Hydroxide Ion Catalysis of the Mono- and Bis(triglycinato)cuprate(II) Interconversion and the Preference for Cis *vs.* Trans N-Peptide Bonding to Copper(II)

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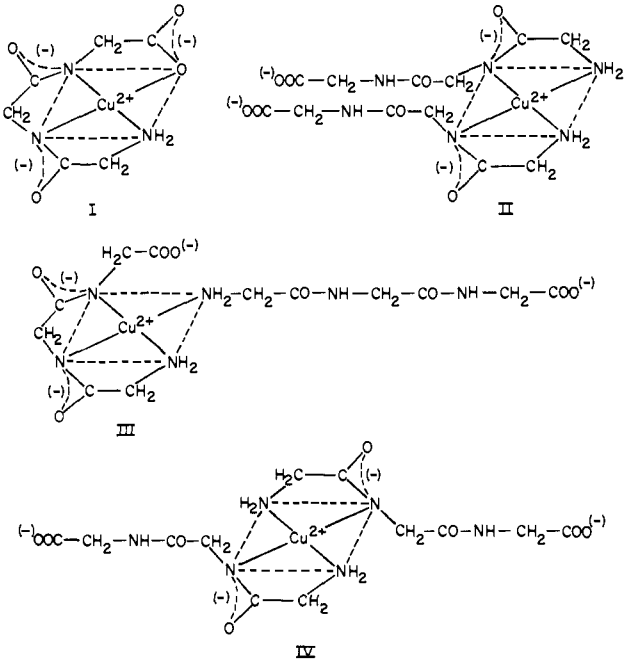
Abstract: The $Cu(H_{-2}GGG)^-$ complex (where GGG^- is the triglycinate ion and the protons are ionized from two peptide nitrogens coordinated to copper(II)) reacts with excess GGG^- to form $Cu(H_{-1}GGG)_2^{2-}$: $Cu(H_{-2}GGG)^- + GGG^- + OH^- \rightleftarrows Cu(H_{-1}GGG)_2^{2-} + OH^-$ (k_f, k_r). The formation and dissociation rates are catalyzed by hydroxide ion, and the values of the rate constants (25.0°, 0.10 μ) are $k_f = (1.26 \pm 0.02) \times 10^7\ M^{-2}\ sec^{-1}$ and $k_r = (8.8 \pm 0.3) \times 10^4\ M^{-1}\ sec^{-1}$. In the proposed reaction mechanism the hydroxide ion deprotonates an incoming peptide group of one triglycine as the Cu–N(peptide) bond of another triglycine breaks. A combination of absorption and circular dichroism spectrophotometry, kinetic results, and model studies indicate that $Cu(H_{-1}GGG)_2^{2-}$ exists in a planar form in which the two triglycine molecules are arranged around the copper in a cis bidentate configuration. Infrared and Raman results show that the doubly deprotonated bis complex of glycinamide with copper(II) also adopts a cis geometry. It is postulated that strong σ donation from the deprotonated peptide-nitrogen to copper(II) causes a preference for the cis rather than the trans configuration.

Triglycinate ion (GGG^-) reacts with copper(II) in neutral solutions to form a complex in which the ligand is tetradentate, $Cu(H_{-2}GGG)^-$ (structure I).[1,2] With the addition of excess triglycinate ion in basic solution Dobbie and Kermack[1] found potentiometric and spectrophotometric evidence for a bis complex. They proposed the formula $CuH_{-2}(GGG)_2^{2-}$ and suggested that both "symmetric" (II) and asymmetric (III) structures were present in solution. Structure III was estimated to constitute 50% or more of the bis complex. Another bis structure which is more symmetric and has less steric hindrance also might be postulated (IV). Österburg and Sjöberg[3] carried out a potentiometric study in 3 M $NaClO_4$ and also found evidence for a bis complex ($CuH_{-2}(GGG)_2^{2-}$). Other binuclear complexes were detected at higher copper(II) concentrations.



I

II

III

IV

Recent work in this laboratory[4] has shown that excess triglycine catalyzes the exchange reaction between $Cu(H_{-2}GGG)^-$ and EDTA. Rapid formation of the bis complex is proposed in the mechanism for the catalysis. In the present work the equilibrium constant for reaction 1 is determined from spectrophotometric

$$Cu(H_{-2}GGG)^- + GGG^- + OH^- \underset{k_r}{\overset{k_f}{\rightleftarrows}} Cu(H_{-1}GGG)_2^{2-} + OH^- \quad (1)$$

data and the kinetics of the forward and reverse reaction are determined by stopped-flow methods. Our studies indicate that only one bis species is present in solution and that this species must have both ligands in at least bidentate coordination. A comparison of the reactions of a number of tripeptides indicates that the cis isomer (structure II) must be present.

Experimental Section

Triglycine (chromatographically homogeneous) was obtained from Mann Research Laboratories (New York, N. Y.). The L-alanine substituted tripeptides (glycylglycyl-L-alanine (GGA), glycyl-L-alanylglycine (GAG), and L-alanylglycylglycine (AGG)) were obtained from the Cyclo Chemical Division of Travenol Laboratories (Los Angeles, Calif.). A 9.85×10^{-2} M Cu(II) stock solution, prepared from twice recrystallized $Cu(ClO_4)_2$ and standardized against EDTA, was used. The copper(II)–triglycine solutions were freshly prepared before each series of kinetic runs. Ionic strength was maintained at 0.10 M with $NaClO_4$. The ionic strength of the solutions used to determine the stability constant of $Cu(H_{-1}GGG)_2^{2-}$ was 0.10 M $NaClO_4$ plus that of the added triglycine (μ_{max} = 0.17). Hydrogen ion concentrations were calculated from pH measurements by the relationship $-\log [H^+] = pH - 0.11$,[5] and hydroxide ion concentrations were calculated from $pK_w = 13.78$.

Glycinamide·HCl was obtained from the Cyclo Chemical Co. The preparation of $Cu(H_{-1}Ga)_2$ (where Ga is glycinamide) followed the method of ref 6 with the exception of using $CuCl_2 \cdot 2H_2O$ instead

(1) H. Dobbie and W. O. Kermack, *Biochem. J.*, **59**, 257 (1955).
(2) H. C. Freeman and M. R. Taylor, *Acta Crystallogr.*, **18**, 939 (1965).
(3) R. Österburg and B. Sjöberg, *J. Biol. Chem.*, **243**, 3038 (1968).
(4) G. R. Dukes, G. K. Pagenkopf, and D. W. Margerum, *Inorg. Chem.*, 10, 2419 (1971).
(5) R. G. Bates, "Determination of pH," Wiley, New York, N. Y., 1964, p 92.
(6) T. Komorita, J. Hidaka and Y. Shimura, *Bull. Chem. Soc. Jap.*, **42**, 168 (1969).

Table I

A. Acid Dissociation Constants of the Tripeptides, 25°

Ligand	pK_a	Ref
Glycylglycylglycine (GGG)	7.88	*a*
Glycylglycyl-L-alanine (GGA)	8.02	*b*
Glycyl-L-alanylglycine (GAG)	8.08	*b*
L-Alanylglycylglycine (AGG)	8.05	*b*

B. Log β Values of the Copper–Tripeptide Complexes

Species	Log β	μ, M	Ref
$Cu(H_{-1}GGG)$	−0.01	0.1 (KNO_3)	*a*
$Cu(H_{-2}GGG)^-$	−6.67	0.1 (KNO_3)	*a*
	−7.03	0.16 (KCl)	*b*
$Cu(H_{-2}GGG)OH^{2-}$	−18.7	0.1 (KNO_3)	*a*
$Cu(H_{-1}GGG)_2^{2-}$	−4.43	0.10→0.17 ($NaClO_4$)	Spectrophotometric[c]
	−4.51	0.1 ($NaClO_4$)	Kinetics[c]
$Cu(H_{-2}GGA)^-$	−6.91	0.16 (KCl)	*b*
$Cu(H_{-2}GAG)^-$	−6.76	0.16 (KCl)	*b*
$Cu(H_{-2}AGG)^-$	−7.01	0.16 (KCl)	*b*

[a] H. Hauer, E. J. Billo, and D. W. Margerum, *J. Amer. Chem. Soc.*, **93**, 4173 (1971). [b] G. F. Bryce and F. R. N. Gurd, *J. Biol. Chem.*, **241**, 1439 (1966). [c] This work.

of $Cu(C_2H_3O_2)_2 \cdot H_2O$. The fine purple crystals obtained were washed with ether and dried over P_2O_5.

Kinetic runs were followed spectrophotometrically at 550 nm using a modified Durrum-Gibson stopped-flow spectrophotometer with a 2.0-cm cell path. The photomultiplier output was interfaced to a Hewlett-Packard 2115A general purpose digital computer as described elsewhere.[7] All reactions in this study were run under first-order or pseudo-first-order conditions and each rate constant is the average of at least four kinetic runs. The standard deviations were calculated from the deviation of the individual rate constants from the mean. [The standard deviations of the rate constants calculated from the data points (100–250) for the individual runs are much smaller.]

The stability constant of $Cu(H_{-1}GGG)_2^{2-}$ was determined spectrophotometrically using a Cary Model 14 spectrophotometer thermostatted at 25.0° with cells of 10.0-cm light path. Circular dichroism spectra were obtained on a Cary Model 60 spectropolarimeter equipped with a circular dichroism accessory. Measurements were made at room temperature using a cell of 1.00 cm. Results are reported in terms of $\epsilon_L - \epsilon_R$, the difference in molar absorbance (based on $[Cu]_{total}$) between the left and right circularly polarized beams.

Infrared spectra of $Cu(H_{-1}Ga)_2$ were measured with a Beckman IR-12 spectrophotometer as Nujol suspensions between polyethylene windows. The Raman spectra were obtained with an instrument built around a Spex Model 1400 double monochromator and a Coherent Radiation Model 52 argon laser (5145 and 4880 Å). Signal detection and amplification are described elsewhere.[8] The spectra were taken of powder samples in thin capillary tubes by the transillumination technique. To verify that the Raman spectral peaks seen were due to the complex and not to artifacts caused by the nature of the sample, spectra were taken at both the 5145 and 4880 Å lines of the argon laser and only the coincident bands considered. The band positions (Raman and infrared) reported in this work are the average of four different spectra.

Attempts to obtain the Raman spectra of aqueous solutions of $Cu(H_{-1}Ga)_2$, using both the 5145 and 4880 Å lines, were unsuccessful due to the solution absorption of both the exciting and Raman-shifted light.

The tripeptides used in this study, their acid dissociation constants, and the log β values for their copper complexes (where β is a cumulative stability constant with proton loss, *i.e.*, for $Cu(H_{-1}GGG)$, $\beta = [Cu(H_{-1}GGG)][H^+]/[Cu^{2+}][GGG^-]$) are listed in Table I. The constants are in terms of concentrations of all the species. The concentrations of the various species in solution were calculated by use of the species distribution program COMICS.[9]

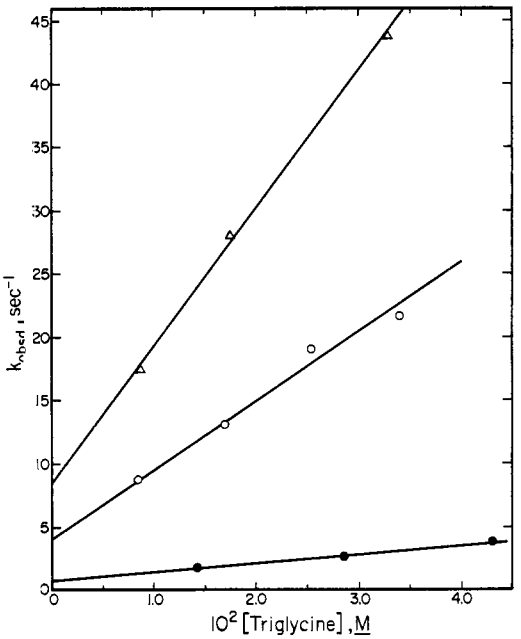


Figure 1. Dependence of k_{obsd} on pH and [GGG] at 25.0°, μ = 0.10 ($NaClO_4$). Experimental points are given and the solid lines are the linear least-squares lines: ●, pH 8.52; ○, pH 9.45; △, pH 10.00.

Results and Discussion

Kinetics of Hydroxide Catalyzed Mono- and Bis-(triglycinato)cuprate(II) Interconversion. 1. Reversible Conditions. The kinetics of the reaction shown in eq 1 have been determined under pseudo-first-order reversible conditions. The experimentally determined rate expression is $-d[Cu(H_{-2}GGG)^-]/dt = k_{obsd}[Cu(H_{-2}GGG)^-]$ where $k_{obsd} = (k_f[GGG^-] + k_r)[OH^-]$ as shown in Figure 1. At a given hydroxide ion concentration, a series of at least three reactions were run with varying triglycine concentrations. Values of $k_f' = k_f[OH^-]$ and $k_r' = k_r[OH^-]$ were resolved from these data either graphically or by the method of simultaneous equations. The data used to obtain these values are given in Table II. Figure 2 demonstrates

(7) B. G. Willis, J. A. Bittikoffer, H. L. Pardue, and D. W. Margerum, *Anal. Chem.*, **42**, 1430 (1970).
(8) W. M. Scovell, G. C. Stocco, and R. S. Tobias, *Inorg. Chem.*, **9**, 2682 (1970).
(9) D. D. Perrin and I. G. Sayce, *Talanta*, **14**, 833 (1967).

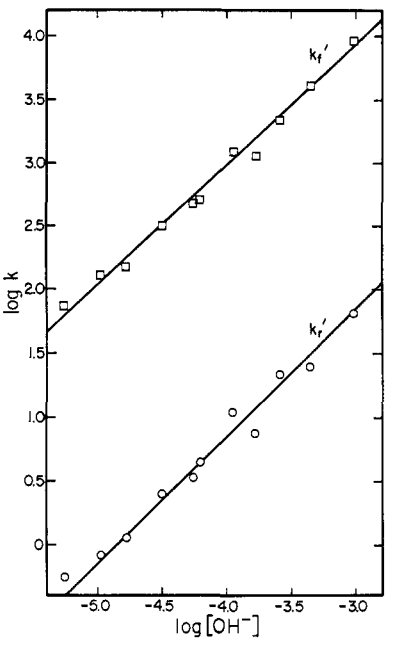


Figure 2. Demonstration of first-order dependence of k_f' and k_r' upon [OH⁻] at constant triglycine concentration at 25.0° and μ = 0.10 ($NaClO_4$). Experimental points are given and the solid lines are drawn with a slope of 1.0: ○, k_r'; □, k_f'.

Table II. Triglycine [GGG⁻] and pH Dependence of the Observed First-Order Rate Constant for the Reaction of $Cu(H_{-2}GGG)^-$ with Excess GGG⁻

pH[a]	10^2[triglycine], M	k_{obsd}, sec^{-1}
8.53	1.43	1.68 ± 0.01
8.51	2.86	2.65 ± 0.03
8.53	4.30	3.85 ± 0.03
8.78	1.70	2.90 ± 0.02
8.80	2.54	4.19 ± 0.03
8.77	3.39	5.05 ± 0.04
9.00	0.88	2.50 ± 0.03
9.00	1.76	3.65 ± 0.04
9.00	2.64	5.10 ± 0.02
9.28	0.848	5.27 ± 0.01
9.26	1.70	7.7 ± 0.1
9.27	2.54	10.6 ± 0.1
9.46	0.848	8.75 ± 0.07
9.46	1.70	13.0 ± 0.1
9.46	2.54	19.0 ± 0.1
9.43	3.39	21.6 ± 0.1
9.51	1.43	10.0 ± 0.1
9.53	2.86	17.1 ± 0.2
9.51	4.30	23.4 ± 0.4
9.84	0.848	20.8 ± 0.2
9.80	1.70	30.4 ± 0.3
9.80	2.54	41.8 ± 0.3
10.00	0.88	17.4 ± 0.2
10.00	1.76	28.0 ± 0.8
10.00	3.28	43.8 ± 0.8
10.21	0.424	31.5 ± 0.5
10.17	0.848	39.6 ± 0.8
10.16	1.27	51 ± 2
10.43[b]	0.424	43 ± 2
10.41[b]	1.27	75 ± 1
10.42[b]	2.12	112 ± 3
10.76[b]	0.424	104 ± 8
10.76[b]	1.27	182 ± 5
10.77[b]	2.12	310 ± 20

[a] 25.0 ± 0.1°, μ = 0.10 ($NaClO_4$). [$Cu(H_{-2}GGG)^-$] = 9.85 × 10^{-4} M except where noted. [b] [$Cu(H_{-2}GGG)^-$] = 4.92 × 10^{-4} M.

the first-order dependence of k_f' and k_r' upon hydroxide ion concentration. A linear least-squares treatment of

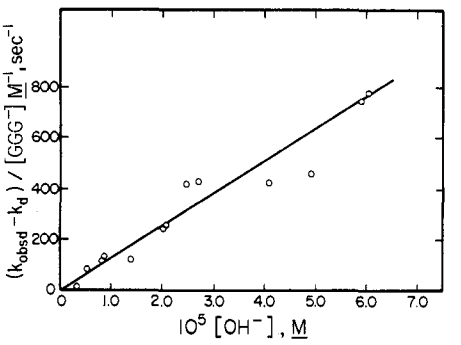


Figure 3. The effect of hydroxide ion concentration on the rate constant of the triglycine-catalyzed exchange reaction between $Cu(H_{-2}GGG)^-$ and EDTA at constant excess triglycine at 25.0° and μ = 0.10 ($NaClO_4$). The points are experimental and the slope of the least-squares line equals k_1 (eq 2 and 4).

k_f' and k_r' *vs.* [OH⁻] yields k_f = (1.26 ± 0.02) × 10^7 M^{-2} sec^{-1} and k_r = (8.8 ± 0.3) × 10^4 M^{-1} sec^{-1}.

2. Forward Reaction Using EDTA as a Scavenger. The proposed[4] rate-determining step in the triglycine-catalyzed exchange reaction between $Cu(H_{-2}GGG)^-$ and EDTA below pH 9.7 is the formation of the bis(triglycine) complex with subsequent rapid attack by $EDTA^{4-}$ (eq 2 and 3). This reaction is not reversible

$$Cu(H_{-2}GGG)^- + GGG^- + OH^- \xrightarrow{k_1} Cu(H_{-1}GGG)_2^{2-} + OH^- \quad (2)$$

$$Cu(H_{-1}GGG)_2^{2-} + EDTA^{4-} \xrightarrow{\text{rapid}} \text{products} \quad (3)$$

because EDTA acts as a scavenger for $Cu(H_{-1}GGG)_2^{2-}$. The experimentally observed rate expression for the EDTA exchange reaction with excess triglycine (pH 8.2–9.6) is $-d[Cu(H_{-2}GGG)^-]/dt = k_{obsd}[Cu(H_{-2}GGG)^-]$ where $k_{obsd} = k_d + k_1[GGG^-][OH^-]$. The k_d value is 0.12 sec^{-1}.[10] The rate constant expression may be rearranged to yield eq 4, which is plotted in

$$(k_{obsd} - k_d)/[GGG^-] = k_1[OH^-] \quad (4)$$

Figure 3. This gives a k_1 value of 1.3 × 10^7 M^{-2} sec^{-1}, which is in excellent agreement with the value determined for k_f *via* reversible kinetics. The use of EDTA as a scavenger to prevent reversibility has been reported previously in the study of the proton transfer reactions of $Cu(H_{-2}GGG)^-$[10,11] and $Ni(H_{-2}GGG)^-$,[12] and in the ligand exchange reactions of $Ni(H_{-2}GGG)^-$.[13] EDTA acts as a scavenger in these reactions because a tertiary nitrogen (as in EDTA) is sterically hindered from directly attacking the planar site vacated by the carboxylate group[14] but will rapidly attack a species which does not present these steric difficulties (*i.e.*, $Cu(H_{-1}GGG)$, $Ni(H_{-1}GGG)$, and $Cu(H_{-1}GGG)GGG^-$).

3. Reverse Reaction Using trien as a Scavenger. Triethylenetetramine (trien), on the other hand, is a very effective nucleophile in its reaction with $Cu(H_{-2}GGG)^-$ (k = 1.1 × 10^7 M^{-1} sec^{-1}).[14] In contrast to its reaction with $Cu(H_{-2}GGG)^-$, the reaction of trien with $Cu(H_{-1}GGG)_2^{2-}$ to form $Cu(trien)^{2+}$ is much

(10) G. K. Pagenkopf and D. W. Margerum, *J. Amer. Chem. Soc.*, **90**, 6963 (1968).
(11) G. K. Pagenkopf and D. W. Margerum, *ibid.*, **90**, 501 (1968).
(12) E. J. Billo and D. W. Margerum, *ibid.*, **92**, 6811 (1970).
(13) E. J. Billo, G. F. Smith, and D. W. Margerum, *ibid.*, **93**, 2365 (1971).
(14) G. K. Pagenkopf and D. W. Margerum, *ibid.*, **92**, 2683 (1970).

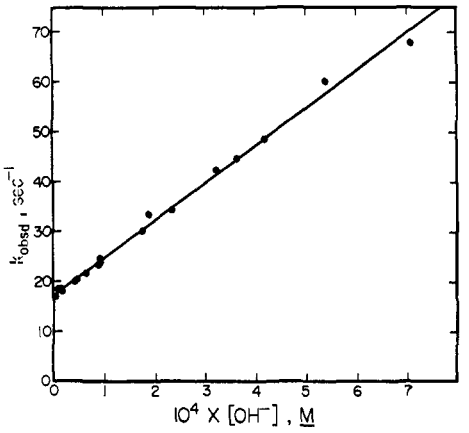


Figure 4. Dependence of k_{obsd} on hydroxide ion concentration for the reaction of trien with $Cu(H_{-1}GGG)_2^{2-}$ at 25.0° and μ = 0.10 ($NaClO_4$): $[Cu]_T = 9.85 \times 10^{-4}$ M, $[GGG]_T = 0.09$ M, $[trien]_T = 1.06 \rightarrow 5.95 \times 10^{-3}$ M. The points are experimental, the slope of the linear least-squares lines is k_{OH} (eq 7), and the intercept is k_2 (eq 5).

slower. The latter reaction is first order in hydroxide ion and exhibits no dependence on the trien concentration under conditions ranging from stoichiometric concentration to a sixfold excess of trien. The experimental rate expression which fits the observed kinetics behavior is $-d[Cu(H_{-1}GGG)_2^{2-}]/dt = (k_2 + k_{OH}\cdot[OH^-])[Cu(H_{-1}GGG)_2^{2-}]$. The observed first-order rate constant, k_{obsd}, equals $k_2 + k_{OH}[OH^-]$ and its hydroxide ion dependence is shown in Figure 4.

The k_2 path may be explained by eq 5 and 6 where

$$H_2O + Cu(H_{-1}GGG)_2^{2-} \xrightarrow{k_2} Cu(H_{-1}GGG)GGG^- + OH^- \quad (5)$$

$$Cu(H_{-1}GGG)GGG^- + trien \xrightarrow{rapid} products \quad (6)$$

k_2 is the rate-determining step (the subsequent trien attack on $Cu(H_{-1}GGG)GGG^-$ is rapid). The value found for k_2 is (17.5 ± 0.4) sec^{-1}, which is in good agreement with the value of 16.7 sec^{-1} reported for this reaction in ref 4. The pH dependent path of the overall reaction may be represented by eq 7 and 8 where k_{OH}

$$Cu(H_{-1}GGG)_2^{2-} + OH^- \xrightarrow{k_{OH}} Cu(H_{-2}GGG)^- + GGG^- + OH^- \quad (7)$$

$$Cu(H_{-2}GGG)^- + trien \xrightarrow{rapid} products \quad (8)$$

is the rate-determining step. The value determined for k_{OH} from the slope of Figure 4 is (7.50 ± 0.15) × 10^4 M^{-1} sec^{-1} which is in general agreement with the value found for k_r.

In this reaction, therefore, trien is acting as a scavenger for $Cu(H_{-2}GGG)^-$ and, as in the case of the EDTA reaction (eq 2 and 3), reversibility is prevented. Thus, the rate constants for the reactions with EDTA (eq 2 and 3) and with trien (eq 7 and 8) acting as scavengers to prevent reversibility provide excellent supporting evidence for the values of k_f and k_r determined under reversible conditions.

4. Proposed Mechanism. The hydroxide ion catalysis and the relatively slow rate of the reaction (eq 1) indicate that the bis complex is not III or any other species involving monodentate triglycine. The rate of dissociation of a monodentate H_2NR group can be estimated from the water exchange rate constant of Cu-

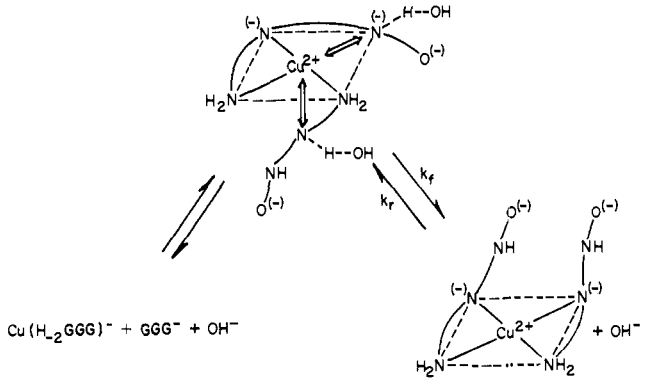


Figure 5. Proposed mechanism for the hydroxide ion catalyzed mono- and bis(triglycinato)cuprate(II) interconversion.

(II)[15] and the stability constants for the copper ammonia complexes.[16] The monoammine dissociation rate constant would be ~10^4 sec^{-1} and the tetraammine complex would be expected to dissociate much faster. Therefore, the rate of loss of a monodentate triglycinate group is expected to be many orders of magnitude faster than the rates observed in this work. In addition, there would be no reason for hydroxide ion catalysis if the equilibrium was between species I and III. Therefore the bis complex must involve two triglycine molecules each coordinated to copper(II) in at least a bidentate manner.

The mechanism proposed for reaction 1 is *via* an associative type path. Hydroxide ion deprotonates the incoming peptide nitrogen as it displaces the terminal peptide nitrogen of the other triglycine molecule (Figure 5). It should be pointed out that structure III, although eliminated as a stable species, is proposed as a transitory intermediate in the formation of II. Since the protonated peptide nitrogen is a very poor nucleophile, the catalytic role of hydroxide ion is the deprotonation of the incoming peptide nitrogen thereby allowing it to act as a much more powerful nucleophile.

5. The Stability Constant of $Cu(H_{-1}GGG)_2^{2-}$. The stability constant of $Cu(H_{-1}GGG)_2^{2-}$ was determined at pH 10 under conditions where $Cu(H_{-2}GGG)^-$ and $Cu(H_{-1}GGG)_2^{2-}$ are the two major copper species in solution. When excess GGG^- is added to $Cu(H_{-2}GGG)^-$, at pH 10, the absorbance drops dramatically accompanied by a small decrease in λ_{max} (ϵ 155 M^{-1} cm^{-1} at λ_{max} 555 nm for $Cu(H_{-2}GGG)^-$ and ϵ 63 M^{-1} cm^{-1} at λ_{max} 545 nm for $Cu(H_{-1}GGG)_2^{2-}$). The stability constant ($K_1 = [Cu(H_{-1}GGG)_2^{2-}]/[Cu(H_{-2}GGG)^-][GGG^-]$) was determined graphically from eq 9

$$\frac{A_1 - A_o}{A_0 - A_\infty} = \frac{[GGG]_T}{[Cu]_T} K_1 [Cu]_T \quad (9)$$

where A_1 = absorbance when all copper is in the form $Cu(H_{-2}GGG)^-$, A_∞ = absorbance when all copper is in the form $Cu(H_{-1}GGG)_2^{2-}$ and A_o = observed absorbance. The average value found for K_1 at six different wavelengths over the range 520–570 nm is 175 ± 13 M^{-1} at μ = 0.10–0.17. This is in reasonable agreement with the value determined kinetically ($K_1 = k_f/k_r$ = 143 ± 6 M^{-1} at μ = 0.10) and the value de-

(15) G. Mass, *Z. Phys. Chem. (Frankfurt am Main)*, 60, 138 (1968).
(16) L. G. Sillen and A. E. Martell, Ed., "Stability Constants," The Chemical Society, London, 1964.

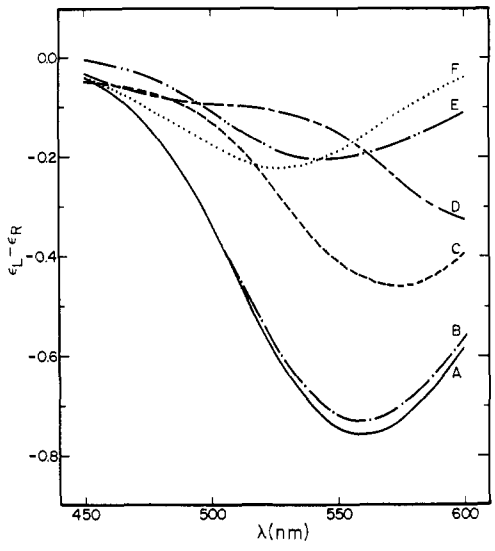


Figure 6. Circular dichroism spectra of copper(II) complexes of L-alanine containing tripeptides. Measurements were taken on solutions which were equimolar in copper and tripeptide (A, C, E) and on solutions in which the tripeptide concentration was 0.10 *M* (B, D, F). All solutions were 5.4×10^{-3} *M* in copper(II) and were adjusted to pH 10 with NaOH. All measurements were taken at room temperature, $\mu = 0.10$ ($NaClO_4$ + tripeptide): A, $Cu(H_{-2}GAG)^-$; B, Cu(II) + 0.1 *M* GAG; C, $Cu(H_{-2}GGA)^-$; D, Cu(II) + 0.1 *M* GGA; E, $Cu(H_{-2}AGG)^-$; F, Cu(II) + 0.1 *M* AGG.

termined potentiometrically by Österberg and Sjöberg in 3 *M* $NaClO_4$ (112 M^{-1}).[2]

Evidence for the Preference of Cis *vs.* Trans N-Peptide Bonding in Copper(II)–Oligopeptide Complexes. 1. Configurational Preference in $Cu(H_{-1}tripeptide)_2^{2-}$ Complexes. There are two possible bis bidentate isomers with square-planar coordination to copper(II). Structure II has a cis arrangement of the ligands while structure IV has a trans configuration. A variety of evidence, both thermodynamic and kinetic, has been obtained which indicates that a definite configurational preference is exhibited by the $Cu(H_{-1}tripeptide)_2^{2-}$ complexes.

A. Absorption and CD Spectra. To determine which of these two configurations is preferred in solution the visible absorption and circular dichroism spectra of the copper(II) complexes of some alanine-substituted tripeptides were measured under 1:1 ([Cu(II)]:[tripeptide$^-$]) conditions and under conditions where $Cu(H_{-1}GGG)_2^{2-}$ is formed. The spectral characteristics observed for the 1:1 complexes are in excellent agreement with those reported by Bryce and Gurd.[17] The values listed in Table IB indicate that the alanine substituents do not exert any major influence on the stability of the $Cu(H_{-2}tripeptide)^-$ complexes. The absence of any marked effect of the alanine substituent is seen again in the similarity of the visible absorption spectra of these 1:1 complexes. However, when the spectra of the complexes of these tripeptides were measured under conditions where $Cu(H_{-1}GGG)_2^{2-}$ forms (0.1 *M* tripeptide, pH 10), it became apparent that AGG and GGA form bis complexes whereas GAG does not. Reference to Table III reveals that under these conditions similar visible absorption maxima are observed (with the exception of GAG) for all of the copper–tripeptide complexes. Figure 6 demonstrates the virtual absence of any effect on the circular dichroism spectra upon changing from $Cu(H_{-2}GAG)^-$ (curve A) to conditions under which $Cu(H_{-1}GGG)_2^{2-}$ would be formed (curve B), while rather large effects are seen for GGA (curve C to curve D) and AGG (curve E to curve F).

(17) G. F. Bryce and F. R. N. Gurd, *J. Biol. Chem.*, 241, 1439 (1966).

Table III. Visible Absorption Characteristics for Copper(II) Complexes of L-Alanine Containing Tripeptides under Conditions Where Bis Complexes Are Expected to Form

Tripeptide	λ_{max}	ϵ_{max}	Tripeptide	λ_{max}	ϵ_{max}
GGG	545	63	AGG	545	79
GGA	548	80	GAG	550[a]	165[a]

[a] These values are identical with the λ_{max} and ϵ_{max} of the 1:1 complex. $[Cu]_T = 5.40 \times 10^{-3}$ *M*, $[tripeptide]_T = 0.10$ *M*, 25.0°, $\mu = 0.1$.

B. Kinetics. As further evidence, a kinetics study was performed to see if GAG would catalyze the exchange reaction between $Cu(H_{-2}GAG)^-$ and EDTA. The results given in Table IV show no evidence for catalysis. The proposed mechanism for triglycine catalysis of the exchange reaction between $Cu(H_{-2}GGG)^-$ and EDTA requires the formation of the bis complex[4] and therefore the absence of catalysis by GAG supports the proposal that the bis complex of GAG with copper(II) does not form.

Table IV. The Absence of Effect of GAG on Rate of Exchange Reaction between $Cu(H_{-2}GAG)^-$ and EDTA

pH[a]	$[GAG]_T$, *M*	k_{obsd}, sec^{-1}
10.02	6.00×10^{-4}	0.0242
10.02	2.00×10^{-3}	0.0282
10.01	1.00×10^{-2}	0.0241

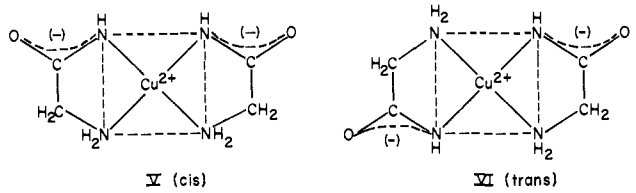
[a] $[Cu]_T = 3.95 \times 10^{-5}$ *M*, $[EDTA]_T = 2.00 \times 10^{-4}$ *M*, $\mu = 0.10$ ($NaClO_4$), 25.0 ± 0.1°.

The reason for the unusual behavior of GAG compared to the other tripeptides is indicated by the examination of CPK atomic models. With the amine terminal groups in a cis configuration the doubly deprotonated bis complex with GAG exhibits serious steric crowding due directly to the L-alanine residues. However, if the amine terminal groups were in a trans arrangement there would be no steric hindrance to formation of doubly deprotonated bis complexes for any of the three L-alanine containing tripeptides. Nevertheless, the previously cited evidence indicates that the bis complex of GAG does not form. It is therefore proposed that the doubly deprotonated bis(tripeptide) complexes of copper(II) preferentially exist in a configuration in which the amine terminal (and N-peptide) groups are cis to one another. The questions which then arise are why is the cis configuration preferred to the trans and if GAG cannot form a cis complex why does it not form a trans complex? A possible answer to these two questions is that the strong σ-donor ability of a deprotonated peptide nitrogen weakens the bonding of copper(II) to another deprotonated peptide nitrogen in the trans position. This not only causes a preference for the cis configuration but also is sufficient to render the mono complex of GAG ($Cu(H_{-2}GAG)^-$) more stable than the trans bis complex. The competition with the mono complex is

important because it is possible to have deprotonated N-peptide groups trans to one another when the structure forces this arrangement. For example, $Cu(H_{-3}GGGG)^{2-}$ has such a structure but the last proton is not ionized until pH 9. The point to be made from the present observations is that the deprotonated N-peptide groups prefer not to be trans to each other.

Attempts were made to prepare the bis complex of GAG under different conditions to test the possibility that the cis isomer is a kinetically preferred rather than a thermodynamically preferred product. The complex was prepared at pH 5.6 where the species distribution shows that the concentration of $Cu(GGG)_2^\circ$ is at a maximum. The solution was allowed to stir for 10–20 min and then was rapidly (<10 sec) raised to pH ~10. The visible absorption spectrum of the solution was measured and found to match that of the 1:1 complex to within ~5%. In addition, long standing (3–4 days) did not cause significant spectral changes.

2. The Configuration of Bis(glycinamidato)copper(II) ($Cu(H_{-1}Ga)_2$). Glycinamide forms a doubly deprotonated bis complex with copper(II) ($Cu(H_{-1}Ga)_2$).[6,18] The two possible square-planar geometrical isomers are shown as structures V (cis) and VI (trans). In contrast to the tripeptides and diglycine (discussed later) there should be no steric interference for the cis structure. On the basis of electrostatic repulsion of the deprotonated peptide nitrogen groups in the cis structure, the trans geometry would appear to be favored. However, a strong σ donor is postulated to weaken the bonding of a metal ion to a trans group.[19] Therefore, two strong donors would prefer not to assume a trans configuration if an alternate geometry were available.



V (cis)

VI (trans)

Using the four Cu–N bonds as a basis set, the cis structure belongs to the C_{2v} point group which has no center of symmetry, and the trans geometry belongs to the D_{2h} point group which is centrosymmetric. Therefore, the Cu–N stretching vibrations which are coincident in the ir and Raman in the C_{2v} point group but mutually exclusive in the D_{2h} point group may be used as the basis for the assignment of the geometry of the solid complex.

A normal coordinate analysis has been performed on bis(glycinato)copper(II) and the copper–nitrogen stretching frequencies were assigned to vibrations occurring at 454 and 471 cm^{-1} in the cis isomer and at 477 cm^{-1} in the trans isomer.[20] A comprehensive study of the infrared spectra of bis(amino acidato)copper(II) complexes recently appeared in which the assigned copper–nitrogen stretching frequencies vary from 411 to 493 cm^{-1}.[21] In addition, the Cu–N stretching frequency in $K_2Cu(biuret)_2$ is assigned at 405 cm^{-1}.[22] Therefore, it seems reasonable to assume that the range 400–500 cm^{-1} should include all Cu–N stretching vibrations for $Cu(H_{-1}Ga)_2$. The results shown in Table V reveal that in the 400–500-cm^{-1} region there are only two infrared-active bands and they are both coincident with Raman-active bands. This indicates that $Cu(H_{-1}Ga)_2$ exists in the cis geometry in the solid state. It also indicates that the preference for cis N-peptide bonding may be a quite general and important phenomenon in copper(II)–oligopeptide complexes.

Table V. Observed Spectra (600–200 cm^{-1}) of Solid $Cu(H_{-1}Ga)_2$ and Glycinamide·HCl

$Cu(H_{-1}Ga)_2$ (Raman)	$Cu(H_{-1}Ga)_2$ (ir)[a]	Ga·HCl (ir)[a]
		205 s
249	250 sh	
265		265 m
	295 sh	290 sh
320		
332	333 m	
380		
404	401 s	
436		440 sh
464	466 s	475 m
		500 w
534	530 s	540 s

[a] The bands are strong (s), medium (m), weak (w), or shoulders (sh).

3. Additional Evidence. In addition to the evidence just cited for $Cu(H_{-1}tripeptide)_2^{2-}$ complexes and $Cu(H_{-1}Ga)_2$, other evidence has been found which supports the proposed preference for cis rather than trans N-peptide bonding.

In particular, in $Cu(H_{-1}GG)_2^{2-}$, a complex in which the N-peptide groups are in a trans configuration, the reported Cu–N(peptide) bond lengths are 1.97 Å[23] which is significantly longer than that reported for glycylglycinatocopper(II) trihydrate (1.89 Å)[24] and the average Cu–N(peptide) bond distance of 1.92 Å as reported in an extensive review by Freeman.[25] This significant difference in bond lengths supports the proposed importance of the preference for cis N-peptide bonding in copper(II) oligopeptide complexes.

That $Cu(H_{-1}GG)_2^{2-}$ adopts a trans configuration while $Cu(H_{-1}GGG)_2^{2-}$ prefers a cis arrangement may be explained by examination of molecular models which reveal that the carboxylate groups of the two diglycine molecules are very close to one another in the cis configuration and would be expected to generate significant electrostatic repulsive forces which are absent in the trans configuration. This could be enough to make the cis isomer unstable with respect to the trans isomer. Since *cis*-$Cu(H_{-1}GGG)_2^{2-}$ would be expected to have "normal" Cu–N(peptide) bond lengths, one would expect to see a significant difference between the pK_a values for the deprotonation of the second peptide

(18) S. P. Datta and B. A. Rabin, *Trans. Faraday Soc.*, **52**, 1117 (1956).
(19) C. H. Langford and H. B. Gray, "Ligand Substitution Processes," W. A. Benjamin, New York, N. Y., 1965, p 25.
(20) R. A. Condrate and K. Nakamoto, *J. Chem. Phys.*, **42**, 2590 (1965).
(21) A. W. Herlinger, S. L. Wenhold, and T. V. Long, II, *J. Amer. Chem. Soc.*, **92**, 6474 (1970).
(22) B. B. Kedzin, P. X. Armendarez, and K. Nakamoto, *J. Inorg. Nucl. Chem.*, **30**, 849 (1968).
(23) A. Sugihara, T. Ashida, Y. Sasada, and M. Kakudo, *Acta Crystallogr., Sect. B*, **24**, 203 (1968).
(24) H. C. Freeman and B. Strandberg, unpublished observation appearing in H. C. Freeman, *Advan. Protein Chem.*, **22**, 257 (1967).
(25) See reference given in footnote 24.

nitrogen in $Cu(H_{-1}GGG)GGG^-$ and $Cu(H_{-1}GG)GG^-$. The pK_a of $Cu(H_{-1}GGG)GGG^-$ (8.7) is, in fact, 2.4 log units lower than that of $Cu(H_{-1}GG)GG^-$ (11.1)[26] thus confirming the prediction.

Conclusions

(1) An equilibrium exists in solution between mono- and bis(triglycinato)cuprate(II) and the interconversion is catalyzed by hydroxide ion. (2) Protonated peptide nitrogen groups are very poor nucleophiles and hydroxide ion catalyzes the interconversion by deprotonating the incoming peptide nitrogen, thereby enhancing its nucleophilic character. (3) The bis(triglycinato)cuprate(II) complex involves both triglycine molecules bonding in a square-planar cis bidentate configuration. (4) The preference for the cis rather than the trans configuration for the copper complexes appears to be due to the strong σ-donor ability of deprotonated peptide nitrogen atoms. This effect may be important in the bonding of copper(II) in polypeptide complexes of biological significance.

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(26) M. Sheinblatt and E. D. Becker, *J. Biol. Chem.*, **242**, 3159 (1967).

Effect of Potentially Coordinating Amino Acid Side Chains on the Cobalt(III) Promoted Hydrolysis of Peptides and Esters Containing Trifunctional Amino Acids

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Abstract: The cobalt(III) promoted hydrolysis of the diethyl ester of L-aspartic acid and dipeptides containing aspartic acid, glutamic acid, and methionine is reported. $Co(trien)(OH)(H_2O)^{2+}$ promotes the hydrolysis of glycyl-L-aspartic acid and glycyl-L-glutamic acid to form the free amino acids and $Co(trien)(gly)^{2+}$, but, contrary to previous studies of the simpler dipeptides N-terminal in glycine, an intermediate of considerably greater stability is formed. From deuterium isotopic exchange of the glycine methylene protons, infrared spectra in D_2O, and circular dichroism spectra, it is postulated that the dipeptides are chelated through the N-terminal amine and amide carbonyl. Interaction of the complex with the potentially hydrogen-bonding side chain on the penultimate trifunctional amino acid is invoked as a factor in the stabilization of the intermediate. This is substantiated by the relative ease of hydrolysis of glycyl-DL-methionine which has a side chain of comparable bulk but with little tendency to hydrogen bond. If the trifunctional amino acid is N-terminal as in α-L-aspartylglycine and L-aspartic acid diethyl ester, essentially no hydrolysis is observed when the "two-site" complex $Co(trien)X_2$ is used. However, the three-site complex $Co(dien)X_3$ promotes hydrolysis with the formation of $Co(dien)(L\text{-}Asp)^+$ where the amino acid is coordinated as a tridentate.

It has been known for some time that certain metal ion complexes promote hydrolysis of simple esters and peptides.[1-3] Of particular interest have been the studies with cobalt(III) initiated by Buckingham and Collman.[2] Tetraaminecobalt(III) complexes with two cis reactive coordination sites, such as *cis*-$[Co(en)_2(OH)(H_2O)]^{2+}$,[4] *cis*-$\beta$-$[Co(trien)(OH)(H_2O)]^{2+}$, and *cis*-$[Co(tren)(OH)(H_2O)]^{2+}$,[4] have been found to effect the hydrolysis of bidentate amino acid residues from amino acid esters, amides, and simple peptides. In aqueous solution it was found that the cationic chelates promote hydrolysis by chelation of the N-terminal amino acid forming $[Co(N_4)(\text{amino acid})]^{2+}$ chelates, where N_4 represents the tetradentate amine ligand. The N-terminal specificity of the reaction was shown by the characterization of the amino acid complexes and residual amino acid or peptide formed. In the case of some simple peptides it was shown that the N-terminal bidentate amino acid can be cleaved in a sequential manner.[2]

Thus far only simple di- and tripeptides and, in a few cases, tetrapeptides of the general formula $NH_2CHR\text{-}CONHR'$, where R′ is the remainder of the peptide and R the side chain, have been studied, but in all cases investigated R was a noncoordinating group. For these peptides, tetradentate cobalt(III) amine chelates were used to cleave the N-terminal bidentate amino acid since they can provide two cis coordinating sites for the interaction of the metal ion with the N-terminal, bidentate amino acid.

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(1) D. A. Buckingham and J. P. Collman, *Inorg. Chem.*, **6**, 1803 (1967); E. Kimura, S. Young, and J. P. Collman, *ibid.*, **9**, 1183 (1970).

(2) D. A. Buckingham, J. P. Collman, D. A. R. Harper, and L. G. Marzilli, *J. Amer. Chem. Soc.*, **89**, 1082 (1967); J. P. Collman and D. A. Buckingham, *ibid.*, **85**, 3039 (1963).

(3) M. D. Alexander and D. H. Busch, *ibid.*, **88**, 1130 (1966).

(4) The following abbreviations are used throughout this paper: en = ethylenediamine, dien = diethylenetriamine, trien = triethylenetetramine, tren = 4-(2-aminoethyl)diethylenetriamine.