Ethylenediamine and Diethylenetriamine Reactions with Copper(II)–Triglycine

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Abstract: Ethylenediamine reacts with the doubly deprotonated copper(II)-triglycine complex ($CuH_{-2}L^-$) to give a mixed complex with only one hydrogen ionized from the peptide nitrogens, $CuH_{-1}Len$. The kinetics of formation of $CuH_{-1}Len$ are first order in [$CuH_{-2}L^-$] and first order in unprotonated en with a rate constant of 4.8×10^6 M^{-1} sec^{-1} (25.0°, 0.1 M $NaClO_4$). The proton adds after the rate-determining step. In the presence of excess en the mixed complex reacts by a dissociation step ($k_d{}^{CuH_{-1}Len} = 1.3$ sec^{-1}) and by en attack ($k_{en}{}^{CuH_{-1}Len} = 4.5 \times 10^3$ M^{-1} sec^{-1}) to give $Cu(en)_2{}^{2+}$. Diethylenetriamine also forms a stable mixed complex, $CuLdien^+$, but with trien mixed complexes are not observed in equilibrium or in kinetic studies. The rate constants for the reaction of en, dien, and trien with $CuH_{-2}L^-$ are nearly the same. The deprotonated-peptide-nitrogen bond to copper is kinetically much slower to dissociate than the amine bond to copper, but is readily activated by an amine chelate.

Two mechanisms have been reported[3,4] for ligand-exchange reactions with $CuH_{-2}L^-$ (where L^- is the glycylglycylglycinate ion and protons are ionized from the two peptide nitrogens coordinated to copper). In one mechanism a proton is transferred to a peptide nitrogen before the exchanging ligand is able to react. In the other mechansim the exchanging ligand acts as a nucleophile and the rate-determining step occurs before protons are added to the peptide nitrogens. Triethylenetetramine (trien) reacts with $CuH_{-2}L^-$ by the nucleophilic mechanism and produces $Cu(trien)^{2+}$ and L^- with no observable reaction intermediates. In the present work diethylenetriamine (dien) and ethylenediamine (en) are shown to form stable mixed complexes with copper–triglycine. They react by the nucleophilic mechanism and their kinetic behavior indicates several distinct steps which occur in the polyamine displacement of triglycine. It is possible to characterize the reaction path in some detail owing to the extreme basicity of the deprotonated-peptide nitrogen when not bonded to copper. The pH dependence of the observed kinetics serves to indicate if any copper–peptide-nitrogen bonds are broken before the rate-determining step.

Experimental Section

Diethylenetriamine (Aldrich) was purified by distillation under reduced pressure. Ethylenediamine was used as received (Baker Analyzed reagent). The polyamine solutions were standardized by acid–base titration or by a mole ratio method with copper(II). The copper(II)–triglycine complex was prepared from twice-recrystallized copper perchlorate and from triglycine (chromatographically homogeneous, Mann Research Laboratories, N. Y.). The copper–triglycine solutions were freshly prepared before each series of reactions. Boric acid or sodium tetraborate was used as a buffering agent (total borate = 0.02 M). All work was at 25.0 ± 0.1°. Ionic strength was maintained at 0.10 M with $NaClO_4$. The hydrogen ion concentrations were calculated from pH measurements by the relationship $-\log [H^+] = pH - 0.10$.[5,6] The value used for pK_w was 13.75, determined by $-\log [H^+]$ measurements on solutions of known hydroxide ion concentration in 0.10 M $NaClO_4$.

Kinetic runs were followed spectrophotometrically at 230–240 nm or at 550 nm on a Durrum-Gibson stopped-flow instrument (Durrum Instrument Corp., Palo Alto, Calif.) with a 2-cm cell path and equipped with a calibrated per cent transmittance offset. Each rate constant is the average of at least three determinations.

Potentiometric titrations were performed using a Beckman research pH meter equipped with Sargent electrodes. Buffer solutions of pH 4.01, 6.86, and 9.18 were used to standardize the electrodes. An atmosphere of nitrogen was maintained over the thermostated solution during the titration. The titrant was 0.1 M NaOH (carbonate free) and was delivered from a calibrated micrometer syringe of 2-ml capacity. The initial concentrations of copper, triglycine, polyamine, and perchloric acid are listed in Table I. The ligands were titrated individually as well as in mixtures with copper.

Table I. Summary of Conditions Used for Potentiometric Titrations[a]

Initial concentrations, $M \times 10^3$					$-\log [H^+]$	No. of data
Cu_T	en_T	$dien_T$	L_T	$HClO_4$	range	points
			2.132	0.000	5.5–10.0	27
			2.132	2.112	3.0–10.3	50
2.012			2.132	0.000	4.3–10.6	43
1.008			1.068	0.000	4.5–10.1	41
	2.114			5.290	2.9–10.8	23
1.008	2.114			4.232	4.0–10.4	28
		1.982		5.280	4.0–11.0	63
		0.993		5.280	4.0–10.5	35
		2.124		5.280	4.3–10.8	56
1.008		1.064		5.280	3.2–10.7	33
1.008		2.124		5.280	3.2–10.5	50
1.008		1.064	1.068	4.224	4.0–10.7	38
1.008		1.064	2.132	4.224	3.2–10.7	53
1.008		1.067	1.037	4.232	3.9–10.7	39
1.008		1.067	4.140	2.116	3.7–10.6	49
2.012		2.130	4.140	0.000	6.2–10.7	37
1.008	1.059		1.037	1.060	4.8–10.7	26
1.008	1.059		1.037	2.116	4.1–10.5	35

[a] Initial volume = 50.0 ml, μ = 0.10 M ($NaClO_4$), 25.0°, titrant = 0.1050 M NaOH.

Equilibrium constants for the copper–triglycine and copper–dien complexes and for systems containing all three components were calculated using a modified version of the computer program SCOGS.[7] The program is capable of calculating, from pH titration

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(3) G. K. Pagenkopf and D. W. Margerum, *J. Amer. Chem. Soc.*, **90**, 6963 (1968).

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(7) I. G. Sayce, *Talanta*, **15**, 1397 (1968).

data, association constants for any of the species formed in systems containing up to two metals and two ligands. Refinement of initial estimates of the constants is accomplished by minimizing the squares of the titrant volume residuals. Modifications to the program (which was run on a CDC 6500 computer) included the use of concentration constants rather than mixed constants involving hydrogen ion activity. Other equilibrium constants were calculated by conventional (*i.e.*, $\bar{n}$) methods.

The log β and log $^*\beta$ values which were determined and used in the calculations are given in Table II; β is the cumulative stability constant using the free ligand and aquocopper ion or hydrogen ion as reactants, while $^*\beta$ refers to similar constants in which protons are ionized. Thus, for a species such as $CuH_{-1}Len$,$^*\beta$ = $[CuH_{-1}Len][H^+]/([Cu^{2+}][L^-][en])$. In order to reproduce the experimental conditions used in the evaluation of the mixed stability constants all of the equilibrium constants were redetermined in the present work. The triglycine constants are in general agreement with those reported at similar ionic strength by other workers,[8-10] and in close agreement with the values of Brunetti, Lim, and Nancollas.[11] The constant for $CuH_{-2}L(OH)^{2-}$ corresponds to a pK_a value of 12.0, in agreement with the pK_a value found by Koltun, Roth, and Gurd[9] but differing from the pK_a value of 10.9 reported by Kim and Martell.[10] The ethylenediamine protonation and copper stability constants are in excellent agreement with those reported by Näsänen and coworkers,[12] although they differ from previously reported values.[13] The constants for the dien and copper-dien species are in general agreement with earlier values[14,15] but $Cu(dien)_2H^{3+}$ is a species which also must be considered.[16]

Table II. Triglycine and Polyamine Equilibrium Constants Determined for Separate and Mixed Complexes with Copper; 25.0°, μ = 0.10 *M* ($NaClO_4$)

Species	Log β (or $^*\beta$)[a,b]
HL	7.88 ± 0.01
H_2L^+	11.00 ± 0.04
CuL^+	5.12 ± 0.01[c]
$CuH_{-1}L$	0.01 ± 0.003[c]
$CuH_{-2}L^-$	−6.67 ± 0.005[c]
$CuH_{-2}L(OH)^{2-}$	−18.7 (pK_a = 12.0 ± 0.1)[d]
Hen^+	9.83 ± 0.005
H_2en^{2+}	16.88 ± 0.01
$Cu(en)^{2+}$	10.40 ± 0.003
$Cu(en)_2{}^{2+}$	19.36 ± 0.004
$Cu(en)OH^+$	2.7 ± 0.1
$Hdien^+$	9.80 ± 0.02
H_2dien^{2+}	18.76 ± 0.01
H_3dien^{3+}	22.96 ± 0.01
$Cu(dien)^{2+}$	16.02 ± 0.003[e]
$Cu(dien)_2{}^{2+}$	20.88 ± 0.008[e]
$Cu(dien)OH^+$	7.02 ± 0.009[e]
$Cu(dien)_2H^{3+}$	29.08 ± 0.03[e]
$CuLdien^+$	18.92 ± 0.05[f]
$CuLen^+$	14.88 ± 0.05[g]
$CuH_{-1}Len$	8.05 ± 0.03[g]

[a] β is a cumulative stability constant, *i.e.*, for H_2L^+, β = $[H_2L^+]/([H^+]^2[L^-])$. $^*\beta$ is a cumulative constant with proton loss, *i.e.*, for $Cu(en)OH^+$, $^*\beta$ = $[Cu(en)OH^+][H^+]/([Cu^{2+}][en])$. The constants are in terms of concentrations of all the species. [b] The precision is for one standard deviation. [c] Standard deviation of the titer in SCOGS was 8 μl, combining the two titrations given in Table I for solutions containing Cu^{2+} and L^-. [d] Spectrophotometric determination. [e] Standard deviation of the titer in SCOGS was 6.5 μl, combining the two titrations given in Table I for solutions containing Cu^{2+} and dien. [f] The individual titrations in Table I for solutions with Cu^{2+}, dien, and L^- gave standard deviations of the titer in SCOGS ranging from 14 to 31 μl and gave log β values ranging from 18.90 to 18.95. [g] Standard deviation of the titer in SCOGS was 8 μl, combining the two titrations given in Table I for solutions containing Cu^{2+}, en, and L^-.

Results

Diethylenetriamine. The potentiometric titrations give evidence of a mixed complex of copper–triglycine and dien. In Figure 1 an equimolar mixture of Cu(II), triglycine, and dien is titrated with base. Curve 1 is the expected titration curve if no mixed complex formed using all the constants in Table II except that for the mixed complex. Curve 2 includes these constants and the constant evaluated for $CuLdien^+$. The points along curve 2 are those actually observed and the solid line is obtained from the constants given in Table II. Calculations also were attempted for two additional mixed complexes, namely $CuH_{-1}Ldien$ and $CuH_{-2}Ldien^-$. However, only the $CuLdien^+$ complex was needed to fit the data and $\beta_{CuLdien} = [CuLdien^+]/[Cu^{2+}][L^-][dien] = 10^{18.92}$.

The absorption spectrum of $Cu(dien)^{2+}$ compared to a 1:1:1 mixture of copper:triglycine:dien (2×10^{-3} *M*) at pH 8.3 also gave evidence of a mixed complex.

Figure 2A shows the effect of acidity on the species distribution of the dien complexes when $Cu_T \cong dien_T \cong L_T \cong 6.5 \times 10^{-4}$ *M*. These concentrations were used in some of the kinetic studies.

Kinetic studies of the reaction between $CuH_{-2}L^-$ and dien at pH 8–10 required fast time scans with the stopped-flow apparatus. At 550 nm (a wavelength sensitive to the disappearance of $CuH_{-2}L^-$) and nearly equimolar reactants (6.48×10^{-4} *M* $CuH_{-2}L^-$ and 6.20×10^{-4} *M* dien) good second-order rate plots were found at the higher pH. The calculated species distribution of the equilibrium products are shown in Figure 2A. At $-\log [H^+] = 9.54$ the observed second-order rate constant was 1.6 (±0.1) × 10^6 M^{-1} sec^{-1}. When the pH was lowered between 8 and 9 more than one reaction could be detected. Attempts to study the reactions using much lower concentrations (4.3×10^{-5} *M*) at 260 nm, where $Cu(dien)^{2+}$ has an absorption peak, also indicated the presence of some kinetic intermediates containing copper, triglycine, and dien. The stepwise kinetics of the dien reaction were not investigated further because the ethylenediamine data which follow made it possible to construct a detailed mechanism for polyamine reactions. A rate constant of $\sim 4 \times 10^6$ M^{-1} sec^{-1} can be estimated for the reaction of unprotonated dien with $CuH_{-2}L^-$.

Ethylenediamine. The stability constants for the copper–ethylenediamine complexes and for copper–triglycine predict that an equimolar mixture of Cu(II), L^-, and en will give $Cu(en)_2{}^{2+}$ and $CuH_{-2}L^-$ as reaction products at pH 8–10. However, potentiometric titration data indicate that the complex $CuH_{-1}Len$ also is present in solution. The SCOGS program was used with assigned constants for the 11 species of triglycine, en, and copper given in Table II. A good fit was found

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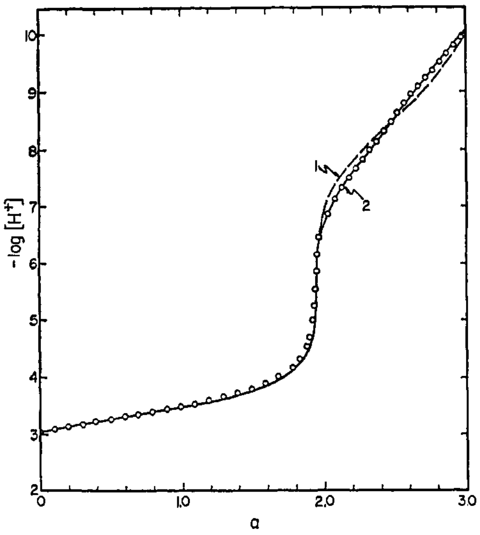


Figure 1. Potentiometric titration of a mixture of 1.01 × 10^{-3} M Cu(II), 1.07 × 10^{-3} M triglycine, 1.06 × 10^{-3} M dien, and 4.22 × 10^{-3} M $HClO_4$ in 50.0 ml of 0.105 M NaOH where a equals moles of base added per mole of copper. Curve 1 is calculated using all the constants except that for the mixed complex, while curve 2 includes the constant for $CuLdien^+$. The points are experimental.

using two additional constants for $CuH_{-1}Len$ and $CuLen^+$. The log β values calculated for these mixed complexes also are given in Table II and the stepwise constants with protonated ethylenediamine are $[CuH_{-1}Len]/[CuH_{-2}L^-][Hen^+] = 10^{4.98}$ and $[CuLen^+]/[CuH_{-2}L^-][H_2en^{2+}] = 10^{4.67}$.

The $CuH_{-2}Len^-$ species was considered in the calculations but evidence was not found for an appreciable concentration of this complex in the titration experiments. The species distribution at 3.24 × 10^{-4} M concentrations of total copper, triglycine, and ethylenediamine are given in Figure 2B.

The rate of the reaction between en and $CuH_{-2}L^-$ was followed at 230 nm with en in 20–40-fold excess over the total copper concentration of 2 × 10^{-5} M. At pH 8.4 two first-order reactions were observed, one 100–200 times faster than the other. In accord with the titration data $CuH_{-1}Len$ would be the most likely observable intermediate and a strong candidate for the first product. The final product with the large excess of en present is $Cu(en)_2^{2+}$.

The faster reaction had a half-life of 5–10 msec and a small change in transmittance in the ultraviolet region. Therefore, it was examined in more detail in the visible region (550 nm) without the use of a large excess of en. A single reaction was observed where en was in twofold or greater concentration over $CuH_{-2}L^-$. However, with a smaller en ratio the transmittance passed through a maximum as seen in Figure 3.

An absorption spectrum at pH 8.4 of a mixture of copper, triglycine, and en identical with that used in the potentiometric titration permitted the determination of the molar absorptivity of $CuH_{-1}Len$. The values at 550 nm for $CuH_{-2}L^-$, $Cu(en)_2^{2+}$, and $CuH_{-1}Len$ are 150, 64, and 70 M^{-1} cm^{-1}, respectively. The disappearing maximum as a function of en ratio in the kinetic data at 550 nm can now be understood. Below 2:1 ratios of en_T/Cu_T the $CuH_{-1}Len$ complex first forms, causing an increase in % T, and then partially disproportionates to $Cu(en)_2^{2+}$ and $CuH_{-2}L^-$, causing a decrease in % T. Above 2:1 ratios $CuH_{-1}Len$ again forms but is converted by a second reaction (observable in the ultraviolet) to $Cu(en)_2^{2+}$ with so little a net change in % T that the second reaction is not easily detected at 550 nm.

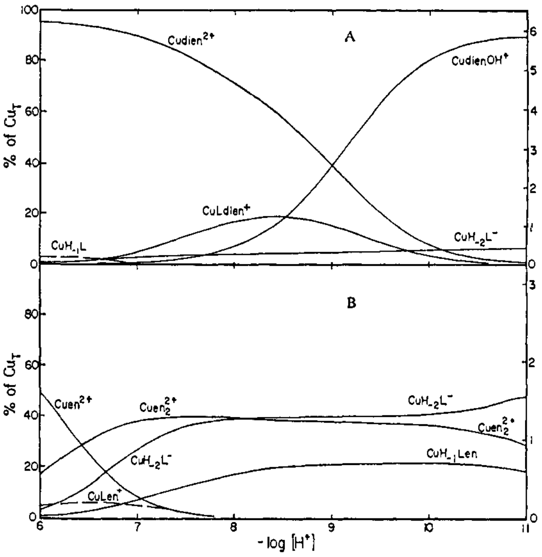


Figure 2. Species distribution of copper–triglycine–polyamine complexes as a function of −log [H^+]. The right-hand ordinate gives molarity × 10^4: (A) total copper = 6.48 × 10^{-4} M, total dien = 6.20 × 10^{-4} M, total triglycine = 6.64 × 10^{-4} M; (B) total copper = 3.24 × 10^{-4} M, total en = 3.24 × 10^{-4} M, total triglycine = 3.24 × 10^{-4} M.

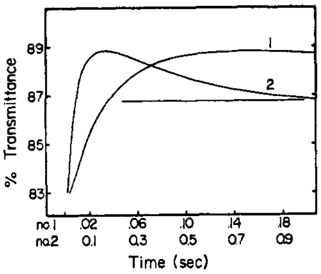


Figure 3. Stopped-flow trace of the ethylenediamine reaction with copper–triglycine at −log [H^+] = 8.21, 550 nm, 25.0°, μ = 0.1 M $NaClO_4$, $[en]_T$ = 3.40 × 10^{-4}, $[CuH_{-2}L^-]_{initial}$ = 3.24 × 10^{-4}. Curve 1 refers to the 0–0.2-sec time scale and curve 2 refers to the same reaction mixture observed on the 0–1-sec time scale.

Table III summarizes the kinetic data for the faster reaction studied at 550 nm. The reactions were first order in $CuH_{-2}L^-$ and first order in en_T and k_{obsd} is based on these reactants. The rate increases with in-

Table III. Kinetics for the Reaction of Ethylenediamine with $CuH_{-2}L^-$ to Give $CuH_{-1}Len$[a]

$10^4[en_T]$, M	−Log [H^+]	$10^{-5}k_{obsd}$, M^{-1} sec^{-1}
0.85	8.24	1.0[b]
1.70	8.27	1.3 ± 0.2
3.40	8.31	1.4[b]
4.25	8.21	1.4[b]
5.10	8.28	1.3[b]
3.40	8.72	3.0 ± 0.1
4.25	8.72	2.9 ± 0.1
8.50	8.72	3.6 ± 0.2

[a] 25.0°, μ = 0.1 M ($NaClO_4$), 550 nm, $[CuH_{-2}L^-]_i$ = 3.24 × 10^{-4} M. [b] Single runs.

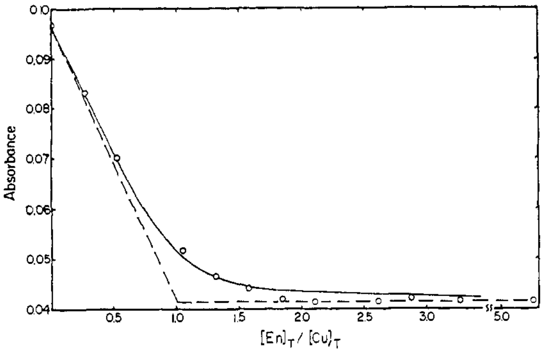


Figure 4. Kinetic mole ratio plot of the absorbance of the intermediate species with en. $[CuH_{-2}L^-]_{initial} = 3.24 \times 10^{-4}$, $[en]_T = (0.85–17.0) \times 10^{-4}$, $-\log[H^+] = 8.29$, $\mu = 0.1$ *M* NaClO$_4$, 25.0°, 550 nm.

creasing pH in proportion to the amount of unprotonated en, and in terms of this species the value for $k_{en}^{CuH_{-2}L}$ is $(4.8 \pm 0.4) \times 10^6$ M^{-1} sec^{-1}.

The kinetic data leave little doubt that en adds in a stepwise manner to copper–triglycine. In order to prove that the observed kinetic intermediate was the same as that found potentiometrically under equilibrium conditions, a kinetic mole ratio plot was used. The absorbances of the maxima in reactions such as that in Figure 3 are plotted against $[en]_T/[Cu]_T$ in Figure 4. The plot shows that a 1:1 complex has formed and that the apparent equilibrium constant for the complex is $(7.8 \pm 1.4) \times 10^4$ M^{-1} at $-\log[H^+] = 8.27$. Similar plots at $-\log[H^+] = 9.06$ gave an apparent constant of $(6.6 \pm 0.7) \times 10^4$ M^{-1} and at $-\log[H^+] = 8.71$ a value of $(6.3 \pm 0.1) \times 10^4$ M^{-1}. These equilibrium constants can be resolved into a single constant with a log value of 4.9 ± 0.1 for the formation of $CuH_{-1}Len$ from $CuH_{-2}L^-$ and Hen^+. This is in excellent agreement with the value found potentiometrically and therefore the product of the first reaction is identified as $CuH_{-1}Len$. As an additional check the reaction was followed in the presence of indicator on the stopped-flow using equimolar reactants (3.24×10^{-4} *M*) each at pH 8.4 and without buffer. Chlorophenol red (pK_a = 5.90 ± 0.05) at 435 nm and thymol blue (pK_a = 8.81 ± 0.02) at 430 nm both indicated that only small pH changes took place in the formation of the first product. At this pH the main ethylenediamine species is Hen^+. A large release of H^+ would be required to produce $CuH_{-2}Len^-$ as a product and large uptake of H^+ would be needed to give $CuLen^+$, while the small change observed agrees with $CuH_{-1}Len$ as a product.

Although $CuH_{-1}Len$ is the first observable product the pH dependence of the kinetics requires the reactant to be en and not Hen^+. Therefore the reaction mechanism is

$$CuH_{-2}L^- + en \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} (CuH_{-2}Len^-) \qquad (1)$$

$$(CuH_{-2}Len^-) + H_2O \xrightarrow{k_2} CuH_{-1}Len + OH^- \qquad (2)$$

where $CuH_{-2}Len^-$ is a reaction intermediate and $k_{en}^{CuH_{-2}L} = k_1k_2/(k_{-1} + k_2)$. Additional evidence that the concentration of $CuH_{-2}Len^-$ never becomes appreciable under the conditions given in Table III was provided by the indicator experiments.

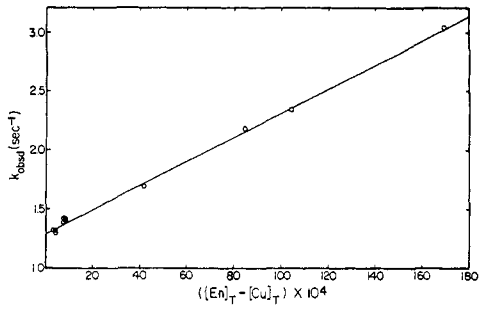


Figure 5. Observed first-order rate constants for the conversion of $CuH_{-1}Len$ to $Cu(en)_2^{2+}$ as a function of the excess en concentration, $-\log[H^+] = 8.22$, 25.0°, $\mu = 0.10$ *M* NaClO$_4$.

The slower reaction observed in the ultraviolet region with excess en is the conversion of $CuH_{-1}Len$ to $Cu(en)_2^{2+}$. The reactions were first order in the copper complex and increased with increasing en as shown in Figure 5. The rate expression and en dependence are given in eq 3 and 4. The observed rate constants at pH 8.3 and 9.3 are given in Table IV. These data gave en-independent rate constants of 1.29 and 1.24 sec^{-1} for

$$d[Cu(en)_2^{2+}]/dt = k_{obsd}([Cu_T] - [Cu(en)_2^{2+}]) \qquad (3)$$

$$k_{obsd} = k_d^{CuH_{-1}Len} + k_{en}^{CuH_{-1}Len}[en] \qquad (4)$$

pH 8.3 and 9.2, respectively. The dependence in en_T was 102 ± 2 M^{-1} sec^{-1} at $-\log[H^+] = 8.22 \pm 0.03$ and 878 ± 3 M^{-1} sec^{-1} at $-\log[H^+] = 9.22 \pm 0.04$. This fits a dependence in free en with negligible Hen^+ contribution. The value of the rate constant, $k_{en}^{CuH_{-1}Len}$, is $(4.48 \pm 0.02) \times 10^3$ M^{-1} sec^{-1}.

Table IV. Observed First-Order Rate Constants for the Reaction of $CuH_{-1}Len$ with Excess Ethylenediamine[a]

Initial [Cu$_T$] × 10^5 *M*	Initial [en$_T$] × 10^4 *M*	$-$Log [H$^+$]	k_{obsd}, sec^{-1}
2.16	4.47	8.17	1.32 ± 0.06
2.16	4.47	8.24	1.30 ± 0.05
2.16	8.94	8.23	1.40 ± 0.06
2.16	8.94	8.25	1.41 ± 0.05
4.32	4.25	8.20	1.32 ± 0.04
4.32	8.50	8.18	1.42 ± 0.07
4.32	8.50	8.26	1.39 ± 0.05
4.32	42.5	8.20	1.69 ± 0.07
4.32	85.0	8.26	2.16 ± 0.06
4.32	106	8.25	2.3 ± 0.1
4.32	170	8.21	3.0 ± 0.1
2.16	4.47	9.20	1.72 ± 0.02
2.16	8.94	9.23	2.2 ± 0.1
4.32	8.74	9.14	1.78 ± 0.08
4.32	8.94	9.25	2.2 ± 0.1
4.32	17.5	9.17	2.4 ± 0.1
4.32	42.5	9.23	4.72 ± 0.07
4.32	425	9.23	38 ± 4
4.32	850	9.28	76 ± 3

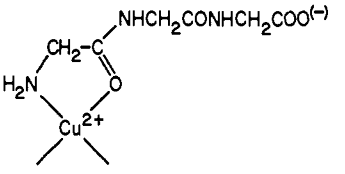
[a] 25.0°, $\mu = 0.1$ *M* (NaClO$_4$), 230 nm.

Discussion

There is a regular progression in the reaction of polyamines with copper-triglycine in that en forms both

$CuH_{-1}Len$ and $CuLen^+$ while dien forms only $CuLdien^+$ and trien forms no stable mixed complex but gives only $Cu(trien)^{2+}$. This is consistent with four strong coordinate bonds to copper in a square-planar configuration and a maximum triglycine coordination of $4 - n$ when a polyamine of n nitrogens is used.

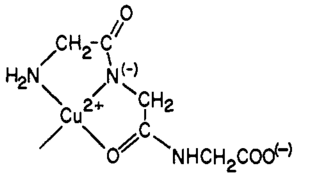
Measurements of $\Delta H°$ and $\Delta S°$ values for copper–triglycine and other complexes has led to the conclusion[11] that CuL^+ in solution is chelated through the amine nitrogen and the peptide oxygen (structure I). This bonding also is found in crystalline solids.[17] It is well established that coordination of the peptide nitrogen occurs in the deprotonated complexes.[17] Structures II and III represent the triglycine species in solution. A comparison of the log K values in Table V (reactions 5, 6, and 7) shows that the addition of L^- to $Cu(en)^{2+}$ is only 0.64 unit less than the addition of L^- to $Cu_{aq}{}^{2+}$, but the addition of L^- to $Cu(dien)^{2+}$ is 2.22 units less. These facts suggest structures IV and V for $CuLen^+$ and $CuLdien^+$, where triglycine in V cannot form a chelate with copper in the coordination plane and is therefore held much less strongly than in IV.



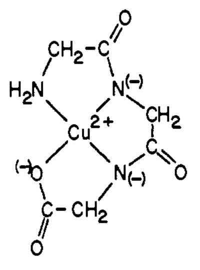
I (CuL^+)

Table V. Comparison of Equilibrium Constants for the Reaction of Various Species

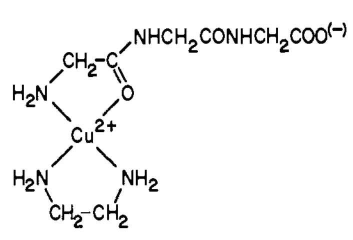
	Reaction	Log K
5	$Cu^{2+} + L^- \rightarrow CuL^+$	5.12
6	$Cu(en)^{2+} + L^- \rightarrow CuLen^+$	4.48
7	$Cu(dien)^{2+} + L^- \rightarrow CuLdien^+$	2.90
8	$Cu^{2+} + L^- \rightarrow CuH_{-1}L + H^+$	0.01
9	$Cu(en)^{2+} + L^- \rightarrow CuH_{-1}Len + H^+$	−2.35
10	$Cu^{2+} + en \rightarrow Cu(en)^{2+}$	10.40
11	$CuL^+ + en \rightarrow CuLen^+$	9.76
12	$Cu(en)^{2+} + en \rightarrow Cu(en)_2{}^{2+}$	8.96
13	$CuH_{-1}L + en \rightarrow CuH_{-1}Len$	8.04
14	$Cu^{2+} + dien \rightarrow Cu(dien)^{2+}$	16.02
15	$CuL^+ + dien \rightarrow CuLdien^+$	13.80
16	$Cu(dien)^{2+} + dien \rightarrow Cu(dien)_2{}^{2+}$	4.86
17	$CuH_{-2}L^- + H_2dien^{2+} \rightarrow CuLdien^+$	6.83
18	$CuH_{-2}L^- + Hen^+ \rightarrow CuH_{-1}Len$	4.89
19	$CuH_{-2}L^- + H_2en^{2+} \rightarrow CuLen^+$	4.67



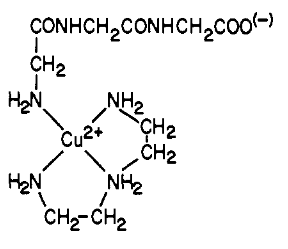
II ($CuH_{-1}L$)



III ($CuH_{-2}L^-$)

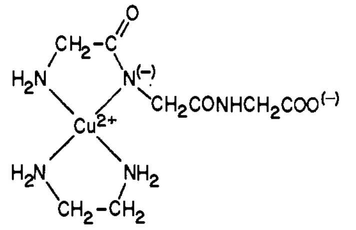


IV ($CuLen^+$)



V ($CuLdien^+$)

Structure VI is proposed for the $CuH_{-1}Len$ complex. In this case the planarity of the triglycine segment bonded to copper will tend to prevent the second peptide oxygen from reaching an axial coordination position and en blocks its in-plane copper coordination. This is reflected in the fact that the coordination of en to copper has a much bigger effect on the stability of the $H_{-1}L^{2-}$ segment of triglycine (compare structures II and VI) than it does on the L^- segment (compare structures I and IV). A comparison of reactions 8 and 9 in Table V shows that the stability of the $H_{-1}L^{2-}$ segment is 2.36 log units greater with aquocopper than it is with $Cu(en)^{2+}$. On the other hand, a comparison of reactions 5 and 6 shows that the stability of the L^- segment is only 0.64 log unit greater with Cu^{2+} than it is with $Cu(en)^{2+}$.



VI ($CuH_{-1}Len$)

In reactions 10, 11, 12, and 13 there is a regular decrease in the ability of en to add to Cu^{2+}, CuL^+, $Cu(en)^{2+}$, and $CuH_{-1}L$. For the first three of these reactions this is consistent with the greater donor strength of the amine group compared to water or to the peptide oxygen. The copper–N(peptide) bond is significantly shorter than the copper–amine bonds,[17] and the negative peptide nitrogen may be a better donor group than the amine. However, the diminished stability of en in reaction 13 compared to reaction 12 must also take into account the need to break a copper–O(peptide) bond in structure II in order to form the mixed complex in structure VI. Hence the effect of donor strength of the negative peptide nitrogen and the amine nitrogen cannot be directly evaluated from these data.

The difference in the log K values for reactions 14 and 15 *vs.* the difference for reactions 10 and 11 is consistent with the proposed structures.

The log K value for reaction 18 indicates that $CuH_{-1}Len$ is a stable complex relative to the main reactant species present in solution in the kinetic studies. This constant was obtained from the kinetic mole ratio plots as well as from the potentiometric titrations.

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(17) H. C. Freeman *Advan. Protein Chem.*, 22, 257 (1967).

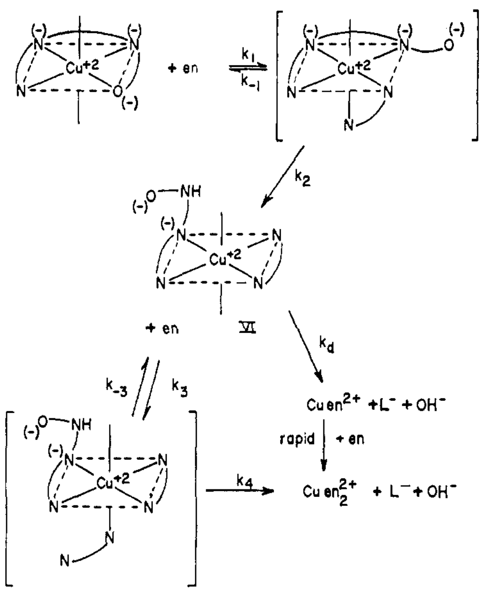

Figure 6. Proposed mechanism for the reactions of ethylenediamine with copper–triglycine ($CuH_{-2}L^-$). $H_{-2}L^{3-}$ is represented by $N-N^--N^--O^-$ and en is represented by N–N. Water may be coordinated to any of the structures but is not shown.

Table VI summarizes the rate constants for polyamine and ammonia reactions with copper–triglycine. The rate constants leading to the first peptide-bond cleavage from copper are the same order of magnitude for trien, dien, and en, but the rate constant for ammonia is a factor of 10^5 smaller. The presence of a diamine chelate ring in the attacking nucleophile greatly accelerates the dissociation of the N(peptide) bond from copper. Chelation appears to be an extremely important factor in the acceleration of several other square-planar substitution reactions including those of the $Ni(CN)_4^{2-}$ and the nickel(II)–triglycine complexes.[18,19]

Table VI. Summary of Copper–Triglycine Rate Constants[a] for Reactions with Rate-Determining Step before or during Peptide-Nitrogen Bond Cleavage from Copper

	First Cleavage
$k_{\text{trien}}^{CuH_{-2}L}$	$(11 \pm 4) \times 10^6\ M^{-1}\ sec^{-1}$ (ref 4)
$k_{\text{dien}}^{CuH_{-2}L}$	$\sim 4 \times 10^6\ M^{-1}\ sec^{-1}$
$k_{\text{en}}^{CuH_{-2}L}$	$(4.8 \pm 0.4) \times 10^6\ M^{-1}\ sec^{-1}$
$k_{NH_3}^{CuH_{-2}L}$	$29\ M^{-1}\ sec^{-1}$ (ref 4)
$k_d^{CuH_{-2}L}$ (in the presence of EDTA)	$0.12\ sec^{-1}$ (ref 3)
$k_{H_2O}^{CuH_{-2}L}$	$3.2 \times 10^{-5}\ M^{-1}\ sec^{-1}$ (ref 3)
	Second Cleavage
$k_{\text{en}}^{CuH_{-1}Len}$	$(4.48 \pm 0.02) \times 10^3\ M^{-1}\ sec^{-1}$
$k_d^{CuH_{-1}Len}$ (in the presence of en)	$1.27 \pm 0.03\ sec^{-1}$

[a] 25.0°, $\mu = 0.1\ M\ (NaClO_4)$.

The rate constant for the reaction of H_2O with $CuH_{-2}L^-$ to give $CuH_{-1}L$ and OH^- is very small. The Cu–N(peptide) bond cleavage appears to be very sluggish when not assisted by acids or by a nucleophilic reactant other than the solvent. The first-order rate constant $k_d^{CuH_{-2}L}$ was obtained in the presence of EDTA and is attributed to a molecular rearrangement of $CuH_{-2}L^-$ to a form that can react with EDTA as opposed to a solvent dissociation of the Cu–N(peptide) bond. Similarly, the first-order constant $k_d^{CuH_{-1}Len}$ was obtained in the presence of en. This constant may correspond to a rate-determining step in the solvent dissociation of the $H_{-1}L^{2-}$ group in $CuH_{-1}Len$ as shown in Figure 6. Alternatively, the $k_d^{CuH_{-1}Len}$ rate constant could correspond to the first-order rearrangement of structure VI to a form which would react rapidly with en.

Ethylenediamine reacts 10^3 faster with $CuH_{-2}L^-$ than it does with $CuH_{-1}Len$. This may seem surprising at first because with $CuH_{-2}L^-$ the copper–carboxylate bond must be broken before the copper–N-(peptide) bond, while this is not the case with CuH_{-1}-Len. The general reaction mechanism in Figure 6 is postulated for the ethylenediamine reaction with copper–triglycine. The species in brackets are proposed as kinetic intermediates and are not observed directly in the kinetic studies. The concentration of $CuH_{-1}Len$ (VI) builds up and decays. The rate constant $k_{\text{en}}^{CuH_{-2}L}$ corresponds to $k_1k_2/(k_{-1} + k_2)$ and the rate constant $k_{\text{en}}^{CuH_{-1}Len}$ corresponds to $k_3k_4/(k_{-3} + k_4)$. The factor of 10^3 can be accounted for in terms of much larger value expected for k_1/k_{-1} compared to k_3/k_{-3}. In the first intermediate, en can form one bond in the equatorial plane of the copper–peptide bonds and can chelate to the out-of-plane position during an associative-type reaction accompanied by N(peptide) bond cleavage. In the second intermediate (following k_3), neither in-plane coordination nor chelation of en is available. In the latter case k_{-3} would be expected to be much greater than k_4 and k_3/k_{-3} should be of the same order of magnitude as the stability constant for the addition of NH_3 to $Cu(NH_3)_4^{2+}$ ($K_5 = 0.28$). Accordingly, k_4 is about 10^4 sec^{-1}, which is 10^4 times greater than $k_d^{CuH_{-1}Len}$ and again indicates the accelerating effect of en on the rate of copper–N(peptide) bond dissociation.

In the reaction of trien with $CuH_{-2}L^-$ no kinetic intermediates were observed; dien at higher pH also reacts rapidly to break both copper–N(peptide) bonds of triglycine. The nature of the two reactions observed at pH 8–9 with dien was not established. Several kinetic intermediates are possible when the main reactant is H_2dien^{2+} or $Hdien^+$ because of the lack of a free polyamine group adjacent to a copper–N(peptide) bond. Apparently as long as there are free polyamine groups available in the attacking ligand, the sequential copper–N(peptide) cleavage steps are rapid. The proposed mechanism for the polyamines parallels that given for en in Figure 6 except that an intermediate of the $CuH_{-1}Len$ type would not be expected to build up in concentration if there is a free amine group of the polyamine which can replace the copper–N(peptide) group.

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