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Effect of Hydroxide Ion on the Kinetics of Triethylenetetramine Displacement of Tripeptides from Copper(II) Complexes

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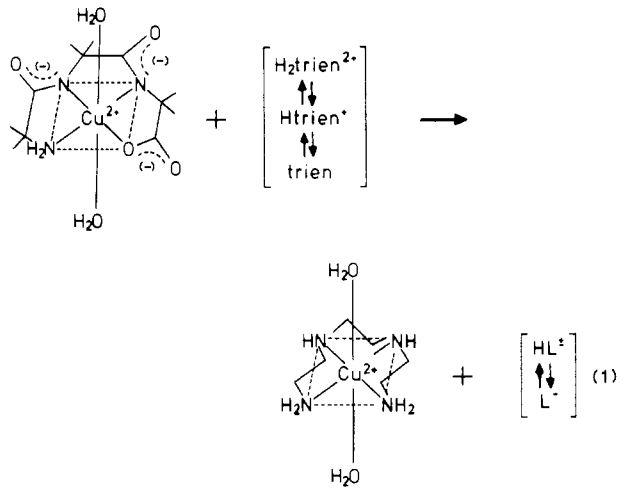
Triethylenetetramine (trien) reacts rapidly with doubly deprotonated (tripeptido)cuprate(II) complexes, $Cu(H_{-2}L)^-$, to give $Cu(trien)^{2+}$ and the free tripeptide, where L = GAG, GGA, GAibG, and Aib_3 (G, glycyl; A, L-alanyl; Aib, α-aminoisobutyryl). The reactions are first order in each reactant, and the second-order rate constants (M^{-1} s^{-1}, 25.0 °C, μ = 1.0 M) decrease greatly with the number of methyl groups in the second and third residues: 8.4×10^4 (GAG), 7.9×10^4 (GGA), 43 (GAibG), and 0.13 (Aib_3). When L is GAG, GGA, and GAibG, the values of the second-order rate constants (for $[trien]_T$ and $[Cu(H_{-2}L)^-]_T$) increase above p[H^+] 9 and reach maximum values at p[H^+] 10.7 ± 0.7. The increase occurs because trien and $Htrien^+$ are more reactive than H_2trien^{2+}. The rate constants decrease in higher base due to the formation of $Cu(H_{-2}L)(OH)^{2-}$, which is less reactive than $Cu(H_{-2}L)^-$ with trien. Values for the stability constants of the hydroxide adduct, K_{OH} (M^{-1}, 25.0 °C, μ = 1.0 M), are 41, 10, and 4 for L = GAG, GAibG, and GGA, respectively. A hydroxide adduct is not observed for the Aib_3 complex ($K_{OH} < 0.02$ M^{-1}). The $Cu(H_{-2}Aib_3)^-$ complex behaves differently because the reaction with trien is more sterically hindered. An acid-assisted H_2trien^{2+} path contributes to the observed rate below p[H^+] 10, and the rate begins to increase below p[H^+] 9.5. However, a proton-transfer step between H_3O^+ and $Cu(H_{-2}Aib_3)^-$ ($k_H = 2.5 \times 10^6$ M^{-1} s^{-1}) can limit the reaction so that it no longer depends on the $[trien]_T$ concentration. Above p[H^+] 10, the reaction is first order in both $[trien]_T$ and $[Cu(H_{-2}Aib_3)^-]$, and the rate increases to a plateau at p[H^+] 12. At higher p[H^+] levels the rate is assisted by OH^-, in contrast to the inhibition found for the other tripeptides. A third-order rate constant (0.41 M^{-2} s^{-1}) is found for the path due to $[trien][OH^-][Cu(H_{-2}Aib_3)^-]$.

Introduction

Doubly deprotonated (tripeptido)cuprate(II) complexes, $Cu(H_{-2}L)^-$, react with triethylenetetramine (trien) to form $Cu(trien)^{2+}$ and release the tripeptide (eq 1). This substitution



(1)

reaction is so fast with the triglycine complex, $Cu(H_{-2}G_3)^-$, that even with stopped-flow methods it had to be studied with dilute reactants under second-order conditions over a limited pH range (pH 7.6-9.2).[1] It was possible to resolve individual second-order rate constants (M^{-1} s^{-1}, 25.0 °C, μ = 0.1 M) for the reaction of $Cu(H_{-2}G_3)^-$ with trien ($k_T = 1.1 \times 10^7$), $Htrien^+$ ($k_{HT} = 5.1 \times 10^6$), and H_2trien^{2+} ($k_{H_2T} = 1.2 \times 10^5$), based on the trien protonation constants. Complexes that contained one or two L-alanyl (A) or L-leucyl residues reacted 2 or 3 orders of magnitude slower than the triglycine complex.[2] In the present work we examine the kinetics of the trien substitution reaction from pH 9 to 13 with copper(II) complexes of GAG, GGA, GAibG, and Aib_3, where Aib is an α-aminoisobutyryl residue. Steric effects of A or Aib in the second or third residues of the tripeptide decrease the rate to such an extent that it is possible to study the reactions at high pH and to use pseudo-first-order conditions with excess trien concentrations.

The observed rate constants for the copper(II) complexes of GAG, GGA, and GAibG do not level off at high pH as expected from previously resolved rate constants,[1,2] based on the conversion of all protonated trien species to the more reactive unprotonated ligand. The stepwise protonation constants of trien (25.0 °C, μ = 1.0 M) are $10^{10.02}$ M^{-1}, $10^{9.39}$ M^{-1}, $10^{7.00}$ M^{-1}, and $10^{4.00}$ M^{-1}.[3] Instead, the rates reach maxima at p[H^+] 10.7 ± 0.7 and decrease in more basic solutions due to the formation of hydroxide complexes, $Cu(H_{-2}L)(OH)^{2-}$. Spectrophotometric as well as kinetic evidence is given for the hydroxide complexes.

The rate and pH dependence of the $Cu(H_{-2}Aib_3)^-$ complex differ greatly from those of the other tripeptide complexes. $Cu(H_{-2}Aib_3)^-$ is 5 orders of magnitude slower to react than the GAG or GGA complexes. Its reaction with trien is acid assisted below p[H^+] 9.5 and base assisted above p[H^+] 12.

The effect of hydroxide ion on the kinetics of these substitution reactions is important in distinguishing its role from the enormous changes in rate caused by the steric effects of methyl groups in the tripeptide residues.[4,5]

Experimental Section

Ligand Synthesis. Starting materials were α-aminoisobutyric acid, (benzyloxycarbonyl)glycine (CBzG), benzyl glycinate tosylate (GOBz·TsOH) and *N,N'*-dicyclohexylcarbodiimide (DCC). *N*-(*tert*-butoxycarbonyl)-α-aminoisobutyric acid (BOCAib).[6] CBzAib and AibOBz·TsOH were prepared from Aib according to the standard procedure for protecting amino acids.[7] Aib_3 was synthesized as previously described.[8]

Glycyl-α-aminoisobutyrylglycine (GAibG). The dipeptide AibGOBz was obtained from the DCC coupling[6] of BOCAib with GOBz and deblocking of the BOCAibGOBz product by reacting it with trifluoroacetic acid (TFA).[9] The blocked tripeptide CBzGAibGOBz was synthesized from CBzG and AibGOBz by use of the DCC coupling procedure.[7] The free tripeptide GAibG was isolated by the catalytic hydrogenation[7,10] of

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CBzGAibGOBz. Anal. Calcd for GAibG, $C_8H_{15}N_3O_4$: C, 44.22; H, 6.97; N, 19.34. Found: C, 44.52; H, 7.00; N, 19.12. Mass spectrum (chemical ionization with isobutane): m/e 218 (M + H). ^{1}H NMR spectral data (vs TMS): δ 1.51 (s), 3.76 (s), 3.81 (s).

Reagents. Chromatographically homogeneous tripeptides of diglycyl-L-alanine (GGA) and glycyl-L-alanylglycine (GAG) were purchased from Biosynthetika (Oberdorf, Switzerland). Triethylenetetramine was prepared by distillation of commercially available trien (Aldrich) under reduced pressure. An aqueous stock solution was prepared by weight. The purity of the amine was checked by refractive index measurements and by the absence of significant absorbance above 250 nm. These tests indicate the absence of commonly encountered impurities. A stock $Cu(ClO_4)_2$ solution was prepared from the twice recrystallized salt and standardized with Na_2H_2EDTA (murexide indicator).[11] Sodium perchlorate solutions were prepared by the reaction of Na_2CO_3 with $HClO_4$. The resulting solution was boiled for several hours to remove the dissolved CO_2 and was filtered. The concentration of the $NaClO_4$ solution was determined gravimetrically. The ionic strength of all solutions was maintained at 1.0 M with $NaClO_4$.

The doubly deprotonated tripeptide complexes of copper(II) were prepared in solution by the slow addition of NaOH to a solution of $Cu(ClO_4)_2$, and the tripeptide (1:1.1 mol ratio) until a pH of 10–10.5 was attained. A color change from blue to violet was observed as the pH was increased, and $Cu(H_{-2}L)^-$ was formed. These solutions were freshly prepared for each series of experiments.

Methods. All pH values measured with Corning Model 476051 combination glass electrodes and an Orion Model 601 pH meter were corrected to the corresponding –log [H$^+$] (i.e. p[H$^+$]) values at 25.0 °C and μ = 1.0 M ($NaClO_4$) on the basis of electrode calibration titrations with standard solutions of NaOH and $HClO_4$. Gran plots[12] were used in the calibration of the electrodes. The value used for pK_w at 25.0 °C and μ = 1.0 M is 13.79.[13]

The reactions of trien with the $Cu(H_{-2}$tripeptide$)^-$ complexes were run under pseudo-first-order conditions ($[trien]_T > 10[Cu(II)]_T$) at 25.0 °C. A Durrum stopped-flow spectrophotometer interfaced to a Hewlett-Packard 2100S computer or a Perkin-Elmer 320 spectrometer interfaced to a Perkin-Elmer 3600 data station was used to observe the reaction kinetics. Experimental rate constants (k'_{obsd}) larger than 100 s^{-1} were corrected for mixing effects[14] in accord with eq 2, where k_m is 1700 s^{-1}.

$$k_{obsd} = k'_{obsd}/[1 - (k'_{obsd}/k_m)] \quad (2)$$

Depending on the tripeptide used, monitoring wavelengths between 240 and 280 nm were chosen. All of the reactions were pseudo-first-order in $Cu(H_{-2}$tripeptide$)^-$ for at least 4 half-lives and conformed to the rate expression in eq 3, where $[Cu(H_{-2}L)^-]_T = [Cu(H_{-2}L)^-] + [Cu(H_{-2}L)(OH)^{2-}]$. Each value of k_{obsd} is the average of at least four replicates. Under all conditions, the UV/vis spectrum of the final product was consistent with the quanitative formation of $Cu(trien)^{2+}$ and/or $Cu(trien)(OH)^+$. Even at high $[trien]_T$ no $(trien)_2$ complex was evident.

$$-d[Cu(H_{-2}L)^-]_T/dt = k_{obsd}[Cu(H_{-2}L)^-]_T. \quad (3)$$

Results and Discussion

Replacement of glycyl residues by L-alanyl or α-aminoisobutyryl residues markedly reduces the reactivity of the copper(II) complex with trien. Each methyl group in the second or third residue reduces the rate of the reaction by a factor of approximately 100. Thus, $Cu(H_{-2}Aib_3)^-$ reacts 8 orders of magnitude more slowly than $Cu(H_{-2}G_3)^-$. The slower rate permits the reaction to be studied from p[H$^+$] 9 to 13. The observed first-order rate constants depend on the concentrations of excess $[trien]_T$, where $[trien]_T = [H_2trien^{2+}] + [Htrien^+] + [trien]$. From previous studies,[1,2] the pH dependence of the k_{obsd} values would be expected to reach a plateau above p[H$^+$] 11, where $[trien] \simeq [trien]_T$. Instead, the k_{obsd} values for the GGA, GAG, and GAibG complexes have maximum values between p[H$^+$] 10–11.5 and then decrease as the p[H$^+$] values increase further. On the other hand, the reactivity of $Cu(H_{-2}Aib_3)^-$ reaches a plateau at p[H$^+$] 11–12, and then k_{obsd} increases at higher p[H$^+$] values.

Hydroxide Ion Complexes, $Cu(H_{-2}L)(OH)^{2-}$. As the hydroxide ion concentration increases, the absorbance of the GAG and GGA complexes at 540 nm decrease (Figure 1). This is accompanied by shifts of the λ_{max} values from 550 nm at p[H$^+$] 10 to λ_{max} values of 574 nm for $Cu(H_{-2}GAG)^-$ and 578 nm for $Cu(H_{-2}GGA)^-$ in high base concentrations. These spectral shifts are attributed to the formation of hydroxide complexes (eq 4), where hydroxide

$$Cu(H_{-2}L)^- + OH^- \overset{K_{OH}}{\rightleftharpoons} Cu(H_{-2}L)(OH)^{2-} \quad (4)$$

ion replaces a carboxylate group in an equatorial coordination site of copper(II). The same type of spectral shift is seen for $Cu(H_{-2}G_3)^-$ (λ_{max} = 553 nm, ϵ = 159 M^{-1} cm^{-1})[15] compared to $Cu(H_{-2}G_2a)(OH)^-$ (λ_{max} = 575 nm, ϵ = 81 M^{-1} cm^{-1}),[16] where G_2a is glycylglycinamide. The latter complex, with no carboxylate group to displace, is formed by loss of a proton from an equatorially coordinated water molecule with a pK_a value of 9.82.[16] The G_3 complex also forms a hydroxide complex, $Cu(H_{-2}G_3)(OH)^{2-}$, but the pK_a value is shifted to 12.0 (K_{OH} = 56 ± 12 M^{-1})[17] because of the need to displace the carboxylate group. Data plotted in Figure 1 correspond to the formation of $Cu(H_{-2}GAG)(OH)^{2-}$ (K_{OH} = 41 ± 1 M^{-1}) and $Cu(H_{-2}GGA)(OH)^{2-}$ (K_{OH} = 4 ± 1 M^{-1}) (Table I). A simplex curve-fitting program[18] is used for these data and is also used to fit similar data for complexes of GAibG (K_{OH} = 10 ± 1 M^{-1}), and A_3 (K_{OH} = 2.5 ± 0.6 M^{-1}).[19] An L-alanyl group in the third peptide residue gives smaller K_{OH} values than a glycyl group in the third residue. When Aib is in the third residue a hydroxide adduct does not form. Thus, $Cu(H_{-2}Aib_3)^-$ shows no spectral shift even in 4.5 M NaOH, and therefore the K_{OH} value for this complex must be less than 0.02

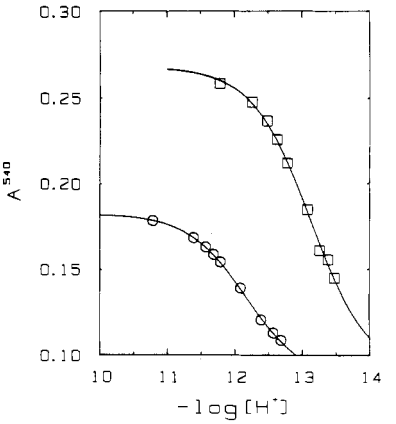

Figure 1. Spectrophotometric evidence for hydroxide complexes of $Cu(H_{-2}GAG)^-$ (○) and $Cu(H_{-2}GGA)^-$ (□). The solid lines correspond to K_{OH} values of 41 M^{-1} and 4 M^{-1}, respectively. Conditions: absorbance at 540 nm; 1.0 cm cell; pK_w = 13.79; μ = 1.0 M.

Table I. Equilibrium Constants for the Hydroxide Adduct with Copper(II) Tripeptides[a]

	K_{OH},[b] M^{-1}	
tripeptide	spectrophotometric data	kinetic data
GGG	56 ± 12[c]	
GAG	41 ± 1[d]	62 ± 12
GAibG	10 ± 1[e]	12 ± 6
GGA	4 ± 1[f]	5 ± 4
AAA	2.5 ± 0.6[g]	
Aib_3	<0.02	

[a] Conditions: 25.0 °C, μ = 1.0 M ($NaClO_4$), pK_w = 13.79. [b] $K_{OH} = [Cu(H_{-2}L)(OH)^{2-}]/([Cu(H_{-2}L)^-][OH^-])$. [c] Reference 17; μ = 0.1 M ($NaClO_4$). [d] ϵ_A^{540} = (159 ± 8) M^{-1} cm^{-1}; ϵ_B^{540} = (74 ± 1) M^{-1} cm^{-1}; A = $Cu(H_{-2}L)^-$; B = $Cu(H_{-2}L)(OH)^{2-}$. [e] ϵ_A^{530} = (142 ± 1) M^{-1} cm^{-1}; ϵ_B^{530} = (67 ± 1) M^{-1} cm^{-1}. [f] ϵ_A^{540} = (150 ± 2) M^{-1} cm^{-1}; ϵ_B^{540} = (50 ± 2) M^{-1} cm^{-1}. [g] Reference 19.

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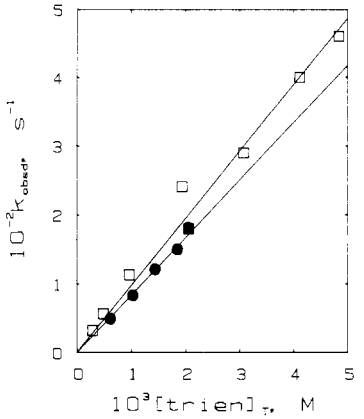

Figure 2. Dependence of the observed first-order rate constant (25.0 °C, μ = 1.0 M) for the reaction of Cu(H$_{-2}$GGA)$^-$, initially 5.72 × 10^{-5} M, with excess concentrations of trien: (●) p[H$^+$] 11.10 ± 0.06; (□) p[H$^+$] 11.80 ± 0.02.

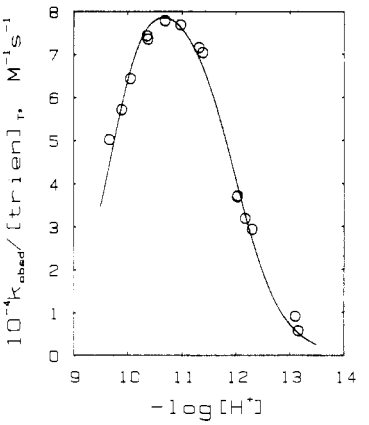

Figure 3. Dependence of second-order rate constants (25.0 °C, μ = 1.0 M) on p[H$^+$] for the reaction of Cu(H$_{-2}$GAG)$^-$ with trien. The solid line is a fit of eq 5 with K_{OH} = 62 M^{-1}, k_{HT} = 9 × 10^4 M^{-1} s^{-1}, and k_T = 8.4 × 10^4 M^{-1} s^{-1}.

M^{-1}. The spectral shifts for the Cu(H$_{-2}$L)(OH)$^{2-}$ complexes (λ_{max} = 565 nm for GAibG, 574 nm for GAG, and 578 nm for GGA) are consistent with an equatorially rather than axially coordinated hydroxide ion. The methyl groups in the Aib$_3$ complex would not sterically hinder axial coordination of hydroxide ion. In the case of the corresponding nickel(III) complexes, axial adducts of NH$_3$, pyridine, or imidazole form as readily with Aib$_3$ as with G$_3$a or A$_3$.[20] However, an axial hydroxide adduct is not observed with Ni(III) or Cu(II) peptide complexes. An L-alanyl or Aib residue in the third position does cause steric hindrance for equatorial coordination and this accounts for the decrease in the K_{OH} values as the third residue is changed from G to A to Aib. This steric effect is also reflected in the kinetic behavior with trien. The K_{OH} values determined from the hydroxide ion effect on the trien reaction rate agree with the spectrophotometric values for the GAG, GAibG, and GGA complexes (Table I).

Cu(H$_{-2}$GAG)$^-$ and Cu(H$_{-2}$GGA)$^-$ Kinetics. The reactions of Cu(H$_{-2}$GGA)$^-$ as a function of excess trien concentration at p[H$^+$] values of 11.2 and 11.8 gave excellent first-order plots. The k_{obsd} values increase with the total trien concentration (Figure 2) and show that the reaction is first order in [trien]$_T$. Earlier studies[2] with Cu(H$_{-2}$GAG)$^-$ under second-order conditions at p[H$^+$] 8–9 also indicated that the reaction was first order in each reactant.

The k_{obsd} values for the reactions of GAG and GGA complexes increase with the basicity of the solution up to p[H$^+$] $\simeq$ 11 and then decrease. Figures 3 and 4 show the dependence of the second-order rate constant (k_{obsd}/[trien]$_T$) as the basicity of the solution changes. The kinetic data correspond to the mechanism in Scheme I, where the reactivities of H$_2$trien^{2+} with Cu(H$_{-2}$L)$^-$

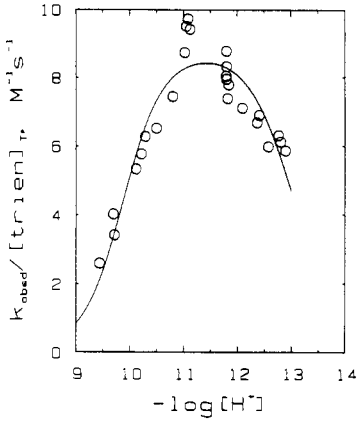

Figure 4. Dependence of the second-order rate constant on p[H$^+$] for the reaction of Cu(H$_{-2}$G$_2$A)$^-$ with trien (25.0 °C, μ = 1.0 M). The solid line corresponds to a fit of eq 5 with K_{OH} = 5 M^{-1}, k_{HT} = 3 × 10^4 M^{-1} s^{-1}, and k_T = 7.9 × 10^4 M^{-1} s^{-1}.

Scheme I

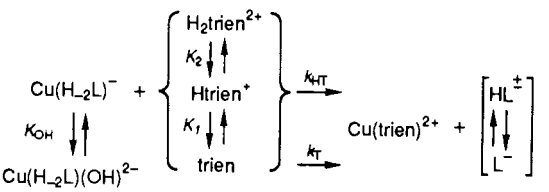

Table II. Resolved Rate Constants for the Reaction of Cu(H$_{-2}$tripeptide)$^-$ with trien[a]

complex	k_{HT}, M^{-1} s^{-1}	k_T, M^{-1} s^{-1}	k_4,[b] M^{-2} s^{-1}
Cu(H$_{-2}$GAG)$^-$	(9 ± 1) × 10^4	(8.4 ± 0.5) × 10^4	
Cu(H$_{-2}$G$_2$A)$^-$	(3 ± 1) × 10^4	(7.9 ± 0.3) × 10^4	
Cu(H$_{-2}$GAibG)$^-$	217 ± 9	43 ± 3	
Cu(H$_{-2}$Aib$_3$)$^-$	0.12 ± 0.03	0.127 ± 0.004	0.41 ± 0.06

[a] Conditions: 25.0 °C, μ = 1.0 M; resolved from eq 5 with pK_1 = 10.02, pK_2 = 9.39, and pK_w = 13.79. K_{OH} values are given in Table I. [b] The k_4 rate constant is from the resolution of eq 13.

and of trien with Cu(H$_{-2}$L)(OH)$^{2-}$ are negligible under the conditions used. This leads to the rate expression in eq 5, which

$$\frac{k_{obsd}}{[\text{trien}]_T} = \frac{[H^+]}{[H^+] + (K_wK_{OH})} \frac{k_{HT}K_2[H^+] + k_TK_1K_2}{K_1K_2 + K_2[H^+] + [H^+]^2} \quad (5)$$

is fit with a simplex program[18] to give the parameters summarized in Table II. K_1 and K_2 are acid dissociation constants for Htrien$^+$ and H$_2$trien^{2+}, respectively. The values of K_{OH} from the fit of the kinetic data are 62 ± 12 M^{-1} for the GAG complex and 5 ± 4 M^{-1} for the GGA complex, in reasonable agreement with the spectrophotometric values (Table I). The lack of reactivity of the Cu(H$_{-2}$L)(OH)$^{2-}$ complex is consistent with the need for trien (or Htrien$^+$) to coordinate in an equatorial position before the rate-determining step. Previous studies[2,17] have shown that trien attack occurs at the carboxylate end with the rate step being the cleavage of the Cu–N(peptide) bond adjacent to the carboxylate terminal.

Cu(H$_{-2}$GAibG)$^-$ trien Dependence. The reaction rate with trien is slower with Aib in the second residue, so that high concentrations of trien (0.2 M) can be tested without exceeding the capabilities of the stopped-flow method. While the reaction is first order in trien at lower concentrations (below 0.025 M), there is evidence of saturation kinetics at higher trien concentrations as shown in Figure 5. The behavior is consistent with the formation of a mixed-ligand complex Cu(H$_{-2}$GAibG)(trien)$^-$, where trien replaces the carboxylate group and coordinates to an equatorial site. As the equilibrium in eq 6 is shifted to the right, the reaction order

$$\text{Cu(H}_{-2}\text{L)}^- + \text{trien} \overset{K_T}{\rightleftharpoons} \text{Cu(H}_{-2}\text{L)(trien)}^- \quad (6)$$

$$\text{Cu(H}_{-2}\text{L)(trien)}^- \xrightarrow{k_3} \text{products} \quad (7)$$

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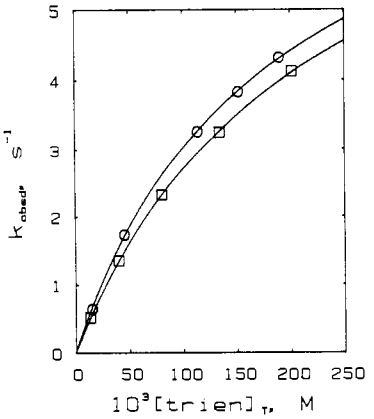

Figure 5. Observed first-order rate constants (25.0 °C, μ = 1.0 M) for the reaction of $Cu(H_{-2}GAibG)^-$, initially 8.95×10^{-5} M, the excess trien: (○) p[H⁺] 11.73; (□) p[H⁺] 11.84.

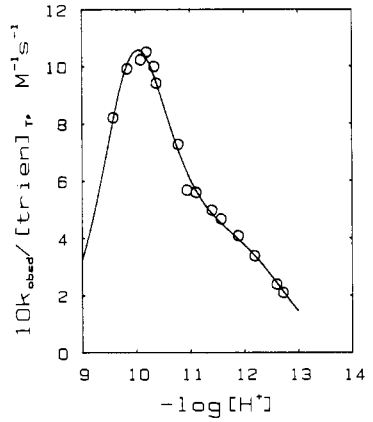

Figure 6. Dependence of the second-order rate constants on p[H⁺] for the reaction of $Cu(H_{-2}GAibG)^-$ with trien (0.0257 M). The solid line corresponds to a fit of eq 5 with K_{OH} = 12 M^{-1}, k_{HT} = 217 M^{-1} s^{-1}, and k_T = 43 M^{-1} s^{-1}.

in the free trien concentration decreases. Data at p[H⁺] values of 11.6–11.9 are also influenced slightly by the competitive equilibrium to form $Cu(H_{-2}L)(OH)^{2-}$. Hence, the observed rate constant is given in eq 8. A simplex fit gave $K_T = 5.6 \pm 0.4$ M^{-1}

$$k_{obsd} = \frac{k_3[\text{trien}]K_T}{K_{OH}[OH^-] + 1 + K_T[\text{trien}]} \quad (8)$$

and $k_3 = 8.5 \pm 0.6$ s^{-1} for the combined data. The value of K_T is reasonable for a monodentate coordinated trien that has replaced a carboxylate group. A mixed-ligand complex is not observed with $Cu(H_{-2}Aib_3)^-$, because steric hindrance blocks the equatorial site.

pH Dependence of $Cu(H_{-2}GAibG)^-$. The k_{obsd} dependence on p[H⁺] for the reaction of the GAibG complex is studied under conditions where $Cu(H_{-2}GAibG)(trien)^-$ is less than 10% of the total copper peptide complex. The rate constant vs p[H⁺] profile in Figure 6 again corresponds to eq 5, and the K_{OH} value of 12 ± 6 M^{-1} that is determined from the simplex fit of the kinetic data agrees well with the spectrophotometric value of 10 ± 1 M^{-1}. The ratio of k_{HT}/k_T is 5 for the GAibG complex while this ratio is only 0.4 and 1.1 for the GAG and GGA complexes, respectively. The reasons for this variation are not entirely clear, but the Htrien⁺ species has a proton available that might help to react with the Aib deprotonated-N(peptide) group in the rate-determining step.

$Cu(H_{-2}Aib_3)^-$ Kinetics. The reaction between trien and $Cu(H_{-2}Aib_3)^-$ is not only much slower than the reactions with other (tripeptido)cuprate(II) complexes, it also has a different pH dependence. An acid-assisted trien path is found below p[H⁺] 9.5. This reverses the dependence of k_{obsd} on pH for Aib_3 (Figure 7) compared to the GAG, GGA, and GAibG complexes. At p[H⁺] 8.80 and $[\text{trien}]_T = 4.7 \times 10^{-3}$ M, the $k_{obsd}/[\text{trien}]_T$ value is 0.43 M^{-1} s^{-1}. This is twice as large as the corresponding value at p[H⁺] 10.10. At p[H⁺] 8.95 ± 0.15 the k_{obsd} values reach a

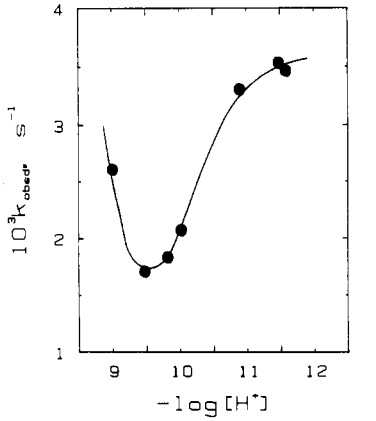

Figure 7. Observed first-order rate constants (25.0 °C, μ = 1.0 M) for the reaction of $Cu(H_{-2}Aib_3)^-$ with $[\text{trien}]_T = 0.026$ M as a function of p[H⁺]. An acid-assisted trien path occurs below p[H⁺] 9.5.

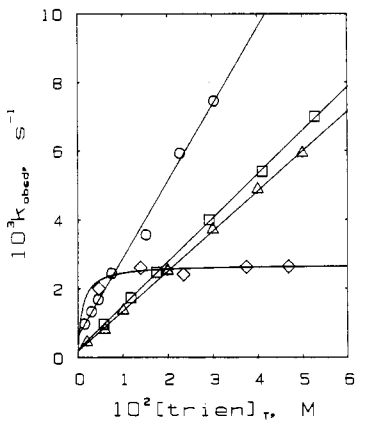

Figure 8. Observed first-order rate constants (25.0 °C, μ = 1.0 M) for the reaction of $Cu(H_{-2}Aib_3)^-$ with trien at p[H⁺] 9.0 (◇), 10.30 (△), 11.16 (□), and 12.79 (○).

limiting value, independent of $[\text{trien}]_T$, as seen in Figure 8. The limiting value of k_{obsd} increases as the concentration of the boric acid buffer increases. This behavior is analogous to the proton-assisted nucleophilic mechanism observed for the reactions of trien with copper(II) and nickel(II) complexes of glycylglycyl-L-histidine.[21,22] Proton transfer to the Cu–N(peptide) bond adjacent to the carboxylate terminal helps to break that bond before the trien substitution process. The proposed mechanism is given in eq 9 and 10. A steady-state approximation for $Cu(H_{-1}Aib_3)$ leads

$$Cu(H_{-2}Aib_3)^- + H^+ \underset{k_{-H}}{\overset{k_H}{\rightleftharpoons}} Cu(H_{-1}Aib_3) \quad (9)$$

$$Cu(H_{-1}Aib_3) + H_2\text{trien}^{2+} \xrightarrow{k_{H_2T}} Cu(\text{trien})^{2+} + HAib_3^{\pm} \quad (10)$$

to eq 11, and k_{obsd} becomes independent of $[H_2\text{trien}^{2+}]$ at high

$$k_{obsd} = \frac{k_H k_{H_2T}[H^+][H_2\text{trien}^{2+}]}{k_{-H} + k_{H_2T}[H_2\text{trien}^{2+}]} \quad (11)$$

concentrations. A more general expression for k_{obsd} at high $[H_2\text{trien}^{2+}]$ includes the path with H_2O (k_d) and buffer acids ($k_{HA}[HA]$) given in eq 12. The values found are $k_d = 2 \times 10^{-4}$

$$k_{obsd} = k_d + k_H[H^+] + k_{HA}[HA] \quad (12)$$

s^{-1}, $k_H = (2.5 \pm 0.1) \times 10^6$ M^{-1} s^{-1}, and $k_{HA} \simeq 5 \times 10^{-2}$ M^{-1} s^{-1} for $B(OH)_3$. Similar values for k_H are found in studies of the reaction of $Cu(H_{-2}Aib_3)^-$ with dilute acid[23] and for the reaction

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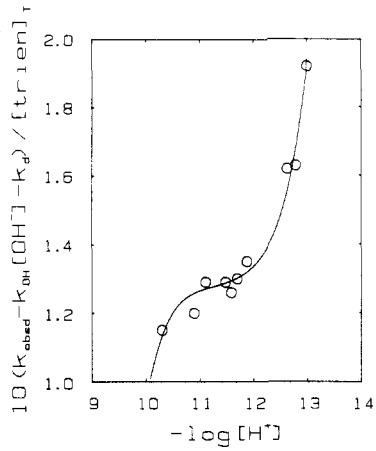


Figure 9. p[H$^+$] dependence of second-order rate constants (25.0 °C, μ = 1.0 M) for the reaction of Cu(H$_{-2}$Aib$_3$)$^-$ with trien. The solid line corresponds to a fit of eq 13 with k_{OH} = 4 × 10^{-3} M^{-1} s^{-1}, k_d = 2 × 10^{-4} s^{-1}, k_{HT} = 0.12 M^{-1} s^{-1}, k_T = 0.127 M^{-1} s^{-1}, and k_4 = 0.41 M^{-2} s^{-1}.

of Cu(H$_{-2}$Aib$_3$)$^-$ with excess terpyridine.[24]

Steric effects are relatively unimportant in proton-transfer reactions. Thus, the k_H value for Cu(H$_{-2}$G$_3$)$^-$ is 1.3 × 10^7 M^{-1} s^{-1},[25] which is only a factor of 5 larger than the k_H value for Cu(H$_{-2}$Aib$_3$)$^-$. This variation is not very significant when compared to the factor of 10^8 found for the relative reactivities of these complexes with trien.[5]

At p[H$^+$] 10.35 and 11.15 the acid-assisted path is negligible and the nucleophilic path predominates because trien and Htrien$^+$ are now more reactive than H$_2$trien^{2+}. A linear dependence between k_{obsd} and [trien]$_T$ is found (Figure 8) with an intercept that corresponds to k_d. As the hydroxide ion concentration is increased further the rate of the trien reaction with Cu(H$_{-2}$Aib$_3$)$^-$ increases, as opposed to the decrease in rate found for the other tripeptides. In 0.10 M NaOH, k_{obsd} has a linear dependence in [trien] with a larger intercept than observed in lower OH$^-$ concentrations (Figure 8). The k_{obsd} value from p[H$^+$] 10 to 13 is expressed by eq 13, where k_{OH} = 4 × 10^{-3} M^{-1} s^{-1} is for a

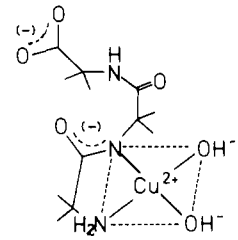
$$k_{obsd} = \frac{(k_{HT}K_2[H^+] + k_TK_1K_2)[trien]_T}{[H^+]^2 + K_2[H^+] + K_1K_2} + k_d + k_{OH}[OH^-] + k_4[OH^-][trien] \quad (13)$$

base-assisted dissociation path, while k_4 is a third-order rate constant for a base-assisted trien path. Figure 9 is a plot of eq 14 that is fitted by the simplex program to give k_{HT} = 0.12 ± 0.03

$$\frac{k_{obsd} - k_d - k_{OH}[OH^-]}{[trien]_T} = \frac{k_{HT}K_2[H^+] + K_1K_2(k_T + k_4[OH^-])}{[H^+]^2 + K_2[H^+] + K_1K_2} \quad (14)$$

M^{-1} s^{-1}, k_T = 0.127 ± 0.004 M^{-1} s^{-1}, and k_4 = 0.41 ± 0.06 M^{-2} s^{-1}.

The k_4 path is interesting because Cu(H$_{-2}$Aib$_3$)$^-$ does not form a hydroxide adduct, and furthermore an adduct such as Cu(H$_{-2}$L)(OH)$^{2-}$ would tend to suppress rather than assist the trien reaction. Trien and OH$^-$ must both act as nucleophiles to displace Aib$_3$ from Cu(II). An intermediate such as Cu(H$_{-1}$Aib$_3$)(OH)$_2^{2-}$



would be less sterically hindered to trien attack than Cu(H$_{-2}$Aib$_3$)$^-$. Other studies[26] show that polyamines can react directly with species such as Cu(OH)$_3^-$ and Cu(OH)$_4^{2-}$. Hence, OH$^-$ can be displaced by trien, but the displacement of a coordinated water is faster.

The Aib$_3$ complex is sterically hindered and is much slower to react with trien. As a result, solvent dissociation paths (k_d + k_{OH}[OH$^-$]) are detected and both acid-assisted and base-assisted trien paths are found.

Conclusions

The rates of trien displacement of tripeptides from copper(II) are very dependent upon the nature of the tripeptide. Methyl group steric effects for 12 tripeptides are discussed in the subsequent paper.[5]

Kinetic saturation effects with excess trien are observed under two sets of conditions. If glycine is the third residue, high concentrations of trien will lead to the formation of a weak mixed-ligand complex, [Cu(H$_{-2}$L)trien]$^-$. If Aib is the third residue, a mixed-ligand complex does not form. However, with [trien]$_T$ in excess below pH 9, the rate-limiting step becomes proton transfer to the peptide nitrogen. The proton-transfer step is accompanied by copper–N(peptide) bond cleavage. This unwraps part of the peptide from the metal ion and permits rapid subsequent reaction with trien.

Hydroxide ion forms Cu(H$_{-2}$L)(OH)$^{2-}$ complexes that inhibit the trien displacement reaction. The Aib$_3$ complex is an exception. It does not form a hydroxide complex, and its reactivity with trien is decreased to such a degree that hydroxide ion assists the rate by acting as a second nucleophile to help displace the peptide. This does not occur with the other tripeptides because the nucleophilic trien path is much more favorable.

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Supplementary Material Available: Tables of rate constants for each copper peptide complex as a function of pH and trien concentrations (8 pages). Ordering information is given on any current masthead page.

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