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Nuclear Magnetic Resonance Studies of the Solution Chemistry of Metal Complexes. V. Cadmium, Zinc, and Lead Complexes of Polyglycine Peptides[1,2]

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The binding of cadmium, zinc, and lead ions by diglycine, triglycine, and tetraglycine in aqueous solution has been investigated by proton magnetic resonance spectroscopy. The sites of metal binding depend on solution pH. At pH <4, each of the metal ions studied binds to the C-terminal end of the polyglycine peptide while the amino nitrogen is still protonated. At higher pH, binding is to both the C-terminal end and the N-terminal end. From chemical shift measurements, formation constants were determined for binding of each of the metal ions at the C-terminal end and at the N-terminal end of each of the polyglycine peptides studied. Probable donor groups involved in binding at the two ends are discussed. It is proposed that at pH >4 polynuclear complexes are formed with triglycine and tetraglycine through binding of the two ends of the peptide to different metal ions. The rates of exchange of the peptide protons of triglycine with solvent protons have been measured in metal-containing solutions. The peptide protons exchange more rapidly when the triglycine is complexed by cadmium, zinc, and lead.

Introduction

Considerable interest has been shown in the binding of metal ions by amino acids and peptides as models for their interaction with proteins. Binding of divalent cadmium, zinc, and lead by short-chain polyglycine peptides has been characterized previously in terms of formation constants, determined primarily by the pH titration method.[4-7] The complexes detected and characterized by this method involve complexation at the amino group of the N-terminal glycine residue. Simultaneous binding through a donor atom of the neighboring peptide linkage has also been proposed.[7-9] Coordination to the carboxylic acid group of the C-terminal glycine residue has not been characterized, however, presumably because the complexes are weak and difficult to detect by the pH titration method.

Coordination of the C-terminal end of polypeptides to these metal ions is of interest in view of the biological activity of carboxypeptidase enzymes. Starting from the C-terminal end, these enzymes digest polypeptide chains to give the constituent amino acids.[10,11] They also catalyze the hydrolysis of esters. Their catalytic activity is attributed to increasing the reactivity of the peptide linkage toward hydrolysis through binding of the C-terminal end of the peptide to the zinc atom at the active site of the enzyme. Replacement of zinc by cadmium, lead, or mercury results in complete loss of catalytic activity toward cleavage of the C-terminal amino acid residue although the resulting enzymes still catalyze the hydrolysis of esters.[12-14]

In the present paper, results of a proton magnetic resonance study of the complexation of cadmium, zinc, and lead by diglycine, triglycine, and tetraglycine are reported. These systems have been investigated in an effort to elucidate the binding of these metal ions by simple peptides. Nmr is sensitive at the molecular level to coordination and, thus, can provide information on metal binding at each of the potential coordination sites. From the dependence of the chemical shifts of the methylene protons of the polyglycine peptides on solution conditions, the complexes formed in different pH regions have been identified and their formation constants have been determined. Also, the rates of exchange of the peptide protons of triglycine with solvent protons have been measured for both free and complexed triglycine to provide information on the effect of coordination on the reactivity of the peptide linkages.

Experimental Section

Chemicals.—Diglycine, triglycine, and tetraglycine (Nutritional Biochemicals Corp.) and reagent grade metal nitrate salts were used as received. A stock tetramethylammonium (TMA) nitrate solution was prepared by titration of a 25% aqueous solution of TMA hydroxide (Eastman Organic Chemicals) with HNO_3 to a neutral pH.

pH Measurements.—All pH measurements were made at 25° with an Orion Model 801 pH meter equipped with a standard glass electrode and a fiber-tip, saturated calomel reference electrode. Saturated potassium acid tartrate and 0.01 *M* sodium tetraborate buffers, pH 3.56 and 9.18 at 25°, were used to standardize the pH meter.

Nmr Measurements.—Nmr spectra were obtained on a Varian A-60-D high-resolution spectrometer at a probe temperature of 25 ± 1°. Spectra were recorded at sweep rates of 0.1 or 0.2 Hz/sec for both chemical shift and line-shape measurements; sweep widths of 50 or 100 Hz were used. For line-shape measurements, each spectrum was recorded several times and the individual spectra were averaged.

Solutions used in the nmr studies were prepared in triply distilled water from the requisite amounts of crystalline metal nitrate and peptide. KNO_3 was added to solutions containing only peptide to give an ionic strength comparable to that of solutions containing a metal nitrate. HNO_3 or KOH was added to bring the solutions to the desired pH's. TMA nitrate was added to each solution as a reference compound. Chemical shift measurements are reported relative to the central resonance of the TMA triplet. The central resonance of TMA is 3.17 ppm downfield from the methyl resonance of sodium 3-(trimethylsilyl)-1-propanesulfonic acid (TMS*).

Results

Interpretation of the Spectra.—The chemical shifts

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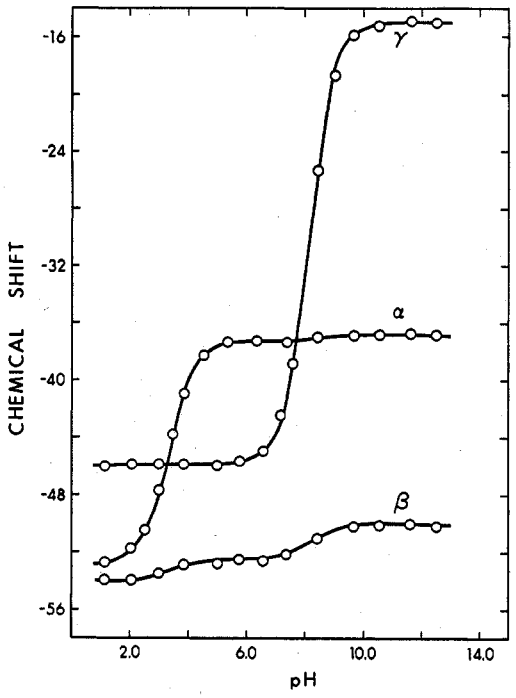

Figure 1.—pH dependence of the chemical shifts of the methylene protons of triglycine. Proton assignment: $^{+}NH_3CH_{2(\gamma)}CONHCH_{2(\beta)}CONHCH_{2(\alpha)}CO_2^{-}$. The smooth curves are the theoretical chemical shift behavior predicted by the acid ionization constants determined in this work and the chemical shifts listed in Table I. Conditions: 0.20 *M* triglycine, 0.60 *M* KNO_3, 25°. Chemical shifts are in hertz (measured at 60 MHz) relative to the central resonance of TMA; negative shifts are downfield from TMA.

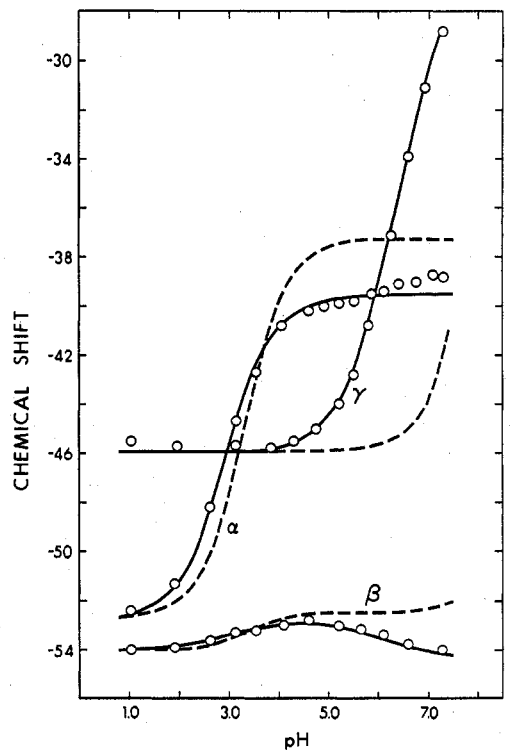

Figure 2.—pH dependence of the chemical shifts of the methylene protons of 0.20 *M* triglycine in the presence of 0.20 *M* $Cd(NO_3)_2$. Proton assignments are as in Figure 1. The smooth curves are the theoretical chemical shift behavior predicted by the equilibrium constants determined in this work and the chemical shifts in Table I; in this prediction it is assumed that coordination at the amino end does not change the concentration of carboxylate-coordinated complex. The dashed curves are the chemical shift behavior of 0.20 *M* triglycine in the presence of 0.60 *M* KNO_3 (25°).

and shapes of the resonances from the methylene protons of diglycine, triglycine, and tetraglycine are pH dependent and metal ion dependent in aqueous solution. Only the interpretation of the spectra for the triglycine system will be described in detail since the diglycine and tetraglycine systems show similar results.

Following the notation of Sheinblatt,[15] the peptide and methylene groups of triglycine are labeled as follows

$$\bar{O}_2CCH_2NHCOCH_2NHCOCH_2\overset{+}{N}H_3$$

peptide group: α β

methylene group: α β γ

Resonances for the α- and β-methylene protons are doublets or singlets, depending on the rate of exchange of the α- or β-peptide protons with solvent protons.[15] The peptide proton–methylene proton coupling constant in all of the systems studied was 5.9 Hz. The resonance for the γ-methylene protons is a single line except at pH <0 where exchange of the amino protons is slow.

The pH dependence of the chemical shifts of the methylene protons, which is due to ionization of the acidic protons, is shown in Figure 1. At pH 1, the amino and carboxylate groups are both protonated. As the pH is increased from 1 to 6, ionization of the carboxylic acid proton to give zwitterionic triglycine results in a large upfield shift for the adjacent α-methylene protons and a much smaller upfield shift for the β-methylene protons. The chemical shift of the γ-methylene protons is not changed within experimental error. A further increase in pH from 6 to 11 ionizes the amino proton which causes a large upfield shift for the adjacent γ-methylene protons and smaller upfield shifts for the β- and α-methylene protons. From pH 1 to 11, averaged resonances are observed rather than separate resonances for each of the species present indicating exchange of the acidic protons is rapid on the nmr time scale.[16,17]

Addition of an equimolar concentration of cadmium nitrate, zinc nitrate, or lead nitrate changes the pH dependence of the chemical shifts of the methylene protons, as shown in Figures 2–4. The shift of the α-methylene resonance accompanying ionization of the carboxylic acid proton occurs over a lower pH range indicating displacement of the carboxylic acid proton by the metal. Also, the chemical shift after the carboxylic acid is completely deprotonated is different in the presence of the metal ions indicating different chemical shifts in the complexed and uncomplexed triglycine. The chemical shift of the γ-methylene protons is pH independent up to approximately pH 4 in the presence of each of the metal ions, at which pH ionization of the carboxylic acic proton is almost complete. As the pH is increased beyond pH 4, the chemical shifts of the γ- and β-methylene protons change indicating the metal ions are displacing the amino

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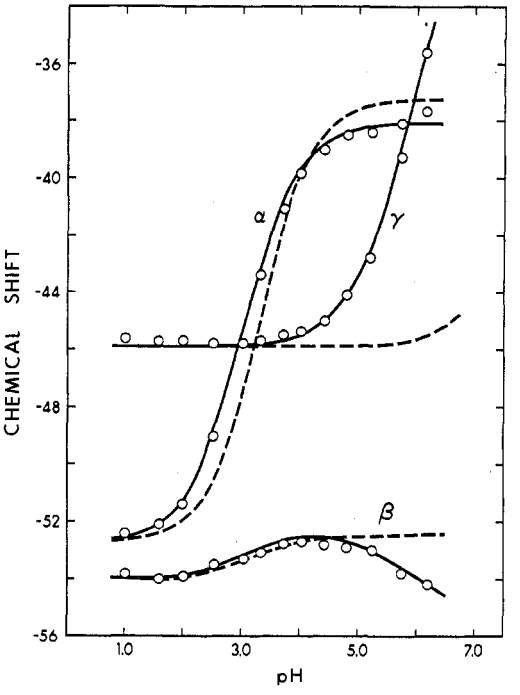

Figure 3.—pH dependence of the chemical shifts of the methylene protons of 0.20 *M* triglycine in the presence of 0.20 *M* $Zn(NO_3)_2$. Proton assignments as in Figure 1. The smooth curves are the theoretical chemical shift behavior predicted by the equilibrium constants determined in this work and the chemical shifts in Table I. The dashed curves are the chemical shift behavior of 0.20 *M* triglycine in the presence of 0.60 *M* KNO_3 (25°).

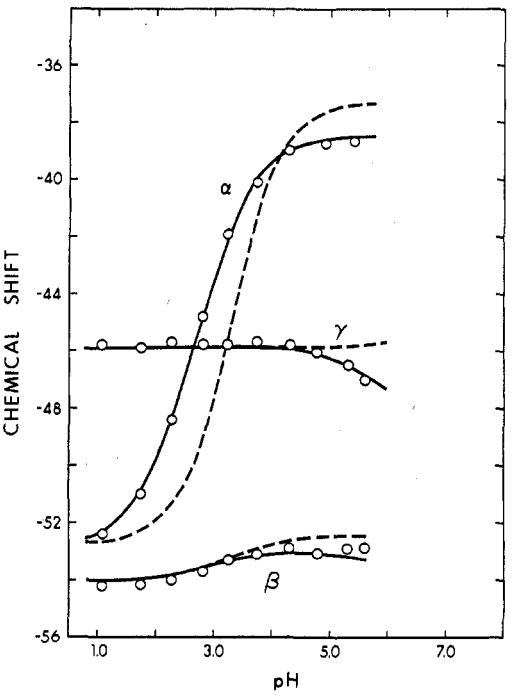

Figure 4.—pH dependence of the chemical shifts of the methylene protons of 0.20 *M* triglycine in the presence of 0.20 *M* $Pb(NO_3)_2$. Proton assignments as in Figure 1. The smooth curves are the theoretical chemical shift behavior predicted by the equilibrium constants determined in this work and the chemical shifts in Table I. The dashed curves are the chemical shift behavior of 0.20 *M* triglycine in the presence of 0.60 *M* KNO_3 (25°).

protons and binding at the amino end of the peptide chain. In the presence of cadmium and zinc, the resonances for the γ-methylene protons shift upfield as the pH is increased whereas lead causes them to shift downfield. With all three metal systems, the resonances for the β-methylene protons shift downfield upon complexation at the amino end. Rapid exchange of triglycine between the free and complexed forms results in averaged resonances for each of the methylene groups.

Owing to precipitation, it was not possible to reach pH's at which the chemical shift of the γ-methylene protons leveled off. Presumably the precipitates are the metal hydroxides. The highest pH's reached before precipitation for solutions containing 0.20 *M* metal nitrate and 0.20 *M* triglycine were 7.3, 6.3, and 5.6 for the cadmium, zinc, and lead systems.

Complexation of each of these metals also causes a decrease in the pH at which the doublet patterns for the α- and β-methylene resonances begin to collapse to single lines, indicating the rate of peptide proton exchange is faster when triglycine is complexed. Because of precipitation, it was not possible to observe complete collapse of the resonances for the α-peptide protons to single lines.

Determination of the Acid Ionization Constants.—Observed chemical shifts in the pH regions over which the carboxylic acid proton and the amino proton are ionized are the sums of the chemical shifts of the protonated and ionized forms, weighted according to the relative concentrations of each species.[17] The chemical shifts of the methylene groups of the peptides in their various protonated forms, obtained from data similar to those in Figure 1, are listed in Table I. Using methods previously described,[17] the acid ionization constants obtained from the chemical shift data are $K_{a1} = 7.42 \times 10^{-4}$ and $K_{a2} = 6.17 \times 10^{-9}$ for diglycine, $K_{a1} = 5.01 \times 10^{-4}$ and $K_{a2} = 7.94 \times 10^{-9}$ for triglycine, and $K_{a1} = 4.90 \times 10^{-4}$ and $K_{a2} = 8.91 \times 10^{-9}$ for tetraglycine where K_{a1} and K_{a2} are defined as

$$K_{a1} = \frac{[H^+][HL^\pm]}{[H_2L^+]} \quad (1)$$

$$K_{a2} = \frac{[H^+][L^-]}{[HL^\pm]} \quad (2)$$

$HL^\pm$ is the zwitterionic form of the peptide. In each case, the ionic strength of the solution was approximately 0.80. For comparison, literature values for triglycine at an ionic strength of 0.16 are $K_{a1} = 5.01 \times 10^{-4}$ and $K_{a2} = 1.10 \times 10^{-8}$.[18]

Determination of the Formation Constants.—Chemical shift data in Figures 2–4 for the α- and γ-methylene protons of triglycine indicate that cadmium, zinc, and lead bind to the C-terminal end of the peptide in the pH range 1–4 while the amino group remains fully protonated. Similar results were obtained for the diglycine and tetraglycine systems. When some complex is formed, the observed chemical shift is the sum of the chemical shifts of the various species present, weighted according to the relative concentration of each species, from which the formation constants can be evaluated. Using methods previously described,[17b,19]

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TABLE I

SUMMARY OF THE CHEMICAL SHIFTS OF THE METHYLENE GROUPS OF THE POLYGLYCINE PEPTIDES[a,b]

Species	Diglycine[c]		Triglycine[d]			Tetraglycine[e]			
	α	β	α	β	γ	α	β	γ	δ
H_2L^+	0.930	0.755	0.879	0.900	0.765	0.873	0.856	0.905	0.765
$HL^{\pm}$	0.654	0.710	0.622	0.875	0.765	0.616	0.830	0.896	0.765
L^-	0.627	0.210	0.615	0.836	0.252	0.615	0.827	0.864	0.250
Cd^+–$O_2CCH_2NH(COCH_2NH)_xCOCH_2\overset{+}{N}H_3$	0.730		0.696	0.890	0.765	0.687		0.900	0.762
$^-O_2CCH_2NH(COCH_2NH)_xCOCH_2NH_2$–$Cd^{2+}$ [f]		0.416	0.615	0.912	0.420	0.614	0.830		0.443
Zn^+–$O_2CCH_2NH(COCH_2NH)_xCOCH_2\overset{+}{N}H_3$	0.717		0.653	0.870	0.765	0.660		0.904	0.762
$^-O_2CCH_2NH(COCH_2NH)_xCOCH_2NH_2$–$Zn^{2+}$ [f]		0.416	0.615	0.928	0.446	0.614	0.830		0.450
Pb^+–$O_2CCH_2NH(COCH_2NH)_xCOCH_2\overset{+}{N}H_3$	0.697		0.653	0.890	0.765	0.649		0.904	0.765
$^-O_2CCH_2NH(COCH_2NH)_xCOCH_2NH_2$–$Pb^{2+}$ [f]		~0.82	0.615	0.900	0.823	0.614			0.807

[a] At 25°. [b] In ppm relative to the central resonance of TMA. Positive shifts are downfield from TMA. [c] $^-O_2CCH_{2(\alpha)}NHCOCH_{2(\beta)}NH_3^+$. [d] $^-O_2CCH_{2(\alpha)}NHCOCH_{2(\beta)}NHCOCH_{2(\gamma)}NH_3^+$. [e] $^-O_2CCH_{2(\alpha)}NHCOCH_{2(\beta)}NHCOCH_{2(\gamma)}NHCOCH_{2(\delta)}NH_3^+$. [f] There is probably some coordination to the carboxylate end also; see text for a more complete description of the amino-coordinated complex.

TABLE II

FORMATION CONSTANTS OF THE CADMIUM, ZINC, AND LEAD COMPLEXES OF DIGLYCINE, TRIGLYCINE, AND TETRAGLYCINE[a]

Metal	Diglycine			Triglycine			Tetraglycine		
	Log K_f^O [b]	Log K_f^N This work[b]	Log K_f^N Lit.	Log K_f^O [b]	Log K_f^N This work[b]	Log K_f^N Lit.	Log K_f^O [b]	Log K_f^N This work[b]	Log K_f^N Lit.
Cd	1.04 ± 0.18[c]	2.76 ± 0.17	2.95[d]	1.03 ± 0.14	2.69 ± 0.12	2.70[d]	1.14 ± 0.13	2.71 ± 0.03	2.70[d]
Zn	0.64 ± 0.24	3.13 ± 0.09	3.6[e]	0.89 ± 0.10	3.10 ± 0.04	3.18[d]	0.75 ± 0.15	2.96 ± 0.07	3.18[d]
Pb	1.30 ± 0.17	~3.0	3.23[f]	1.44 ± 0.15	3.00 ± 0.24	3.02[g]	1.40 ± 0.16	3.00 ± 0.03	3.02[g]

[a] At 25°. [b] Ionic strength ~0.8. [c] Uncertainties represent the range of experimental values. [d] Ionic strength 0.15 (KNO_3).[7] [e] Ionic strength 0.01.[6] [f] Ionic strength →0.4 [g] Ionic strength ~0.5

the formation constants of the carboxylate coordinated complexes, defined as

$$M^{2+} + HL^{\pm} \rightleftharpoons MHL^{2+}; \quad K_f^O = \frac{[MHL^{2+}]}{[M^{2+}][HL^{\pm}]} \qquad (3)$$

were determined from chemical shift data for the α-methylene protons. Values obtained for K_f^O are listed in Table II.

Chemical shifts of the different methylene protons in the carboxylate-coordinated complexes are listed in Table I. Chemical shifts of the α-methylene protons were obtained directly from the chemical shift *vs.* pH curves; the chemical shift of the α-methylene protons in each of the carboxylate-coordinated complexes is the chemical shift at which the curve for the α-methylene protons of the free peptide (indicated by the dashed lines in Figures 2–4 for triglycine) intersects the curve for the peptide solution containing metal nitrate.[17b] Chemical shifts of the other methylene protons in the carboxylate-coordinated complexes were determined from the averaged chemical shifts in the presence of the metal nitrate and the concentrations of each species present. Concentrations were calculated with the formation constants in Table II.

At pH's greater than 4, the chemical shift of the methylene protons adjacent to the amino group (*e.g.*, the γ-methylene protons of triglycine) changes when the solution contains a metal nitrate indicating complexation at the amino end. The chemical shifts of the α-methylene protons indicate that at pH >4 there is still some carboxylate coordination. As discussed later, it is considered unlikely that the carboxylate end and the amino end of a triglycine or tetraglycine molecule are simultaneously coordinated to the same metal ion, when the metal ion is cadmium, zinc, or lead. However, coordination of the amino end of a carboxylate-coordinated peptide molecule to a second metal ion and coordination of a carboxylate-coordinated metal ion to the amino end of a second peptide molecule could presumably take place. Considering this to be the case, complexation to the amino end is represented as

$$M' + L' \rightleftharpoons ML; \quad K_f^N = [ML]/[M'][L'] \qquad (4)$$

where [M′] is the sum of the concentrations of totally aquated metal ion and carboxylate-coordinated metal ion, [L′] is the sum of the concentrations of free and carboxylate-coordinated peptide, both having the amino proton ionized, and [ML] is the concentration of peptide coordinated at the amino end. Some coordination of hydroxide by the metal ions may also be occurring, particularly at the highest pH's attainable before precipitation; however the presence of such coordination could not be detected from the nmr data.

Formation constants for complexation at the amino end were determined from chemical shift data for the methylene protons adjacent to the amino group. The observed chemical shift for these protons is the weighted average of the shifts of the species present, as given by

$$\nu_{obsd} = P_{L''}\nu_{L''} + P_{ML}\nu_{ML} \qquad (5)$$

where L″ represents the sum of the concentrations of free and carboxylate-coordinated peptide and ML is defined as above. P represents the mole fraction of peptide present as the species indicated ($1 = P_{L''} + P_{ML}$). Combination of eq 5 and the equation for mole fractions leads to

$$P_{ML} = \frac{\nu_{obsd} - \nu_{L''}}{\nu_{ML} - \nu_{L''}} \qquad (6)$$

The concentrations used to calculate the formation constants were obtained from these relationships. The concentration of ML equals $P_{ML}[L_t]$. The concentrations of M′ and L″ were calculated from the total metal and peptide concentrations by difference. The concentration of L′ equals $\alpha[L'']$ where $\alpha = K_{a2}/(K_{a2} + [H^+])$.[20]

(20) H. A. Laitinen, "Chemical Analysis," McGraw-Hill, New York, N. Y., 1960, p 35.

The chemical shift of the methylene protons adjacent to the amino group in the amino-complexed peptide is needed in the above calculations. ν_{ML} could not be obtained directly from the data because precipitation of metal hydroxide occurred before all of the amino end was complexed, as shown in Figures 2–4 for the triglycine system. Therefore, a procedure was used which permitted the simultaneous evaluation of ν_{ML} and K_f^N. The chemical shift curves for the methylene protons adjacent to the amino group were extrapolated to high pH values to indicate the region in which ν_{ML} was likely to occur. K_f^N was then calculated at each of the data points using ν_{ML} values in these regions. The final value taken for ν_{ML} was the value which gave the same K_f^N for each of the data points. The chemical shifts obtained in this way are listed in Table I; the formation constants are listed in Table II.

The formation constants obtained in this work and in previous work for coordination of cadmium, zinc, and lead at the amino end of polyglycine peptides were determined by utilizing the competition between metal and protons for the amino nitrogen. The relationships used in evaluating these constants do not correct for competition from the carboxylate end for the metal ions. Therefore, these formation constants must be considered "apparent" formation constants for coordination at the amino end.

Triglycine Peptide Proton Exchange Kinetics.—The kinetics of exchange of the peptide protons of triglycine with solvent protons were measured for both free and complexed triglycine. Sheinblatt has previously reported an nmr study of the exchange kinetics of uncomplexed triglycine at a lower ionic strength than used in this work.[15] Our results differ somewhat from those of Sheinblatt, even when the difference in ionic strength is taken into account, and therefore our experiments will be described.

Two possible reactions leading to exchange of a peptide proton in the pH region where collapse of the methylenic doublet patterns to single resonances takes place when no complexing metal ion is present are[15,21–23]

$$\mathrm{RCONHR'} + \mathrm{H_2O} \underset{}{\overset{k_I}{\rightleftharpoons}} \mathrm{RC\bar{O}NR'} + \mathrm{H_3\dot{O}} \qquad (7)$$

$$\mathrm{RCONHR'} + \mathrm{OH^-} \overset{k_{II}}{\rightleftharpoons} \mathrm{RC\bar{O}NR'} + \mathrm{H_2O} \qquad (8)$$

The kinetic parameter measured by nmr is the average lifetime, τ, of the peptide proton before transfer from triglycine. The rate equation for the above reactions in terms of this parameter is

$$1/\tau = k_I + k_{II}[\mathrm{OH^-}] \qquad (9)$$

Lifetimes were obtained from experimental spectra by comparison with computer-simulated spectra. Spectra were simulated as a function of lifetime using Bloch phenomenological equations modified for transfer of magnetization by two-site chemical exchange. This approach is valid because coupling of the peptide protons to the methylene protons essentially is first order.[24] The treatment of McConnell and Meiboom was used to obtain the modified Bloch equations.[25,26]

The T_2 values used in calculation of spectra were obtained from the widths of the resonances in the low-pH region, 1.09 and 1.36 Hz for the α- and β-methylene protons. These were considered to be the widths corresponding to no exchange for the following reasons. First, the widths are pH independent in the low-pH region indicating that in this pH region exchange *via* the reaction represented by eq 8 is not occurring on the nmr time scale. If exchange is occurring *via* the reaction represented by eq 7, the amount of exchange broadening should be temperature dependent. Variation of the temperature from 10 to 60° at several pH's in this pH range caused no significant change in the width of the methylene proton resonances. Second, the width of the averaged resonance in the high-pH region where exchange is rapid is the sum of the widths in the absence of exchange, weighted according to the relative intensities of each resonance.[16] The widths of the averaged resonances for the α- and β-methylene protons are 1.09 and 1.35 Hz at pH 10.0.

Kinetic data for exchange of the peptide protons are listed in Table III. Evaluation of these data by eq 9 with standard least-squares methods yields the rate constants given in Table IV. The upper limits given for $k_{I\alpha}$ and $k_{I\beta}$ were set assuming an experimental uncertainty of 0.1 Hz in the line widths. The rate constants reported by Sheinblatt are also listed in Table IV. The rate constants obtained in this work at an ionic strength of ~0.8 M differ from those reported by Sheinblatt for a lower ionic strength. Sheinblatt's plot of $1/\tau$ *vs.* $1/a_{H^+}$ ($=[\mathrm{OH^-}]/K_w$) for the α-peptide proton was nonlinear and was resolved into $k_{II\alpha}$ and $k_{II\alpha'}$ for the triglycine zwitterion and the triglycinate anion, respectively. The results in Table III for α-peptide protons in the solutions containing 0.60 M KNO_3 give a linear plot. Further, Sheinblatt reported nonzero values for $k_{I\alpha}$ and $k_{I\beta}$. The study was repeated using a lower ionic strength to determine if the differences between our results and those of Sheinblatt were due to the different ionic strengths. The rate constants obtained at the lower ionic strength, also listed in Table IV, indicate that the differences between the rate constants previously reported and those measured in the present study cannot be attributed solely to ionic strength effects. The nonzero values reported by Sheinblatt for $k_{I\alpha}$ and $k_{I\beta}$ suggest the line widths used for conditions of no exchange in

TABLE III
KINETIC DATA FOR EXCHANGE OF THE PEPTIDE HYDROGENS OF TRIGLYCINE[a,b]

pH	$1/\tau\beta$	pH	$1/\tau\beta$	pH	$1/\tau\alpha$	pH	$1/\tau\alpha$
3.83	0.6	5.02	9.1	6.35	0.5	7.19	11
4.09	1.1	5.18	10	6.38	1.1	7.35	16
4.19	1.2	5.23	15	6.57	2.1	7.38	17
4.30	2.8	5.33	17	6.63	2.0	7.80	35
4.41	2.6	5.47	36	6.75	3.3	7.90	56
4.53	3.4	5.49	31	6.84	4.0	8.03	77
4.62	3.7	5.72	59	6.96	5.3	8.26	125
4.76	5.0	5.92	110	7.06	6.9	8.42	190
4.81	5.9	6.15	150	7.18	12	8.50	200

[a] At 25°. [b] Ionic strength ~0.8; 0.20 M triglycine, 0.60 M KNO_3.

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TABLE IV

RATE CONSTANTS FOR EXCHANGE OF PEPTIDE PROTONS OF TRIGLYCINE[a]

Rate constant	This work[b]	Lit.[c]	This work[d]
$k_{I\alpha}$	≤ 0.3 sec^{-1}	~ 2 sec^{-1}	≤ 0.3 sec^{-1}
$k_{II\alpha}$	6.7×10^7 M^{-1} sec^{-1}	8.4×10^7 M^{-1} sec^{-1}	7.9×10^7 M^{-1} sec^{-1}
$k_{II\alpha'}$		0.8×10^7 M^{-1} sec^{-1}	2.6×10^7 M^{-1} sec^{-1}
$k_{I\beta}$	≤ 0.3 sec^{-1}	5 sec^{-1}	≤ 0.3 sec^{-1}
$k_{II\beta}$	1.1×10^{10} M^{-1} sec^{-1}	6.7×10^9 M^{-1} sec^{-1}	1.0×10^{10} M^{-1} sec^{-1}

[a] At 25°. [b] Ionic strength ~ 0.8; 0.60 M KNO_3, 0.20 M triglycine. [c] Reference 15; 0.15–0.589 M triglycine. [d] 0.20 M triglycine.

the previous work were less than the line widths used in the present work. Sheinblatt's values for $k_{I\alpha}$ and $k_{I\beta}$ also do not appear reasonable when the relationship between the forward and reverse rate constants in eq 7 is considered. Since the rate constant for the reverse reaction cannot exceed the diffusion-controlled limits and pK_A of the peptide hydrogen is greater than 14, $k_{I\alpha}$ and $k_{I\beta}$ must in fact be $<<<0.3$ sec^{-1}.[27]

The linearity of the plot of the kinetic data in Table III for the α-peptide proton indicates that $k_{II\alpha'}$ equals $k_{II\alpha}$ at this ionic strength. Thus $k_{II\alpha'}$ increases with increasing ionic strength (see Table IV) whereas $k_{II\alpha}$ and $k_{II\beta}$ are ionic strength independent. This is consistent with the known effect of ionic strength on rate constants for reaction between like-charged species compared to the effect on reactions between one charged and one neutral species.[28]

Kinetic data for exchange of the α- and β-peptide protons of triglycine in solutions containing a metal nitrate are listed in Tables V and VI, respectively.

TABLE V

KINETIC DATA FOR EXCHANGE OF THE α-PEPTIDE PROTON OF TRIGLYCINE IN METAL-CONTAINING SOLUTIONS[a]

	$1/\tau_\alpha$			
pH	0.20 M triglycine[b] 0.60 M KNO_3	0.20 M triglycine 0.20 M $Cd(NO_3)_2$	0.20 M triglycine 0.20 M $Zn(NO_3)_2$	0.20 M triglycine 0.20 M $Pb(NO_3)_2$
5.07	0.08[c]			0.8
5.19	0.10			1.4
5.30	0.13			1.7
5.43	0.18			2.3
5.50	0.22	2.4		3.0
5.60	0.26	3.5		3.3
5.75	0.30		1.7	
5.95	0.60	4.2	2.3	
6.32	1.3	9.1	5.1	
6.60	2.6	12	10	
6.69	3.1		13	
6.92	7.5	22		
7.09	8.3	31		
7.27	12.3	48		

[a] At 25°. [b] Kinetic data in this column calculated from rate constants in Table IV. [c] In sec^{-1}.

Also listed is the inverse of the lifetime for the peptide protons at these same pH's in the absence of a complexing metal ion. These lifetimes were calculated from the rate constants determined in this work in solutions containing 0.60 M KNO_3. Because of the large number of species present in the pH regions where kinetic data can be obtained, it was not possible to resolve the data in Tables V and VI into rate constants for peptide proton exchange from individual triglycine complexes. The data indicate, however, that the rate of peptide proton exchange is enhanced upon coordination.

TABLE VI

KINETIC DATA FOR EXCHANGE OF THE β-PEPTIDE PROTON OF TRIGLYCINE IN METAL-CONTAINING SOLUTIONS[a]

	$1/\tau_\beta$			
pH	0.20 M triglycine[b] 0.60 M KNO_3	0.20 M triglycine 0.20 M $Cd(NO_3)_2$	0.20 M triglycine 0.20 M $Zn(NO_3)_2$	0.20 M triglycine 0.20 M $Pb(NO_3)_2$
3.98	0.9[c]		2.0	1.7
4.12	1.3	3.0		2.9
4.30	2.0	3.6	2.6	4.0
4.43	2.7	5.5		4.8
4.60	4.0	7.7	5.0	8.3
4.92	8.3	14	11	14
5.21	16	30	20	22
5.35	22	42		
5.44	28		50	32
5.50	32	63		
5.55	35			40
5.60	40			50
5.97	93	135	125	
6.25	178	250		
6.32	209		240	
6.58	390	500	477	

[a] At 25°. [b] Kinetic data in this column calculated from rate constants in Table IV. [c] In sec^{-1}.

Discussion

Structures of the Cadmium, Zinc, and Lead Complexes of Diglycine, Triglycine, and Tetraglycine.—The potential metal binding sites in the polyglycine peptides are the carboxylate group, the amino group, and the peptide linkages. Binding of divalent metal ions to the peptide linkage in aqueous solution has been proposed *via* the carbonyl oxygen,[9,29–37] the protonated peptide nitrogen,[38–40] and the deprotonated peptide nitrogen.[30,36–38,41,42] In the present work, the doublets due to coupling of the methylene protons to the peptide protons were not completely collapsed in metal-containing solutions, indicating that in not more than a fraction of the complexed peptide could the peptide proton be ionized. Mass balance calculations on base added are also consistent with titration of only the amino and carboxylate protons, in agreement with previous studies.[4–7] Therefore, any coordination of

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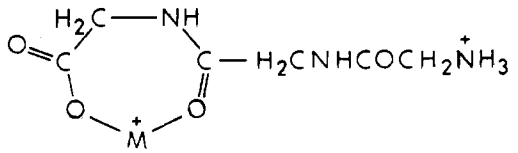
cadmium, zinc, or lead to the peptide linkages of the glycine peptides in the pH regions studied in this work must be to the protonated peptide linkages. Of the two potential coordination sites in the protonated peptide linkage, the carbonyl oxygen is considered the more basic on the basis of nmr studies of protonation of the amide linkage[21] and crystallographic[43,44] and infrared[7,9] studies of binding to the peptide linkage.

Chemical shift data in Figures 2–4 indicate at pH <4 cadmium, zinc, and lead coordinate to the C-terminal end of the peptide chain. The carboxylate group is coordinated as evidenced by displacement of the ionization of the carboxylic acid proton to a lower pH region. The α-peptide linkage might also be simultaneously coordinated *via* the carbonyl oxygen giving a chelate system. The lack of any change in the chemical shift of the methylene protons adjacent to the amino group indicates the amino nitrogen is still protonated. Thus I and II are possible structures

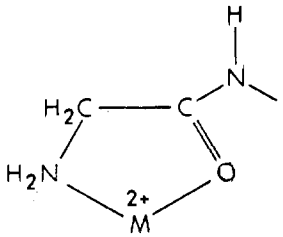
I



II

for the complex formed with triglycine at pH <4. Of these, I is considered the more likely. Coordination to the carbonyl oxygen would be expected to affect the chemical shift of the neighboring β-methylene protons more than observed. Also a seven-membered chelate ring as in II is not normally stable.[45] Further, the formation constants for coordination to the C-terminal end of diglycine, triglycine, and tetraglycine are approximately the same as the formation constants of the acetylglycine complexes of these metal ions (log K_f = 1.22 for the cadmium complex of acetylglycine, log K_f = 0.86 for the zinc complex, and log K_f = 1.38 for the lead complex)[17b] suggesting that coordination to the C-terminal end of the glycine peptides and to acetylglycine involves the same ligand donor groups. A comparison of the formation contants of a series of carboxylic acid complexes of these metals with the basicity of the carboxylic acids suggests there is little chelation involved in the coordination of acetylglycine.[46]

At pH >4, the chemical shift behavior of the methylene protons adjacent to the amino group indicates the metal is binding to the amino end of the peptide chain. The amino nitrogen is coordinated as evidenced by displacement of the ionization of the amino proton to a lower pH region. The peptide linkage adjacent to the N-terminal amino group, known to be a site of metal binding in the dipeptide and tripeptide complexes of certain divalent metal ions,[7,9,29,30,37,43,44] might also be simultaneously coordinated through the carbonyl oxygen atom giving a five-membered chelate



III

ring (III). The downfield shifts of the resonances for the α-methylene protons of diglycine, the β-methylene protons of triglycine, and the γ-methylene protons of tetraglycine upon complexation of cadmium, zinc, and lead at the amino end are somewhat larger than the downfield shift upon protonation of the amino end supporting simultaneous coordination to the carbonyl oxygen of the N-terminal peptide linkage when the amino end is coordinated.

In the pH region where metal is binding to the amino end of the peptide chain, the small change in the chemical shift of the α-methylene protons of triglycine and tetraglycine suggests the carboxylate group is still metal coordinated. An alternative explanation for the lack of a sizable change in the chemical shift of the α-methylene protons might be that their chemical shift is approximately the same in the C-terminal and N-terminal complexes, both of which are different from that in the uncomplexed peptide. If so, the resonance for the α-methylene protons would be predicted to move downfield upon coordination of the N-terminal end rather than remaining constant or moving upfield slightly as observed. Molecular models show that with triglycine the amino nitrogen, the carbonyl oxygens of the α- and β-peptide linkages, and the carboxylate group cannot all be coordinated simultaneously to the same metal ion. However, the amino nitrogen, the carbonyl oxygen of the β-peptide linkage, and the carboxylate group could be, although the 10-membered chelate ring formed by coordination to the latter two groups would be expected to be unstable.[45] Therefore, simultaneous coordination of the amino end and the carboxylate end must occur to different metal ions resulting in polynuclear complexes. Competition by the amino end for the metal coordination sites probably results in less coordination at the carboxylate end which gives rise to the slight upfield shift in the α-methylene resonance.

Evidence has been reported for polynuclear complexes of the type proposed above. Kim and Martell proposed, on the basis of selective broadening of the various methylene resonances of triglycine and tetraglycine by Cu(II), that in neutral solutions Cu(II) forms polynuclear complexes by coordination of the amino and carboxyl ends to different metal ions.[35] At higher pH's, however, the peptide protons are ionized and Cu(II) binds to the negative peptide nitrogens, the amino nitrogen, and, in the case of triglycine, the carboxylate group to form mononuclear complexes.[41] Crystallographic studies have revealed several polynuclear complexes in the solid state.[43] In the complex $[Cu(GlyGly)(Im)_2]ClO_4$, where GlyGly is diglycine with only the peptide nitrogen protonated and Im is imidazole, the amino nitrogen and the carbonyl oxygen

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are bonded to one metal ion and the carboxylate group is bonded to a second metal ion.[44] In the complex $[Cu(GlyGlyGly)Cl]\cdot 1.5H_2O$, where the peptide nitrogens of triglycine are protonated, the amino nitrogen and the carbonyl oxygen of the β-peptide linkage are bonded to one copper ion while the terminal carboxyl group is coordinated to a second copper ion, so that the structure consists of –Cu–peptide–Cu–peptide– chains.[47]

The α-methylene protons of diglycine, however, undergo a downfield shift upon coordination of the amino group which could be due either to binding to the adjacent peptide linkage or to increased coordination at the carboxylate end. Thus no information about the structures of the amino-coordinated complexes can be obtained from the chemical shift data.

Complexation Effects on the Reactivity of the Peptide Linkages of Triglycine.—The reactivity of the peptide linkages of triglycine is affected by coordination to cadmium, zinc, and lead as evidenced by the increased peptide proton exchange rates (Tables V and VI).

Pagenkopf and Margerum[34] observed that coordination of the carbonyl group of the α-peptide linkage of $Cu(H_{-1}L)$, where $H_{-1}L$ is triglycine with the β-peptide nitrogen deprotonated and copper coordinated, labilizes the α-peptide proton. The peptide proton of acetylglycine is also labilized upon coordination to cadmium, zinc, and lead.[17b] In the case of the cadmium complex, the proton exchange rate is 53 times faster than that of free acetylglycine. In the present work, it was not possible to resolve rate constants for specific complexes from the data in Tables V and VI due to uncertainties in the species present. The data indicate, however, that both the α- and β-peptide protons are labilized by coordination to these metal ions, with the apparent degree of labilization being greater for the α-peptide protons over the pH ranges where the kinetics are accessible by nmr. This is likely due to the fact that over these pH ranges coordination to the C-terminal carboxylate group is at a maximum while there is much less coordination at the amino end. Considering only the changes in charge, coordination of a dipositive metal ion to the ionized C-terminal carboxylate group is also predicted to enhance the rate of exchange of the adjacent α-peptide proton more than replacement of the amino proton by a dipositive metal ion is predicted to enhance the rate of exchange of the β-peptide proton.

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Coordination Compounds of Indium. XVII. Force Constant Calculations for Anionic Indium(I) and -(III) Halide Complexes

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A simplified GQVFF model has been used to calculate force constants for the anionic indium(III) halide complexes InX_4^- (X = Cl, Br, or I), $InCl_5^{2-}$, and $InCl_6^{3-}$ and for the recently prepared indium(I) species InX_3^{2-} (X = Cl, Br, or I). The primary stretching force constants for the indium(III) chloro anions decrease linearly with increasing coordination number. The results for the indium(I) complexes are compared with those in the literature for isoelectronic tin(II) and antimony(III) species.

Introduction

One of the interesting features of the coordination chemistry of indium is that three-, four-, five-, and six-coordinate complexes can be prepared, in some cases with the same ligand. In particular, recent preparative work has shown that for anionic halide complexes, coordination numbers four, five, or six can be obtained with chloride, four or six with bromide, and four with iodide. Slight changes in experimental conditions may bring about changes in the coordination number; for example, both the four- and five-coordinate chloride species are stabilized by the tetraethylammonium cation, depending on the solvent from which the complex is recrystallized.[1,2]

A discussion of these phenomena is hampered both by the lack of a reliable model of the bonding involved and by the absence of appropriate energetic data, in particular of bond strengths. As part of a general investigation of these problems, we have now carried out force constant calculations for all those anionic indium(III) halide complexes for which complete vibrational spectra are available. We also report the results of similar calculations for the halide complexes of indium(I) recently prepared by us,[3] and the values obtained are compared with those for some isoelectronic species. In general, the results reveal some interesting empirical relationships among the force constants and the oxidation state of the metal, the nature of the ligand, and the coordination number. These relationships are discussed in terms of the indium–halide bonding interactions.

The calculations were based on a simplified general quadratic valence force field model (GQVFF), which has generally been accepted as a good approximation for expressing the potential energy of small molecules.

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