the coordination geometry.[5] It can be reasonably assumed that such a change will raise the $g_{\parallel}$ value at the same time. Since a value of 120 G for $A_{\parallel}$ is high for a realistic model for type I copper(II), then an environment made up of sulfur(s) and nitrogen(s) atoms is a probable coordination environment. A guess of one sulfur and three nitrogen atoms seems most reasonable based on this work and the recent conclusion of Spiro that a C_3 local symmetry axis existed at the copper center.[8]

One very interesting aspect of this work is simply the observation that the $Cu(SPh_2PNPPh_2S)_2$ complex is redox unstable in the solid state and in solution. At room temperature, the reaction is apparently first order in complex concentration with $K \cong 2 \times 10^{-3}$ min^{-1}, and a half life of ~300 min. It is substantiated that type I copper(II) is involved in a redox mechanism where copper(II) is reduced to copper(I).[23] It is obvious that simple sulfur ligation does not significantly change the Cu(II) → Cu(I) redox couple since square planar CuS_4 complexes are stable. A change in coordination geometry seems required. That fact alone offers a strong argument for the tetrahedral coordination geometry of this complex.

Further studies of this and similar complexes are underway especially with regard to the kinetic properties. Hopefully, a more refined model will include spin Hamiltonian parameters nearer to type I copper(II) as well as an incorporation of the redox couple for these systems.

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Kinetics of Copper(II)–Glycylglycyl-L-histidine Reactions. Acid Decomposition and Proton-Assisted Nucleophilic Displacement by Triethylenetetramine

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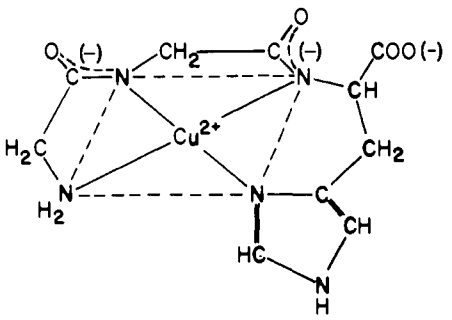
Abstract: The presence of histidine as the third amino acid residue in tripeptide complexes of Cu(II) drastically decreases their susceptibility to nucleophilic attack. The rate constant for triethylenetetramine (trien) reaction with $Cu(H_{-2}glyglyhis)^-$ is only 0.5 M^{-1} s^{-1} whereas the unprotonated ligand reacts more than 10^7 times faster with $Cu(H_{-2}glyglygly)^-$. However, a previously unobserved pathway is found involving the combined reaction of H^+ and H_2trien^{2+} with $Cu(H_{-2}glyglyhis)^-$ (the value of the third-order rate constant is 1.7×10^9 M^{-2} s^{-1}). This proton-assisted nucleophilic reaction becomes the major pathway between pH 6.5 and 8.5 even at low trien concentrations. At higher trien concentrations a rate, limited by proton transfer to the peptide nitrogen, is reached and this reaction is general-acid catalyzed. Acid dissociation reactions in the absence of trien require uptake of two protons and the rate changes from a second-order $[H^+]$ dependence at pH 5 to a zero-order $[H^+]$ dependence at pH 1. This is due to the rapid formation of an "outside" protonated species in which the peptide oxygens have protonation constants of $10^{4.2}$ and $10^{2.3}$. These dissociation reactions are limited by metal–peptide bond cleavage and are not general-acid catalyzed.

The displacement of tripeptides from copper(II) complexes of the type $Cu(H_{-2}tripeptide)^-$ has been shown to occur by two general mechanisms involving either acid attack on the deprotonated ligand or nucleophilic attack on the metal ion.[1-4] When histidine is the third amino acid residue in the tripeptide, as in glycylglycyl-L-histidine (glyglyhis), the reactivity of the complex via both these pathways is reduced greatly and a new reaction pathway is found. This new pathway proceeds by a proton-assisted nucleophilic mechanism with the novel feature that the tripeptide displacement is initiated at a non-terminal position.[5]

Histidine is the third amino acid residue in serum albumin (human, bovine, and rat) and it has been proposed that Cu(II) binds preferentially at the amino terminal of serum albumin with coordination of an amino group, two deprotonated peptide nitrogens, and an imidazole nitrogen[6-9] similar to the coordi-



I

nation of glygly-L-his (structure I). Reactions of $Cu(H_{-2}glyglyhis)^-$ are thus important in helping to understand the mechanism of Cu(II) transfer in the blood. Triethylenetetramine (trien) is used as a therapeutic agent in the treatment of Wilson's disease[10] and a study of the transfer of Cu(II) from glyglyhis to trien is of interest for this reason. However, the primary reason for the choice of trien as a nucleophilic agent in these studies is the strikingly different behavior of the trien reaction with $Cu(H_{-2}glyglyhis)^-$ compared to its reaction with $Cu(H_{-2}glyglygly)^-$ and other tripeptide complexes.[3] The reactions of $Cu(H_{-2}glyglyhis)^-$ exhibit a number of properties which are very different from those of the corresponding reactions of $Cu(H_{-2}glyglygly)^-$ Thus, for the glyglyhis complex: (1) The attack by trien is 10^7-fold less at high pH. (2) As the pH increases from 6 to 9 the rate of reaction with trien decreases, which is the opposite behavior to that of other $Cu(H_{-2}tripeptide)^-$ complexes. (3) As the trien concentration increases at pH 6-8, a limiting rate is found which is independent of trien, but is general-acid catalyzed. (4) The acid dissociation rate with water acting as the nucleophile is much slower and has a $[H^+]^2$ dependence above pH 4. This contrasts with a first-order $[H^+]$ dependence for other tripeptide complexes. (5) The acid decomposition reactions with water as a nucleophile are not general-acid catalyzed. (6) A limiting rate is found below pH 2 where the acid decomposition reaction becomes independent of acidity. All these facts are explained in terms of the participation of three main pathways depending upon the pH and trien concentration: (1) an acid dissociation pathway with outside protonation at low pH, (2) a proton-assisted nucleophilic pathway with trien at pH 6-9, and (3) a nucleophilic pathway with trien above pH 9.

Experimental Section

Chromatographically pure glycyglycyl-L-histidine was obtained from Cyclo Chemical Co. and its purity verified by amino acid and elemental analysis. Stock solutions of copper(II) perchlorate were prepared from the twice recrystallized salt and standardized against EDTA. Triethylenetetramine (trien) was prepared as the free base by reacting the disulfate salt of trien with sodium hydroxide solution. The free base was separated by vacuum (0.2 mmHg) distillation and its purity checked by gas chromatography. The protonation constants used are $\log \beta_1 = 9.92$, $\beta_2 = 19.17$, and $\beta_3 = 26.17$.[11] The total trien concentration is given as $[trien]_T = [trien] + [Htrien^+] + [H_2trien^{2+}] + [H_3trien^{3+}]$.

General acids and their respective protonation constants used are acetic acid, $\log K_H = 4.6$, sodium dihydrogen phosphate, $\log K_H = 6.3$,[11] 2-(*N*-morpholino)ethanesulfonic acid (MES), $\log K_H = 6.0$, *N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid (HEPES), $\log K_H = 7.5$, and tris(hydroxymethyl)aminomethane (Tris or THAM), $\log K_H = 8.0$.[12]

Solutions of $Cu(H_{-2}glyglyhis)^-$ were prepared by reaction of glyglyhis (~2-3% excess over Cu(II)) with standardized $Cu(ClO_4)_2$ solutions and the desired pH was obtained using NaOH. The $Cu(H_{-2}glyglyhis)^-$ complex has been reported[13] to lose a proton from the pyrrole nitrogen of the imidazole ring at pH 10.7 ($\mu = 0.1$ M). However, this value was obtained by extrapolating data from an incomplete titration of the Cu(II) complex and appears to be in error.

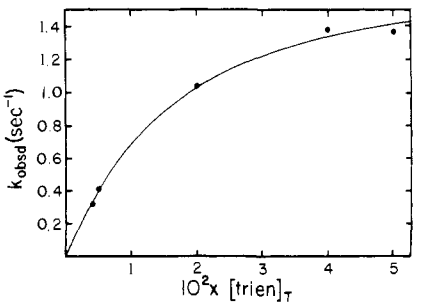


Figure 1. Dependence of k_{obsd} on trien concentration: $[Cu(H_{-2}glyglyhis)^-] = 2.0 \times 10^{-4}$ M, pH 6.9, 1.0 M $NaClO_4$, 25.0 ± 0.1 °C.

We could find no evidence for the deprotonation of the pyrrole nitrogen in the copper complex by titrimetric, kinetic, or spectrophotometric studies.

Stock $NaClO_4$ solutions were prepared by reacting Na_2CO_3 with concentrated $HClO_4$. The $NaClO_4$ solutions were boiled for several hours to expel CO_2. All reactions were run in 1.0 M $NaClO_4$ and at 25.0 ± 0.1 °C. Measurements of pH were made using saturated NaCl in the reference electrode.

Reactions were followed, depending upon their speed, using either a Cary 16 spectrophotometer interfaced to a Varian G-2000 recorder or a Durrum stopped-flow spectrophotometer interfaced to a Hewlett-Packard 2115A (8K, 16 bit) general purpose computer as desribed elsewhere.[14] The absorbance changes were monitored at 250 nm for the buffered acid decomposition reactions of $Cu(H_{-2}glyglyhis)^-$ and with an excess of trien. Excellent first-order plots were obtained for the reactions of $Cu(H_{-2}glyglyhis)^-$ under all conditions used. The observed first-order rate constant is designated as k_{obsd} throughout this work and is defined such that

$$-d[Cu\text{-}glyglyhis]_T/dt = k_{obsd}[Cu\text{-}glyglyhis]_T \quad (1)$$

where $[Cu\text{-}glyglyhis]_T$ represents all forms of Cu(II) complexed to glyglyhis.

Results and Discussion

A. Reactions with Trien. The overall reaction is the transfer of Cu(II) shown in eq 2, where n is 0-3 and p is 0-2. The rate of this reaction was studied as a function of trien concentration, pH, and the catalytic activity of various buffers. When trien acts as a nucleophile as it does in its reactions with $Cu(H_{-2}glyglygly)^-$, $Cu(H_{-2}glyleugly)^-$, $Cu(H_{-2}glyalagly)^-$, $Cu(H_{-2}leuglyleu)^-$, and $CuEDTA^{2-}$, the relative reactivity of the various protonated trien species are trien > $Htrien^+ \gg H_2trien^{2+} \gg H_3trien^{3+}$.[3,15] Thus, the nucleophilic path is characterized by an increasing rate as the pH increases. In the present system, however, a decreasing rate is observed as the pH is increased between pH 6 and 9.

$$Cu(H_{-2}glyglyhis)^- + H_ntrien^{n+} + (2 + p - n)H^+ \rightarrow Cu(trien)^{2+} + H_p(glyglyhis)^{p-1} \quad (2)$$

Trien Dependence (pH 6.4-8.3). The rate of formation of $Cu(trien)^{2+}$ at constant pH is first order with respect to $trien_T$ at low trien concentrations and obeys the expression given in eq 3.

$$d[Cu(trien)^{2+}]/dt = k[Cu(H_{-2}glyglyhis)^-][trien]_T \quad (3)$$

At high concentrations of trien the rate of $Cu(trien)^{2+}$ formation is independent of trien and follows eq 4. Figure 1 shows a typical dependence of k_{obsd} on the trien concentration.

$$d[Cu(trien)^{2+}]/dt = k'[Cu(H_{-2}glyglyhis)^-] \quad (4)$$

Hydrogen Ion Dependence. The hydrogen ion dependence for the displacement of glyglyhis from Cu(II) with trien was determined using high trien (≥0.07 M) concentrations where the reaction rate is independent of trien. The reaction rate was

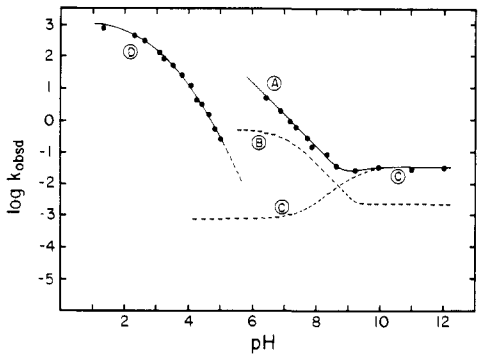


Figure 2. Dependence of k_{obsd} on $[H^+]$ for the reaction of $Cu(H_{-2}glyglyhis)^-$. $[Cu(H_{-2}glyglyhis)^-] = 2.0 \times 10^{-4}$ M or 5.0×10^{-5} M, 1.0 M NaClO, 25.0 ± 0.1 °C. A = Reaction of $Cu(H_{-2}glyglyhis)^-$ with 0.07 M [trien], showing the importance of the proton and nucleophilic pathways with pH. The solid curve was calculated using $k_0 = k_H[H^+] + k_N[trien_T] + k_d$, $k_H = 1.4 \times 10^7$ M^{-1} s^{-1}, k_{Htrien} and $k_{trien} = 0.5$ M^{-1} s^{-1} and $k_d = 7.5 \times 10^{-4}$ s^{-1}. B = Reaction of $Cu(H_{-2}glyglyhis)$ with 3.3×10^{-3} M [trien], showing the calculated contribution of the proton-assisted nucleophilic pathway and nucleophilic pathway at various pH values. C = Reaction of $Cu(H_{-2}glyglyhis)^-$ with 0.07 M trien, showing the behavior of the nucleophilic and water dissociation ($k_0 = k_d + k_N[trien]_T$) pathways with pH if the proton-assisted nucleophilic pathway were not present. D = Dependence of k_{obsd} on $[H^+]$ for the acid decomposition of Cu-glyglyhis. The solid curve was calculated using eq 17, protonation constants $K_1 = 10^{4.2}$ M^{-1} and $K_2 = 10^{2.3}$ M^{-1} and $k_3 = 1 \times 10^3$ s^{-1}.

Table I. Dependence of k_{obsd} on pH at 0.07 M Trien Concentrations[a]

pH	k_{obsd}, s^{-1}	pH	k_{obsd}, s^{-1}
6.41[b,c]	4.68 ± 0.07	8.32[c]	0.081 ± 0.001
6.87[c]	1.83 ± 0.03	8.66[d]	0.0365 ± 0.0004
7.17[c]	0.87 ± 0.03	9.27[d]	0.0240 ± 0.0002
7.37[c]	0.55 ± 0.02	9.97[d]	0.0333 ± 0.0002
7.74[c]	0.260 ± 0.006	11.0[d,e]	0.030
7.89[c]	0.138 ± 0.003	12.0[d,e]	0.033

[a] $[Cu(H_{-2}glyglyhis)^-] = 5 \times 10^{-5}$ M, 25.0 ± 0.1 °C, 1.0 M $NaClO_4$. [b] 0.08 M trien is required to reach the proton-limited rate. [c] k_{obsd} is independent of trien. [d] k_{obsd} depends on trien. [e] k_{obsd} is calculated for 0.07 M trien from variation of trien at lower concentrations.

found to be first order in hydrogen ion concentration (between pH 6.4 and 8.3) and is described by eq 5.

$$d[Cu(trien)^{2+}]/dt = k_H[Cu(H_{-2}glyglyhis)^-][H^+] \quad (5)$$

The value of the rate constant k_H is 1.4×10^7 M^{-1} s^{-1}. Table I lists the observed rate constants obtained for the reaction of $Cu(H_{-2}glyglyhis)^-$ with trien as a function of pH. A plot of log k_{obsd} against pH is shown in Figure 2 (curve A). The predominant contribution to the rate for pH <8.5 is the proton pathway and for pH >8.5 is a nucleophilic pathway with attack by $Htrien^+$ and trien. Thus the plot of log k_{obsd} against pH at high trien begins to deviate from a negative slope of one (first-order hydrogen ion dependence) and levels off at high pH because all the trien is converted to its free base form.

Nucleophilic Attack by Trien. Variation of trien concentration at pH 11 and 12 gives a value for the second-order rate constant for direct nucleophilic attack by trien of 0.5 M^{-1} s^{-1}. Analysis of data at pH <11 gives the rate of nucleophilic attack by $Htrien^+$ which is also ~0.5 M^{-1} s^{-1}. A water dissociation rate constant (k_d) of 7.5×10^{-4} s^{-1} is obtained from the data in Table II as an intercept in a plot of k_{obsd} vs. $trien_T$ at pH 12.

Mechanism. The proposed mechanism for the reaction of $Cu(H_{-2}glyglyhis)^-$ with trien is described by eq 6-9.

Table II. Observed Rate Constants for the Reaction of $Cu(H_{-2}glyglyhis)^-$ with Trien at pH 12.0[a]

10^4[trien], M	$10^4 k_{obsd}$, s^{-1}	10^4[trien], M	$10^4 k_{obsd}$, s^{-1}
5.0	9.5	20.0	18.6
8.0	11.8	50.0	33.4

[a] $Cu(H_{-2}glyglyhis)^-$ 5×10^{-5} M, 25.0 ± 0.1 °C, 1.0 M $NaClO_4$.

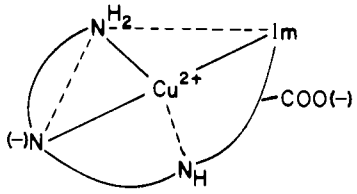
$$Cu(H_{-2}L) + H^+ \underset{k_{-H}}{\overset{k_H}{\rightleftharpoons}} Cu(H_{-1}L) \quad (6)$$

$$Cu(H_{-1}L) + H_2trien^{2+} \xrightarrow{k_{H_2T}} Cu(trien)^{2+} + HL \quad (7)$$

$$Cu(H_{-2}L)^- + trien_T \xrightarrow{k_N} Cu(trien)^{2+} \quad (8)$$

$$Cu(H_{-2}L)^- + H_2O \xrightarrow{k_d} Cu(trien)^{2+} \quad (9)$$

L represents glyglyhis. The proton-assisted nucleophilic path is represented by eq 6 and 7 where $Cu(H_{-1}L)$ (structure II)



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is a steady-state intermediate. This proposed species is much more subject to nucleophilic attack than the initial complex. The rate constant (k_H) is several orders of magnitude less than typical rate constants for reactions of H^+. This behavior is generally observed for the protonation of nitrogen in metal-N(peptide) complexes.[16] Protonation of the peptide oxygen would be expected to be diffusion controlled. The nucleophilic pathway given by eq 8 represents direct attack by trien species without proton assistance. A separate water dissociation path followed by a rapid reaction with trien is shown in eq 9. The above mechanism yields the rate law for the formation of $Cu(trien)^{2+}$ given in eq 10 and the observed rate constant is described by eq 11. Although H_3trien^{3+} predominates below pH 7, the reactive trien species within the pH range of the acid-catalyzed pathway is H_2trien^{2+}. At low trien concentrations within this pH range, the rate is dependent on both hydrogen ion and trien concentrations. This combined dependence is approximated for a given pH by dividing the initial slope of a plot of k_{obsd} vs. trien by the hydrogen ion concentration. At pH 6.9 linear plots of $[H^+]/k_{obsd}$ against $1/[H_2trien^{2+}]$ give values for k_{-H}/k_{H_2T} and k_H. The calculated curve in Figure 1 is obtained from eq 11 using these values.

$$\frac{d[Cu(trien)^{2+}]}{dt} = \frac{k_H k_{H_2T}[Cu(H_{-2}L)^-][H^+][H_2trien^{2+}]}{k_{-H} + k_{H_2T}[H_2trien^{2+}]} + (k_N[trien_T] + k_d)[Cu(H_{-2}L)^-] \quad (10)$$

$$k_{obsd} = \frac{k_H k_{H_2T}[H^+][H_2trien^{2+}]}{k_{-H} + k_{H_2T}[H_2trien^{2+}]} + k_N[trien_T] + k_d \quad (11)$$

Curve A in Figure 2 was calculated from eq 11 for 0.07 M trien concentration. The proton-assisted nucleophilic pathway predominates below pH 8, whereas the nucleophilic path is more important above pH 10. The effect of reducing the trien concentration is seen in curve B where all the k_{obsd} values are reduced. There is no longer a first-order hydrogen ion depen-

Table III. General Acid Rate Constants for the Reaction of $Cu(H_{-2}glyglyhis)^-$ with Acid[a]

Acid	pK_a	Log k_{HA}
H(tris)	8.0	0.8
H(HEPES)	7.5	1.7
$H_2PO_4^-$	6.3	3.2
H(MES)	6.0	2.1
HOAc	4.6	3.8

[a] $[Cu(H_{-2}glyglyhis)^-] = 5 \times 10^{-5}$ M, 25.0 ± 0.1 °C, 1.0 M $NaClO_4$.

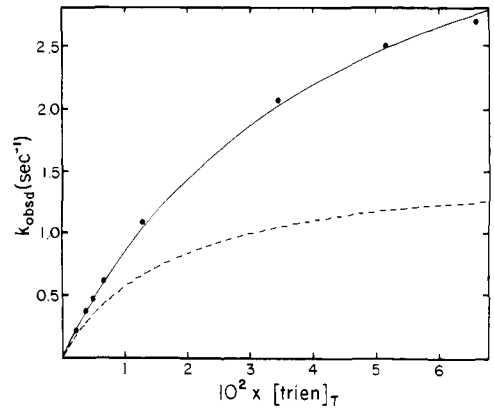


Figure 3. Dependence of k_{obsd} on trien concentration in the presence of 0.1 M HOAc. $[Cu(H_{-2}glyglyhis)^-] = 5.0 \times 10^{-5}$ M, pH 7.0, 1.0 M $NaClO_4$, 25.0 ± 0.1 °C. The dashed curve refers to the reaction in the absence of HOAc.

dence at pH 6 because the trien-limiting rate condition is not in effect and H_3trien^{3+} is not as reactive as H_2trien^{2+} in the proton-assisted pathway. Curve C is hypothetical for 0.07 M trien and represents the predicted behavior of k_{obsd} according to eq 8 and 9 if the proton-assisted nucleophilic pathway were not present.

General Acid Catalysis. The rate of ligand displacement by H_2trien^{2+} on the $Cu(H_{-2}L)^-$ complex is general-acid catalyzed under conditions of high trien concentration. A plot of k_{obsd} against $[trien]_T$ for the reaction with $Cu(H_{-2}L)^-$ in the presence of 0.1 M HOAc at pH 7.0 is given in Figure 3. The dashed line is calculated from eq 11 for the same reaction in the absence of a general acid. As the trien concentration is increased, the rate constant for the reaction in the presence of a general acid is enhanced. The maximum increase occurs when the reaction rate is independent of trien concentration (where proton transfer is rate limiting). Several acids including Tris, $H_2PO_4^-$, MES, HEPES, and acetic acid catalyze the displacement reaction. The catalytic ability of the general acids is dependent on their K_a values[16] except for phosphate which appears high. This could be due to the fact that it is a coordinating acid. The general acid rate constants for the reaction of $Cu(H_{-2}glyglyhis)^-$ with acid are given in Table III. Over the pH range where the proton-assisted nucleophilic pathway is important the presence of a general acid contributes to the rate of formation of the $Cu(H_{-1}L)$ species. Thus, the initial protonation step is described by both eq 5 and 12.

$$Cu(H_{-2}L)^- + HA \underset{k_{A^-}}{\overset{k_{HA}}{\rightleftharpoons}} Cu(H_{-1}L) + A^- \qquad (12)$$

The observed rate constant with a general acid present is given by eq 13. According to eq 13 the reaction is proton catalyzed at low trien concentrations. However, as the trien concentration increases the reaction canges from specific-acid to general-acid catalyzed, in accord with experimental results. Supporting evidence for the mechanism is found by varying the general-acid concentration at high trien concentrations. A variation of phosphate concentration at pH 7.26 and 0.06 M $[trien]_T$ shows an initial first-order dependence becoming zero order on increasing the total phosphate concentration. This again is expected since, as the phosphate concentration is increased, the $k_A[A^-]$ term in eq 13 dominates $k_{H_2T}[H_2trien^{2+}]$ and a rate, independent of general acid but dependent on both $[H^+]$ and [trien], results. The same k_{HA} values for phosphate are obtained by varying either the trien concentration or the phosphate concentration.

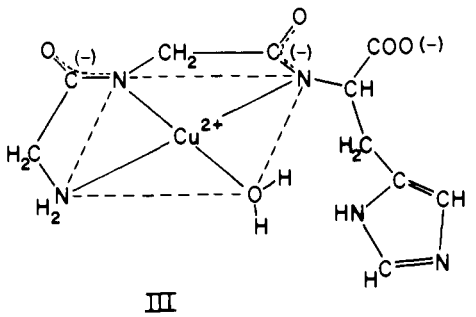
$$k_{obsd} = \frac{(k_H[H^+] + k_{HA}[HA])k_{H_2T}[H_2trien^{2+}]}{k_{-H} + k_A[A^-] + k_{H_2T}[H_2trien^{2+}]} \qquad (13)$$

The general-acid catalysis study shows that the behavior of the $Cu(H_{-2}glyglyhis)^-$ complex, when acting as a base in proton-transfer reactions, is intermediate between that of "normal" bases and bases of carbon acids.[16] The proton-transfer rate for $Cu(H_{-2}glyglyhis)^-$ is less than the diffusion controlled limit. This can be explained by considering both the energy of solvent reorganization (large W_R) required to form the acid-base encounter complex as well as the reorganizational barrier (small $\lambda/4$) necessary to initiate hydrogen bonding after the encounter complex is formed. Detailed discussion of these proton-transfer reactions is given elsewhere.[16]

Proton-Assisted Nucleophilic and Nucleophilic Pathways. There are large differences in the kinetic behavior of $Cu(H_{-2}glyglyhis)^-$ and $Cu(H_{-2}glyglygly)^-$ in their reactions with trien. The nucleophilic attack by trien is a factor of 2.2×10^7 slower for the glyglyhis complex because the availability of an equatorial position for nucleophilic displacement of peptides is extremely important. This is illustrated by studies where steric effects of both nucleophiles[1] and of peptides[3] reduce the reactivity with respect to nucleophilic attack towards Cu(II)-tripeptide complexes. In addition, the rate of nucleophilic attack on a Cu(II)-peptide complex, in which the fourth coordination position is occupied by a peptide nitrogen, is very much smaller than found for the triglycine complex.[4,17]

In $Cu(H_{-2}glyglygly)^-$, only a carboxylate group need be displaced for a nucleophile to gain an equatorial site, whereas the corresponding reaction of $Cu(H_{-2}glyglyhis)^-$ would require an imidazole nitrogen to be displaced. This is more difficult for several reasons. (a) The imidazole nitrogen forms a much stronger bond to copper than does the carboxylate group. (b) The 5,5,6-membered ring in the glyglyhis omplex is more stable than the 5,5,5-membered ring in the glyglygly complex. This effect has been seen in polyamine complexes[18] as well as tripeptide complexes.[19] (c) The trans deprotonated peptide nitrogen may weaken the bonding of the carboxylate group to copper more than it does that of the imidazole nitrogen because of back-bonding in the latter case. Some of these differences between $Cu(H_{-2}glyglygly)^-$ and $Cu(H_{-2}glyglyhis)^-$ are reflected in their stability constants ($K = ([CuH_{-2}tripeptide^-][H^+]^2/[Cu^{2+}][tripeptide^-])$) which for the complex with glyglygly[20] is $10^{-6.7}$ as compared to an estimated value of $10^{-2.2}$ for glyglyhis. The estimated value was obtained from the published protonation constants for Cu-glyglyhis, the first complexation constant of the *N*-acetyl derivative,[21] and the protonation constant for the amino group of glyglyhis.[11] A pathway in which trien displaces the imidazole group in the rate-determining step is suggested for the nucleophilic attack by trien. The rate constant (k_N) has a value of 0.5 M^{-1} s^{-1}.

The trien independent rate constant of 7.5×10^{-4} s^{-1} at high pH is believed to correspond to the solvent assisted imidazole ring opening step. Once imidazole dissociates from Cu(II) to give a species where the coordination about Cu(II) involves three nitrogens (one amine and two peptides as in structure III), the trien attack would be expected to be very fast



III

with a rate constant similar to that of glyglygly (1.1×10^7 M^{-1} s^{-1}), so that a first-order dissociation reaction is observed without a trien dependence. A similar very fast reaction with trien would be expected if the histidine carboxylate group were coordinated rather than the imidazole group.

A rate dependence of the type ($k_d + k_N$[nucleophile])[complex] is typical of the reactions of square-planar complexes. However, neither pathway can account for the increased rate with trien below pH 9. First, H_2trien^{2+} would be expected to be a poorer nucleophile than $Htrien^+$ or trien; second, neither the imidazole nor the carboxylate group would be expected to add a proton above pH 7; third, the H^+ cannot assist the cleavage of the imidazole group from Cu^{2+}. A new pathway which is a combined proton and nucleophilic attack initiated at a nonterminal peptide position is proposed.[5] This combined pathway involves attack by hydrogen ion at an internal peptide position followed by nucleophilic reaction with H_2trien^{2+}. One characteristic of this proton-assisted nucleophilic pathway is that the dependence in trien changes from first order to zero order as the trien concentration increases. This proton-assisted nucleophilic pathway was not observed for $Cu(H_{-2}glyglygly)^-$ because the direct nucleophilic attack is (2.2×10^7)-fold greater than for $Cu(H_{-2}glyglyhis)^-$.

B. Acid Decomposition of $Cu(H_{-2}glyglyhis)^-$. The acid decomposition of $Cu(H_{-2}glyglyhis)^-$ was studied over a wide pH range (pH 1-5). Perchloric, chloroacetic, formic, and acetic acids were used. The rate of decomposition of the Cu(II) complex of glyglyhis is given by eq 1. However, k_{obsd} does not have a simple hydrogen ion dependence. The decomposition rate is independent of hydrogen ion at high acidities and has a first-order dependence on hydrogen ion at pH 2.5-3.5. Above pH 3.5, the hydrogen ion dependence increases and the decomposition rate becomes second order in $[H^+]$ by pH 5.

This complicated dependence of the rate on hydrogen ion concentration indicates that the acid decomposition of the copper complex requires more than one protonation. A change in hydrogen ion dependence from first order to zero order as the hydrogen ion concentration increases has previously been observed for protonation reactions of Ni(II)-peptide complexes.[22] A similar mechanism is proposed except that two protonation reactions precede the rate-determining step for the Cu(II)-glyglyhis complex as given in eq 14-16.

$$Cu(H_{-2}L)^- + H^+ \overset{K_1}{\rightleftharpoons} Cu(H_{-2}L)H \text{ (IV)} \quad (14)$$

$$Cu(H_{-2}L)H + H^+ \overset{K_2}{\rightleftharpoons} Cu(H_{-2}L)\cdot 2H^+ \text{ (V)} \quad (15)$$

$$Cu(H_{-2}L)2H^+ \xrightarrow{k_3} CuL^+ \text{ (VI)} \xrightarrow{\text{rapid}} \text{products} \quad (16)$$

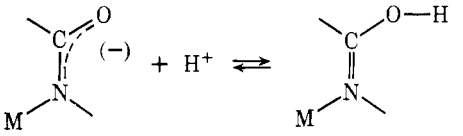
$$k_{obsd} = \frac{k_3K_1K_2[H^+]^2}{1 + K_1[H^+] + K_1K_2[H^+]^2} \quad (17)$$

The corresponding expression for the observed rate constant is given in eq 17. A curve calculated, using eq 17, is shown in Figure 2, curve D, and provides a good fit for the experimentally determined rate constants (Table IV) for the acid decomposition of $Cu(H_{-2}glyglyhis)^-$. The values for the protonation constants obtained kinetically are 4.2 for log K_1 and 2.3 for log K_2. These values represent "outside" protonation constants for the Cu(II)-glyglyhis complex, where the complex has been protonated at a peptide oxygen but the Cu(II)-N(peptide) bond has not been broken. No initial absorbance jump is observed upon mixing. Equation 18 illustrates the formation of an "outside" protonated complex.

Table IV. Observed Rate Constants for the Acid Decomposition of $Cu(H_{-2}glyglyhis)^-$ [a]

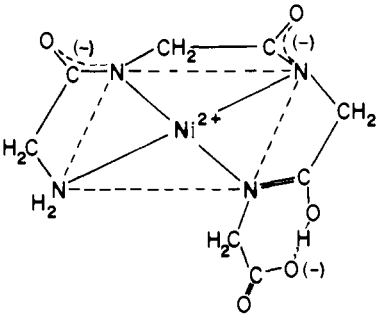
pH	k_{obsd}, s^{-1} [b]	pH	k_{obsd}, s^{-1} [b]
1.33	762 ± 7	4.11	11.20 ± 0.06
2.38	454 ± 36	4.28	4.630 ± 0.002
2.64	323 ± 6	4.44	3.20 ± 0.06
3.13	134 ± 2	4.67	1.29 ± 0.04
3.30	83.1 ± 0.8	4.82	0.507 ± 0.014
3.58	52.2 ± 0.4	5.03	0.258 ± 0.004
3.83	25.5 ± 0.2		

[a] $[Cu(H_{-2}glyglyhis)] = 2.01 \times 10^{-4}$ M or 5×10^{-5} M, 25.0 ± 0.1 °C, 1.0 M $NaClO_4$. [b] Standard deviation from average of three or more runs.



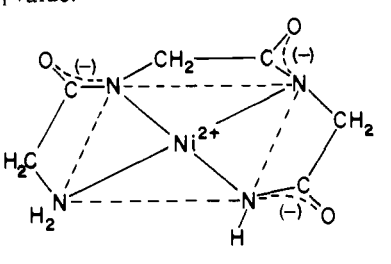
(18)

The "outside" protonation constants obtained for Cu(II)-glyglyhis are similar to those obtained for the Ni(II) complexes[22] of tetraglycine (log K_H = 4.1) and triglycineamide (log K_H = 2.4). It has been suggested that the difference between the protonation constant for nickel(II)-tetraglycine and nickel(II)-triglycineamide is due to internal hydrogen bonding. The free carboxylate from tetraglycine is able to hydrogen bond with the "outside" protonated species of nickel(II)-tetraglycine (structure VII). This stabilizes the "outside"



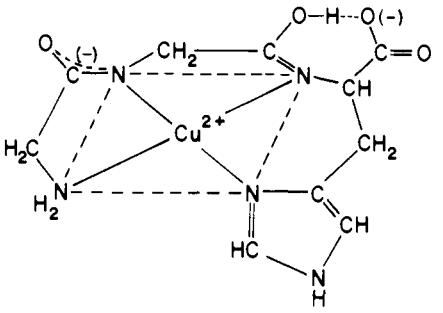
VII

protonate complex resulting in a higher log K_H value (4.2). There is no possibility of hydrogen bonding in the nickel(II)-triglycineamide complex (structure VIII) which has a lower log K_H value.



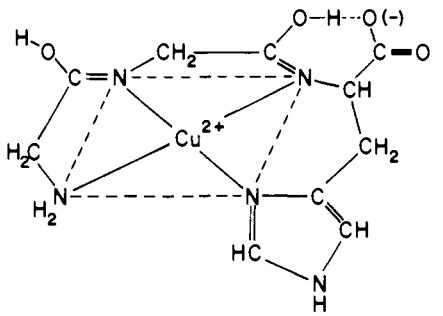
VIII

An "outside" protonated Cu(II)-glyglyhis complex has the capability to hydrogen bond using the free carboxylate group from the histidyl residue to give the same type of ring structure as that formed by nickel(II)-tetraglycine. We therefore propose that the protonation constant $K_1 = 10^{4.2}$ represents the formation of the "outside" protonated complex for Cu(II)-glyglyhis stabilized by hydrogen bonding with the free carboxylate group from the histidine residue (structure IV). The



IV

protonation of the second peptide site for Cu(II)-glyglyhis ($K_2 = 10^{2.3}$) is less favorable because this "outside" protonated species (structure V) cannot form an internal hydrogen bond



V

to increase its stability. This second protonation constant for the nonstabilized "outside" protonated Cu(II)-glyglyhis complex is very close to the protonation constant ($10^{2.4}$) found for nickel(II)-triglycineamide.

There is a distinct difference in the kinetic behavior of the nickel(II)-peptide protonation reactions and those found for Cu(II)-glyglyhis. Two protons are required for Cu(II)-glyglyhis to dissociate under all acid conditions whereas the nickel complexes can dissociate on the addition of only one proton. Although a second proton assists the dissociation of nickel(II)-tetraglycine below pH 3, this additional proton path is not seen for nickel(II)-triglycineamide. Why are two protons required for the dissociation of glyglyhis from Cu(II) whereas only one proton is sufficient for the dissociation of the nickel complexes? The glyglyhis complex is coordinated by an amine and an imidazole nitrogen at the two terminal positions. Imidazole coordination imparts a great increase in stability to the metal complex and forces the protonation and dissociation to be initiated at nonterminal positions. Because acid attack is initiated at internal peptide positions, dissociation of a singly protonated species is not observed and both peptide sites must be protonated before the ligand dissociates. The Cu(H_{-2}glyglyhis) complex adds two protons in a stepwise fashion before the rate-determining step (eq 14-16) so that the K_H values for both peptide groups enter into the resolved rate expression for the acid decomposition. This shows different behavior for the two Ni(II) complexes. The mechanism for the Ni(II) protonation reaction is given in eq 19-21. The rate-determining step occurs after the initial protonation because of the greater reactivity of the monoprotonated complex. Although "outside" protonation could take place at any of the peptide sites for nickel(II)-tetraglycine or -triglycineamide, only the kinetically important protonation will allow the complex to dissociate. The most reactive site is the terminal peptide or amide nitrogen or tetraglycine or triglycineamide. Thus a major difference in acid dissociation reactions between Cu(II)-glyglyhis and nickel(II)-tetraglycine or -triglycineamide is that protonation and ligand dissociation for Cu(II)-glyglyhis is initiated at a nonterminal position whereas protonation and ligand dissociation for the Ni(II) complexes begins at a terminal position.

$$Ni(H_{-n}L)^{-n+1} + H^+ \overset{K_H}{\rightleftharpoons} Ni(H_{-n}L)^{-n+2}H \quad (19)$$

$$Ni(H_{-n})^{-n+2}H \xrightarrow{k} Ni(H_{-n+1}L)^{-n+2} \quad (20)$$

$$Ni(H_{-n+1}L)^{-n+2} \xrightarrow{fast} \text{products} \quad (21)$$

The acid decomposition reactions for nickel(II)-triglycine[23] and copper(II)-triglycine[1,16,24] are general-acid catalyzed. However, the reactions of nickel(II)-tetraglycine and nickel(II)-triglycineamide[22] are not general-acid catalyzed. A proposed general mechanism[22] for the acid decomposition of metal-peptide complexes explains this difference. Metal-peptide reactions are general-acid catalyzed if proton transfer is the rate-limiting step and metal-N(peptide) bond breaking is fast. This is the case for the triglycine complexes of Cu(II) and Ni(II). Reactions of nickel(II)-tetraglycine and triglycineamide are not general-acid catalyzed because metal-N(peptide) bond breaking is the rate-determining step and proton transfer occurs as a rapid preequilibrium. The sluggish acid decomposition rate of palladium(II)-triglycine also falls into this latter category.[25] Bond breaking is likewise the rate-determining step for the acid decomposition reaction of Cu(II)-glyglyhis, and we observe no general acid catalysis. This is in line with the proposed general mechanism[22] for acid decomposition reactions of metal-peptides.

Conclusions

The presence of histidine as the third amino acid residue completely alters the reactivity of the Cu(H_{-2}glyglyhis)$^-$ complex compared to other copper(II)-tripeptide complexes, particularly in reducing direct nucleophilic attack by trien. Previous work has shown the importance of an equatorial position in the reactions of metal-peptides with nucleophiles.[2-4] When nucleophiles can react by displacing either an equatorial carboxylate group or water molecule, the reactions are much faster than when deprotonated peptide groups must be displaced. Thus [Cu(H_{-1}glyglygly)en] and Cu(H_{-3}glyglyglygly)$^{2-}$ react with polyamines much more slowly than Cu(H_{-2}glyglygly)$^-$. In the case of the glyglyhis complex, the attacking nucleophile must displace either an imidazole or an amine nitrogen which is less favorable than displacing a peptide group.

When this direct nucleophilic attack is unfavorable, a previously unobserved pathway is found in which a proton adds to a nonterminal peptide nitrogen (structure II) permitting more faorable nucleophilic attack. Nucleophilic reactions with other metal-peptide complexes are sterically selective against tertiary amines such as EDTA, but this is not the case with the proton-assisted pathway.[5,26] This pathway is also observed with EDTA and histidine as nucleophiles. Furthermore, the same kinetic characteristics are found in H_2trien^{2+} reactions with Cu(II) complexes of human serum albumin and bovine serum albumin.[27]

With excess trien, in the case of Cu(II)-glyglyhis, a proton-limiting rate is obtained and the proton-limiting rate constant agrees with that found for the protonation of copper(II)-triglycine.[16,24] This supports the original suggestion[1] that a proton can add to a peptide nitrogen without dissociation

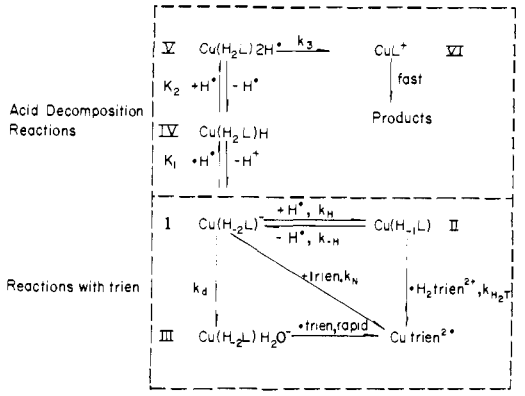


Figure 4. Mechanism for the reaction of $Cu(H_{-2}glyglyhis)^-$ with trien and acid. Structures beside the various protonated forms of Cu-glyglyhis are given in the text.

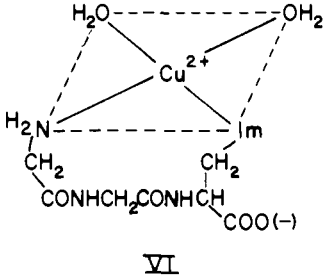
of other groups. Under these conditions, the reaction with $Cu(H_{-2}glyglyhis)^-$ is general-acid catalyzed as was observed also for the protonation reactions of $Cu(H_{-2}glyglygly)^-$.

The overall mechanism for the reaction of Cu(II)-glyglyhis is diagramed in Figure 4 and the rate constants for various steps are summarized in Table V.

The acid pathway with trien depends on an inside protonation where a proton adds directly to a peptide nitrogen prior to trien attack. The acid pathway without trien requires addition of two protons before the rate-determining step. Since two protons are required for the aqueous acid dissociation reaction and H_2trien^{2+} is very effective with only one proton added before the rate-determining step in the proton-assisted nucleophilic reaction, it is suggested that the H_2trien^{2+} attack on $CuH_{-1}L$ (structure II) may be followed by internal proton transfer within $[CuH_{-1}L{\cdot}trienH_2]^{2+}$ to give $[CuL{\cdot}trien-H]^{2+}$.

The inside-protonated complex (structure II) must be less stable with respect to proton addition than the outside-protonated complexes (structures IV and V), otherwise it would have been observed in the acid dissociation studies because the proton-transfer rate constant k_H is large (10^7 M^{-1} s^{-1}). Therefore $k_H/k_{-H} < 10^2$, which means $k_{-H} > 10^5$ s^{-1} and since k_{H_2T}/k_{-H} is 1.2×10^2, $k_{H_2T} > 10^7$ M^{-1} s^{-1}.

Species II and III are steady-state intermediates whereas species IV and V are present in substantial concentrations. In fact, below pH 2 the reactant is almost 100% converted to $[Cu(H_{-2}L){\cdot}2H]^+$ (V). The absence of general-acid catalysis in the reactions with acid shows that proton transfer to the peptide oxygen is rapid and metal-peptide bond breaking becomes rate limiting. The rapidly formed outside protonated species (V) decomposes with a first-order rate constant more than 10^6 times greater than k_d, the partial solvent dissociation reaction to give species III. This is due to weakening of the Cu(peptide) bonds by protonation of the peptide oxygens, as in the case of the bis(glycylglycinato)cobaltate(III) complexes.[28] The relatively large value for k_3 suggests that the intermediate immediately following this step is CuL^+. The CuL^+ complex has both the imidazole and amine groups coordinated in a very large ring (structure VI) so that step k_3 is one in which Cu(II) is "skip roping" over the ligand. If the remaining steps in the acid dissociation path follow the same intermediates as those proposed in titrimetric studies[21] of the formation of the complex (pH 4-6), the next species would be one in which the imidazole and carboxylate groups of the histidyl residue are coordinated and the amine group is protonated.



VI

Table V. Rate and Outside Protonation Constants for Reactions with $Cu(H_{-2}glyglyhis)^-$, 1.0 M $NaClO_4$, 25.0 ± 0.1 °C

Constant	Value
k_d (H_2O, partial dissociation)	7.5×10^{-4} s^{-1}
k_H (proton transfer)	1.4×10^7 M^{-1} s^{-1}
k_N (trien)	0.5 M^{-1} s^{-1}
k_{H_2T}/k_{-H}	1.2×10^2 M^{-1}
$k_H k_{H_2T}/k_{-H}$	1.7×10^9 M^{-2} s^{-1}
k_3 (acid decomposition)	1×10^3 s^{-1}
K_1 (outside protonation)	1.6×10^4 M^{-1}
K_2 (outside protonation)	2.0×10^2 M^{-1}
$k_3 K_1 K_2$	3.2×10^9 M^{-2} s^{-1}

The acid decomposition path (Figure 2, curve D) is competitive with the proton-assisted path for 3×10^{-3} M trien (curve B) at pH 5. However, at pH 6, the acid decomposition pathway is very small and no longer competes. In neutral solution, the proton-assisted pathway predominates even at low nucleophile concentrations.

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