptides by metal ions would not be detected in the pH range 4–8 by potentiometric measurements of hydrogen ion concentration. The order of disappearance of individual peaks is included in the fifth column of Table I by using the peak assignment made in Table III.

As implied previously[6] in the case of triglycine and tetraglycine, the coordination sphere of nickel(II) undergoes a configurational change from octahedral to planar when protons are displaced simultaneously from all the peptide groups, and very strong metal–nitrogen bonds are formed. Martin, *et al.*,[14] postulated a transition of the nickel(II) coordination sphere from an octahedral to a planar configuration in order to explain their potentiometric titration results and the visible color changes of the nickel(II)–tetraglycine system. Recently Mathur and Martin[10] obtained the proton nmr spectra of diamagnetic nickel(II)-tetraglycine solution where 4 equiv of alkali was added. The present investigation also provides evidence for changes in configuration of the nickel(II)–triglycine and –tetraglycine systems. The proton nmr spectra of nickel(II)–triglycine and nickel(II)–tetraglycine illustrated in Figures 4A and 4B show that these complexes are diamagnetic, since the ligand peaks remain sharp and are shifted upfield in the complex when the solution is sufficiently alkaline to ensure complete displacement of peptide protons by the metal ion.

Copper(II) Complexes. The evidence obtained about coordinate bonding in copper(II) complexes of diglycine[4] is confirmed by the proton nmr spectra shown in Table I. The A peak always disappears at metal concentrations lower than that required for the D peak. As already pointed out by Li, *et al.*,[8] this behavior shows that the strongest metal–ligand bonding occurs with the nitrogen atoms of amino and peptide groups. The results of this investigation are in good agreement with the observations of Li and coworkers[8] that the diglycine D peak disappears in the presence of 10^{-4} *M* Cu(II) ion under alkaline conditions, corresponding to the formation of $CuH_{-1}L$.

The proton nmr spectra of copper(II) complexes of triglycine show that the peaks disappear in the order A → B, D in both neutral and basic solutions. As is clearly indicated in parts A and B of Figure 3, the involvement of the terminal amino group in metal coordination again causes the A peak to be more strongly influenced by the metal ion than are the other two peaks. It is, however, interesting to note that the D peak is affected as much as is the B peak in neutral or basic solutions, contrary to what is found for the other cases listed in Table I. The simultaneous disappearance of B and D peaks in basic solution can be explained in the following way. The four functional groups of triglycine (*i.e.*, one amino group, two peptide groups, and one carboxylate group) probably occupy the four corners of the planar copper(II) coordination sphere in the 1:1 chelate at high pH. In the formation of a complex of this type, the barrier for peptide binding, the displacement of the peptide proton, is greater than that for coordination by the completely dissociated carboxylate group. Since the formation of the stable fused chelate ring system required peptide nitrogen coordination as the first step, it is seen that the B- and D-type resonances would disappear simultaneously.

On the other hand, comparison with the proton nmr spectra of tetraglycine in the presence of copper(II) ion shows that the simultaneous disappearance of B and D peaks of triglycine in neutral solution may be due to the fact that the negatively charged carboxylate group and

(14) R. B. Martin, M. Chamberlin, and J. T. Edsall, *J. Am. Chem. Soc.*, **82**, 495 (1960).

the terminal amino group coordinate readily to the positively charged metal ion whereas the metal–peptide coordination before proton displacement is very weak. Strong interaction with the terminal amino group might conceivably influence the B methylene group about as much as somewhat weaker binding to a carboxylate oxygen would affect the D methylene group. The use of molecular models shows that strong coordination of the terminal amino group and the adjacent peptide linkage, and weak coordination of the other peptide group, would not allow coordination of the terminal carboxylate group with the same metal ion. Therefore, if this explanation applies to the effects observed in neutral solution, carboxylate bonding must occur with other metal ions to form polynuclear species. To explain the observed effect it is not necessary to have more than traces of these polynuclear species in solution.

Li and his coworkers[3] also noted the disappearance of A peak at copper(II) concentration of 10^{-4} *M* but made no further study of the disappearance of B and D peaks.

In the case of copper(II)–tetraglycine complexes, the order of disappearance of peaks in basic solution, A → B → C → D, indicates the relative order of formation of coordinate bonds, the metal–amino nitrogen bond forming first, followed by formation of the metal–peptide nitrogen (negative) bonds. In neutral solution the D peak is more strongly affected than are the B and C peaks. This indicates that the negatively charged carboxylate group of the dipolar ion combines readily with the positively charged metal ion. The metal–peptide bonds are relatively very weak in this pH range since displacement induced by copper(II) ion has not yet occurred. Here again an explanation may be found in coordination of terminal amino and carboxylate groups to different metal ions, as suggested above for triglycine. The sequence of tetraglycine donor groups coordinated by Cu(II) ion is terminal amino, carboxylate, negative peptide.

Nickel(II) Complexes. From the potentiometric titrations and aqueous infrared spectral studies,[6] there appears to be some extent of association of the protonated peptide linkage of diglycine with the nickel(II) ion, though the interaction is not so strong as in the copper(II) case. This is supported by the proton nmr spectra of this system, for which both A and D peaks are affected in basic solution. The relative weakness of the coordinate bonds between nickel(II) ion and diglycine is shown by the fact that in neutral solution the A peak remains unchanged at nickel(II) ion concentrations up to 10^{-2} *M*. The change in the D peak under the same conditions is probably due to weak association between the Ni(II) ion and the completely dissociated carboxylate group, even though the degree of formation of the complex may be very low.

The proton nmr spectra of triglycine in the presence of small amounts of nickel(II) ion give different orders of peak disappearance: D → B → (A) in neutral solution and A → B → D in basic solution. As in the case of diglycine, the A peak remains unchanged in neutral solution even at metal ion concentrations as high as $\sim 10^{-2}$ *M*. This corresponds to weak coordination to the ligand at low pH as shown by the titration curve, which is not much below the free ligand curve in the region between $A = 0$ and $a = 1.0$.[6] The effect observed for the D peak again indicates carboxylate group coordination. In basic solution, however, competition with the hydrogen ion for the amino group is greatly decreased so that the coordination tendency of the metal ion toward the amino group finally predominates, and the A peak is the first to disappear.

When the complex ML^+ (where L^- is the triglycine anion, $NH_2CH_2CONHCH_2CONHCH_2COO^-$) loses two protons from its peptide nitrogens, the resulting metal–peptide chelate becomes quite stable, and the coordinative interactions between the metal ion and ligand donor groups are quite strong. These four strong coordinate bonds, a metal–amino nitrogen, a metal–carboxylate oxygen, and two metal–negative peptide nitrogen bonds, are probably arranged around the nickel(II) ion in a planar fashion. The original octahedral configuration of the nickel(II) coordinate bond is distorted under the influence of the planar donor groups, and the structure of the resulting chelate, $NiH_{-2}L^-$, is either planar or tetragonal. Such a structural change is indicated by the potentiometric titration data, whereby the reaction with added base was observed to become instantaneous at "*a*" values larger than 1.0, in contrast to the slow rates observed at "*a*" between 0 and 1.0.[6] Also, the dissociation of the second and third protons in the presence of the metal ion was observed to occur simultaneously, indicating the formation of a very stable complex. Martin[15] reported that the magnetic susceptibility of solid yellow nickel(II)–triglycine complex, obtained from a solution containing a 1:1:3 molar ratio of nickel(II) chloride, triglycine, and sodium hydroxide, is about 1.4 BM, much smaller than the 2.8 BM usually obtained with paramagnetic octahedral nickel(II) complexes. However, the proton nmr spectrum shown in Figure 4A provides direct proof that the aqueous complex under the reaction conditions employed is diamagnetic, and is probably planar or tetragonal. All three methylene peaks shift upfield. Since the proton nmr spectrum of Figures 4A still shows a small degree of broadening compared to the spectra of the analogous nickel(II)–tetraglycine complex, $NiH_{-3}L^{2-}$, it is possible that the diamagnetic complex is in equilibrium with a trace of paramagnetic species in solution. The fact that Martin's[15] value for the magnetic susceptibility was not zero or nearly zero, together with the observation by Mathur and Martin[10] that they were unable to obtain proton nmr spectra of this nickel(II)–triglycine chelate in solution, indicates that their conditions were not exactly right for the formation of the diagmagnetic chelate in the absence of significant concentrations of exchangeable paramagnetic forms. In this connection it is interesting to note that the proton nmr spectra of the Ni(II)–triglycine system at "*a*" values smaller than 3.0 do not show well-defined peaks because of the presence of paramagnetic chelate species. At "*a*" values above 3.0 the nmr peaks were broadened considerably, probably because of exchange of methylene hydrogens with deuterium in strongly basic solution.

The effect of a small amount of nickel(II) ion on the proton nmr spectra of tetraglycine in neutral and acid solutions is similar to the effect of this metal ion on the proton nmr spectra of triglycine. In neutral solution

(15) R. B. Martin, *Federation Proc. Suppl.*, 10, 54 (1961).

the order is D → B,C → (A). The fact that the A peak is not affected at metal ion concentration as high as $\sim 10^{-2}$ *M* again indicates the strong competition between the nickel(II) ion and the proton for the basic amino group in acid and neutral solution. At higher pH, however, there is less hydrogen ion competition, and coordination of metal ion to the deprotonated amino nitrogen and to the deprotonated peptide nitrogen atoms predominates over coordination of the metal ion to carboxylate oxygen, as is shown by the order of peak disappearance, A → B, C → D.

The weak carboxylate-coordinated species, ML^+, of the type detected above for Ni(II)–peptide compl x s in acidic and neutral solutions may also involve coordination (chelation) to the adjacant peptide carbonyl oxygen. Since amide carbonyl groups are very weak donors, this type of chelate ring would be very weak and very easily broken.

The proton nmr spectrum obtained at $a = 4$ (Figure 4B) indicates the diamagnetism and square-planar structure of the species having the formula $NiH_{-3}L^{2-}$, in which all the peptide protons are dissociated. The large double peak can be considered as composed of the B and C peaks, which arise from very similar molecular environments. The other two peaks at 187 and 185 cps are assigned to the A and D methylene groups. Although there is very little basis on which to make a choice between them, the 185-cps peak is assigned to the A methylene group, and the 187-cps peak is assigned to the D group.

Mathur and Martin[10] obtained the proton nmr spectra of the nickel(III) complex, $NiH_{-3}L^{2-}$, from the same experimental solutions and under the same conditions as were used in the present investigation, and their observations of the number of peaks, peak positions, and the assignment of B and C peaks are the same as in the present investigation. The broad peaks obtained in the present investigation for the proton nmr spectra at $a = 3$, and the difference between these spectra and those obtained at $a = 4$, again confirm that the three peptide protons are dissociated in one step and that complete conversion to a planar diamagnetic configuration occurs at $a = 4$.