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Methyl Group Steric Effects on the Kinetics of the Copper(II)–Tripeptide Reactions with Triethylenetetramine

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Rates of reaction of triethylenetetramine (trien) with doubly deprotonated (tripeptido)cuprate(II) complexes are measured as a function of the number and position of methyl groups in the amino acid residues. Twelve tripeptides that consist of glycyl (G), L-alanyl (A), and α-aminoisobutyryl (Aib) residues are compared. The $Cu(H_{-2}Aib_3)^-$ complex reacts 8 orders of magnitude more slowly with trien than does the $Cu(H_{-2}G_3)^-$ complex. Methyl groups on the α-carbon of the second and third amino acid residues (from the amino terminus) decrease the rate of the trien substitution reaction to a much greater extent than methyl groups on the first residue. An empirical correlation between the second-order rate constant (k_{trien}, M^{-1} s^{-1} at 25.0 °C, pH ≃11) and the number and position of the methyl groups is found: $\log k_{trien} = 6.7 - 0.2C_1 - 2.2C_2 - 1.6C_3$, where C_i denotes the number of methyl groups in the ith amino acid residue. The enormous changes in reactivity are attributed primarily to steric effects in these ligand–ligand exchange reactions.

Introduction

Triethylenetetramine (trien) reacts with doubly deprotonated (tripeptido)cuprate(II) complexes, $Cu(H_{-2}L)^-$ (structure I), in

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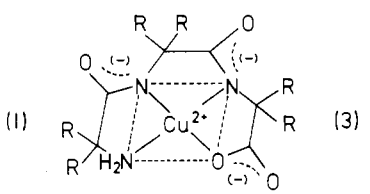

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I

a substitution process, where the tetradentate trien completely replaces the tetradentate tripeptide (eq 1; water molecules axially

$$\text{trien} + Cu(H_{-2}L)^- + 2H_2O \rightarrow Cu(\text{trien})^{2+} + L^- + 2OH^- \quad (1)$$

coordinated to the peptide and the trien complexes are omitted). We find enormous changes in the rates of the reaction with variation of glycyl (G), L-alanyl (A), and α-aminoisobutyryl (Aib) residues in the tripeptide. In order to understand why there is a 10^8-fold range of reactivity, the reactions are studied as a function of the number of methyl groups in the first, second, and third amino acid residues of the tripeptides.

Earlier studies[1] showed the trien replacement of triglycine in $Cu(H_{-2}G_3)^-$ is very rapid. Second-order rate constants (M^{-1} s^{-1} at 25.0 °C) of 1.1×10^7, 5.1×10^6, and 1.2×10^5 were resolved for trien, Htrien$^+$, and H_2trien^{2+}, respectively. Ethylenediamine (en) and diethylenetriamine (dien) also react very rapidly with $Cu(H_{-2}G_3)^-$ (4.8×10^6 M^{-1} s^{-1} for en and 4×10^6 M^{-1} s^{-1} for dien) to form mixed-ligand complexes, $Cu(H_{-1}G_3)$en and Cu-(G_3)dien$^+$.[2] It is evident that the substitution process begins at the carboxylate terminal. Steric effects were observed[3] in trien reactions when L-alanyl and L-leucyl (leu) residues were used in the tripeptide complexes of copper(II). Second-order rate constants (M^{-1} s^{-1}) were 1.3×10^5 (GAG), 6×10^4 (GleuG), and 3.6×10^4 (leuGleu). It was noted that alkyl groups on the α-carbon atoms of the second and third amino acid residues from the N-terminal end of the tripeptide (structure I) were most effective in hindering the trien reaction. In the present work, the effect of the number and position of methyl groups in the amino acid residues of the tripeptides is explored further. Rate constants for the reactions of trien at p[H$^+$] 11.5 ± 0.3 are measured for AGA, GAA, A_3, G_2Aib, A_2Aib, Aib$_2$G, and Aib$_2$A. These values are compared to the rate constants for four other tripeptides (GAG, GGA, GAibG, and Aib$_3$)[4] and to the value for G_3.[1]

The temperature dependence for the reactions at p[H$^+$] 11 gives activation parameters for four of the tripeptides. All four rate constants with trien have large negative activation entropies and small positive activation enthalpies.

Experimental Section

Ligand Synthesis. Starting materials were α-aminoisobutyric acid (Aib), (benzyloxycarbonyl)glycine (CBzG), (benzyloxycarbonyl)alanine (CBzA), benzyl alaninate tosylate (AOBz·TsOH), benzyl glycinate tosylate (GOBz·TsOH), *N*-(*tert*-butoxycarbonyl)alanine (BOCA), 1-cyclohexyl-3-(2-morpholinoethyl)carbodiimide metho-*p*-toluenesulfonate, and *N*,*N*′-dicyclohexylcarbodiimide (DCC). CBzAib and AibOBz·TsOH were prepared from Aib according to the standard procedure for protecting amino acids.[5]

Glycylglycyl-α-aminoisobutyric Acid. The blocked tripeptide $CBzG_2AibOBz$ was synthesized from $CBzG_2$ and AibOBz by using the DCC coupling procedure.[5] The free tripeptide G_2Aib was isolated by the catalytic hydrogenation[5,6] of $CBzG_2AibOBz$.

Di-α-aminoisobutyrylalanine and Di-α-aminoisobutyrylglycine. $CBzAib_2$ was prepared by a procedure described previously.[7] Heating of $CBzAib_2$ with acetic anhydride at 115 °C for 15 min, followed by removal of the resulting acetic acid and excess acetic anhydride under vacuum at 55 °C, gave 2-[1-[(benzyloxycarbonyl)amino]-1-methylethyl]-4,4-dimethyloxazolone.[8,9] Coupling of this oxazolone intermediate with AOBz or GOBz in acetonitrile (95 °C, 12 h) afforded $CBzAib_2AOBz$ or $CBzAib_2GOBz$. Deblocking of these fully protected tripeptides by hydrogenolysis over PdO gave Aib$_2$A and Aib$_2$G, respectively.[5]

Alanylalanyl-α-aminoisobutyric Acid. BOCAAibOBz was prepared from BOCA and AibOBz by using 1-cyclohexyl-3-(2-morpholinoethyl)-carbodiimide metho-*p*-toluenesulfonate as the coupling reagent. Deblocking was achieved by treatment with trifluoroacetic acid to give AAibOBz·CF_3COOH. $CBzA_2AibOBz$ was then prepared from CBzA and AAibOBz·CF_3COOH by the mixed carbonic anhydride procedure using isobutyl chloroformate as the carboxyl activator.[10] Deprotection

(1) Pagenkopf, G. K.; Margerum, D. W. *J. Am. Chem. Soc.* **1970**, *92*, 2683–2686.
(2) Hauer, H.; Billo, E. J.; Margerum, D. W. *J. Am. Chem. Soc.* **1971**, *93*, 4173–4178.
(3) Hauer, H.; Dukes, G. R.; Margerum, D. W. *J. Am. Chem. Soc.* **1973**, *95*, 3515–3522.
(4) Schwederski, B. E.; Margerum, D. W. *Inorg. Chem.*, preceding paper in this issue.
(5) Greenstein, J. P., Winitz, M. *Chemistry of the Amino Acids*; Wiley: New York, 1961; Vol. 2.
(6) Shriner, R. L.; Adams, R. *J. Am. Chem. Soc.* **1924**, *46*, 1683–1693.
(7) Hinton, J. P.; Lee, H. D.; Margerum, D. W. To be submitted for publication.
(8) Leplawy, M. T.; Jones, D. S.; Kenner, G. W.; Sheppard, R. C. *Tetrahedron* **1960**, *11*, 39–51.
(9) Jones, D. S.; Kenner, G. W.; Preston, J.; Sheppard, R. S. *J. Chem. Soc.* **1965**, 6227–6239.
(10) Anderson, G. W.; Zimmerman, J. E.; Callahan, F. M. *J. Am. Chem. Soc.* **1967**, *89*, 5012–5017.
(11) Welcher, F. J. *The Analytical Uses of EDTA*; Van Nostrand: New York, 1963; p 103.

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Table I. Pseudo-First-Order Rate Constants for the Reaction of trien with Cu(H$_{-2}$tripeptide)$^-$ Complexes[a]

complex	−log [H$^+$]	10^2[trien]$_T$, M	k_{obsd}, s^{-1}
Cu(H$_{-2}$AGA)$^-$[b]	11.20	0.0290	17.9 ± 0.2
	11.07	0.0484	31.5 ± 0.2
	11.20	0.0967	63 ± 3
	11.29	0.193	128 ± 4
	11.29	0.309	209 ± 10
Cu(H$_{-2}$GA$_2$)$^-$[c]	11.65	0.268	6.51 ± 0.05
	11.67	0.535	13.0 ± 0.1
	11.67	1.07	25.7 ± 0.3
	11.65	1.61	37.9 ± 0.9
	11.72	2.15	48 ± 2
Cu(H$_{-2}$A$_3$)$^-$[d]	11.40	0.295	2.67 ± 0.04
	11.50	0.585	5.29 ± 0.05
	11.50	1.17	10.2 ± 0.3
	11.40	3.52	30.9 ± 0.5
Cu(H$_{-2}$GGAib)$^-$[d]	11.60	0.268	1.54 ± 0.05
	11.60	0.535	2.99 ± 0.07
	11.66	1.07	6.2 ± 0.1
	11.60	1.61	8.8 ± 0.6
	11.65	2.15	11.8 ± 0.8
Cu(H$_{-2}$A$_2$Aib)$^-$[e]	11.60	0.102	(1.917 ± 0.008) × 10^{-2}
	11.59	0.203	(3.596 ± 0.005) × 10^{-2}
	11.53	0.305	(5.14 ± 0.02) × 10^{-2}
	11.60	0.407	(6.76 ± 0.06) × 10^{-2}
	11.57	0.509	(8.46 ± 0.03) × 10^{-2}
	11.62	0.764	0.124 ± 0.001
Cu(H$_{-2}$Aib$_2$G)$^-$[e]	11.65	0.102	(1.98 ± 0.01) × 10^{-2}
	11.62	0.203	(3.58 ± 0.03) × 10^{-2}
	11.61	0.305	(5.12 ± 0.03) × 10^{-2}
	11.67	0.407	(6.80 ± 0.05) × 10^{-2}
	11.58	0.509	(8.36 ± 0.06) × 10^{-2}
	11.66	0.764	0.1213 ± 0.0007
Cu(H$_{-2}$Aib$_2$A)$^-$[e]	11.88	0.102	(2.49 ± 0.02) × 10^{-3}
	11.83	0.203	(3.70 ± 0.06) × 10^{-3}
	11.79	0.305	(5.12 ± 0.02) × 10^{-3}
	11.59	0.407	(6.38 ± 0.03) × 10^{-3}
	11.64	0.509	(8.23 ± 0.02) × 10^{-3}
	11.81	0.764	(1.14 ± 0.01) × 10^{-2}

[a] Conditions: μ = 1.0 ($NaClO_4$), 25.0 °C. [b] [Cu(H$_{-2}$L)$^-$]$_0$ = 5.0 × 10^{-5} M, λ = 280 nm. [c] [Cu(H$_{-2}$L)$^-$]$_0$ = 5.0 × 10^{-5} M, λ = 234 nm. [d] [Cu(H$_{-2}$L)$^-$]$_0$ = 5.0 × 10^{-5} M, λ = 240 nm. [e] [Cu(H$_{-2}$L)$^-$]$_0$ = 8.95 × 10^{-4} M, λ = 260 nm.

by hydrogenolysis over PdO gave the free tripeptide A_2Aib.

Analyses. Satisfactory elemental analysis and mass spectra were obtained for the tripeptides Aib$_2$G, Aib$_2$A, and A_2Aib. ^{1}H NMR data are also reported for the compounds. ^{1}H NMR spectral data for G_2Aib (vs TMS): δ 1.86 (s), 4.36 (s), 4.39 (s). Anal. Calcd for Aib$_2$A·$^1/_2H_2O$, $C_{11}H_{21}N_3O_4$: C, 49.23; H, 8.26; N, 15.66. Found: C, 49.02; H, 8.53; N, 15.28. Mass spectrum (chemical ionization with isobutane): *m/e* 260 (M + H). ^{1}H NMR spectral data (vs TMS): δ 1.31 (d), 1.47 (s), 1.49 (s), 1.61 (s), 1.63 (s), 4.13 (q). Anal. Calcd for A_2Aib, $C_{10}H_{19}N_3O_4$: C, 48.96; H, 7.80; N, 17.13. Found: C, 48.82; H, 7.93; N, 16.94. Mass spectrum (chemical ionization with isobutane): *m/e* 246 (M + H). ^{1}H NMR spectral data (vs TMS): δ 1.39 (d), 1.43 (s), 1.57 (d), 4.08 (q), 4.36 (q). Anal. Calcd for Aib$_2$G, $C_{10}H_{19}N_3O_4$: C, 48.96; H, 7.80; N, 17.13. Found: C, 48.65; H, 7.99; N, 16.79. Mass spectrum (chemical ionization with isobutane): *m/e* 246 (M + H). ^{1}H NMR spectral data (vs TMS): 1.52 (s), 1.64 (s), 3.76 (s).

Reagents. The chromatographically homogeneous tripeptides tri-L-alanine (A_3), glycyl-L-alanyl-L-alanine (GAA), L-alanylglycyl-L-alanine (AGA), diglycyl-L-alanine (GGA), glycyl-L-alanylglycine (GAG) and L-alanylglycylglycine (AGG) were purchased from Biosynthetika (Oberdorf, Switzerland). Triethylenetetramine was prepared as the free base by the slow addition of trien·2H_2SO_4, which had been recrystallized four times, to a 10 M NaOH solution in isopropyl alcohol. After removal of the Na_2SO_4 precipitate by filtration, the solution was distilled under reduced pressure and the middle third of the trien fraction was collected. Stock solutions of trien, $Cu(ClO_4)_2$, $NaClO_4$, and doubly deprotonated tripeptide complexes of copper(II), Cu(H$_{-2}$L)$^-$, were prepared as described in the previous paper.[4] The ionic strength of all solutions was maintained at 1.0 with $NaClO_4$.

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

tripeptide	k_d, s^{-1}	k_{trien}, M^{-1} s^{-1}
G_3[b]	0.12	(1.1 ± 0.4) × 10^7
GAG[c]	*d*	(8.4 ± 0.5) × 10^4
G_2A[c]	*d*	(7.9 ± 0.3) × 10^4
AGA	*d*	(6.81 ± 0.04) × 10^4
GA_2	*d*	(2.22 ± 0.06) × 10^3
A_3	*d*	(8.75 ± 0.04) × 10^2
GGAib	*d*	(5.4 ± 0.1) × 10^2
GAibG[c]	*d*	43 ± 3
A_2Aib	(3.4 ± 0.4) × 10^{-3}	15.83 ± 0.09
Aib$_2$G	(4.6 ± 0.6) × 10^{-3}	15.4 ± 0.1
Aib$_2$A	(1.0 ± 0.1) × 10^{-3}	1.37 ± 0.03
Aib$_3$[c]	(2.2 ± 0.3) × 10^{-4}	0.127 ± 0.004

[a] Conditions: μ = 1.0 M ($NaClO_4$), 25.0 °C. [b] μ = 0.1 ($NaClO_4$), 25.0 °C.[1] [c] k_{trien} rate constants resolved from pH dependence data.[4] [d] Not determined, because of large uncertainties in the value of k_d.

Table III. Stability Constants of Copper(II)–Tripeptide Complexes[a]

complex	log $\beta_{1,-2,1}$[b]	complex	log $\beta_{1,-2,1}$[b]
Cu(H$_{-2}$G$_3$)$^-$	−6.76	Cu(H$_{-2}$Aib$_3$)$^-$	−4.95
Cu(H$_{-2}$GAibG)$^-$	−5.65		

[a] Hamburg, A. W.; Nemeth, M. T.; Margerum, D. W. *Inorg. Chem.* **1983**, *22*, 3535–3541. [b] $\beta_{1,-2,1}$ = [Cu(H$_{-2}$L)$^-$][H$^+$]^2/[Cu^{2+}][L$^-$].

Methods. Calibration of p[H$^+$] values and stopped-flow rate constants are described in the previous paper.[4] All of the reactions were pseudo-first-order in Cu(H$_{-2}$L)$^-$ for at least 4 half-lives and conformed to the rate expression

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

Each reported observed rate constant, k_{obsd}, is the average of at least four replicates.

Results and Discussion

Pseudo-first-order rate constants (k_{obsd}) were measured for the reaction of excess trien with doubly deprotonated (tripeptido)-cuprate(II) complexes of AGA, GAA, A_3, G_2Aib, A_2Aib, Aib$_2$G, and Aib$_2$A (Table I). The reactions were studied at p[H$^+$] values of 11.5 ± 0.3 in order to have unprotonated trien (its stepwise protonation constants, M^{-1}, are $10^{10.02}$, $10^{9.39}$, $10^{7.00}$, and $10^{4.00}$)[14] and Cu(H$_{-2}$L)$^-$ as the predominant species in solution. Hydroxide complexes, Cu(H$_{-2}$L)(OH)$^{2-}$, do not form when Aib is the third amino acid residue. The stability constant for K_{OH} (eq 3) is less

$$K_{OH} = \frac{[Cu(H_{-2}L)(OH)^{2-}]}{[Cu(H_{-2}L)^-][OH^-]} \quad (3)$$

than 4 M^{-1} when alanine is in the third residue.[4] When glycine is in the third residue a K_{OH} value of 10 M^{-1} was found for the GAibG complex.[4] Hence, the amount of Cu(H$_{-2}$L)(OH)$^{2-}$ (which is less reactive than Cu(H$_{-2}$L)$^-$)[4] present in solution is less than 3% for all complexes except Aib$_2$G, where it is less than 7%. The k_{obsd} values were measured as a function of the trien concentration. For the A_2Aib, Aib$_2$G, and Aib$_2$A complexes, the dependence is given by eq 4, where k_{trien} is the second-order rate constant for

$$k_{obsd} = k_d + k_{trien}[trien] \quad (4)$$

nucleophilic attack by unprotonated trien and k_d is a trien-independent solvent path. (The values are given in Table II.) For the AGA, GAA, A_3, and G_2Aib complexes only k_{trien} values are given. Under the conditions used, the k_d terms contributed very little to the observed rate constants for these complexes, and hence the uncertainty of the k_d values are too large to permit their comparison. The trien-dependent rate constants for all seven complexes are compared in Table II along with those for five other tripeptides where k_{trien} values were resolved from their pH dependence.[4]

(12) Rossotti, F. J. C.; Rossotti, H. *J. Chem. Educ.* **1965**, *42*, 375–378.
(13) Dickson, P. N.; Margerum, D. W. *Anal. Chem.* **1986**, *58*, 3153–3158.
(14) Smith, R. M.; Martell, A. E. *Critical Stability Constants, Vol. 2: Amines*; Plenum Press: New York, 1975; p 105.

Table IV. Comparison of the Positional Dependence of the Methyl Group Steric Effect

peptide	α-methyl substituent distribn C_1	C_2	C_3	log k_{trien} obsd[a]	calcd[b]
G_3	0	0	0	7.04	6.7
GAG	0	1	0	4.92	4.4
GGA	0	0	1	4.90	5.1
AGA	1	0	1	4.83	4.9
GA_2	0	1	1	3.35	2.8
A_3	1	1	1	2.94	2.6
GGAib	0	0	2	2.73	3.5
GAibG	0	2	0	1.63	2.2
A_2Aib	1	1	2	1.20	1.1
Aib_2G	2	2	0	1.19	1.8
Aib_2A	2	2	1	0.14	0.20
Aib_3	2	2	2	−0.90	−1.4

[a] Conditions: see Table II. [b] Calculated from log $k = \beta_0 + \beta_1C_1 + \beta_2C_2 + \beta_3C_3$ and $\beta_0 = 6.7 \pm 0.3$, $\beta_1 = -0.20 \pm 0.26$, $\beta_2 = -2.2 \pm 0.2$, and $\beta_3 = -1.6 \pm 0.2$.

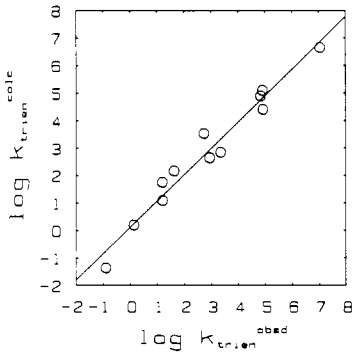


Figure 1. Correlation between calculated k_{trien} values from eq 6 and the observed k_{trien} values (25.0 °C).

The rate constants in Table II for the trien reactions decrease greatly as L-alanine or Aib replace glycine in either the second or third residue of the tripeptide. The k_{trien} values change by a factor of 8.7×10^7 from G_3 to Aib_3, while the k_d values change by only a factor of 5.5×10^2. A comparison of the overall stability constants for three of the complexes is given in Table III. The stability constant of the Aib_3 complex is only a factor of 65 larger than the stability constant for the G_3 complex. Hence, the large kinetic effect that the methyl groups have on the trien rate constant cannot be attributed to changes in the stability of the copper peptide complexes.

Another comparison of interest is the protonation rate constant for the complexes (eq 5). The k_H value for $Cu(H_{-2}G_3)^-$ is 1.3

$$Cu(H_{-2}L)^- + H^+ \xrightarrow{k_H} Cu(H_{-1}L) \qquad (5)$$

$\times 10^7$ M^{-1} s^{-1},[15] while the k_H value for $Cu(H_{-2}Aib_3)^-$ is 2.5×10^6 M^{-1} s^{-1},[4] only a factor of 5 smaller. Obviously methyl groups do little to hinder the process in which H_3O^+ adds a proton to the peptide nitrogen as the Cu(II)–N(peptide) bond breaks.[15-17] The difference in the effect of methyl groups on the k_{trien}, k_d, and k_H values indicates that steric effects are very important in the trien reactions and much less important in the k_d and k_H pathways.

Correlation of k_{trien} Values with Methyl Groups. Table IV shows the effect that the number and position of α-methyl groups have on the trien rate constants. Equation 6 gives a simple mathematical model where the independent variable C_i refers to the

$$\log k_{trien} = \beta_0 + \beta_1C_1 + \beta_2C_2 + \beta_3C_3 \qquad (6)$$

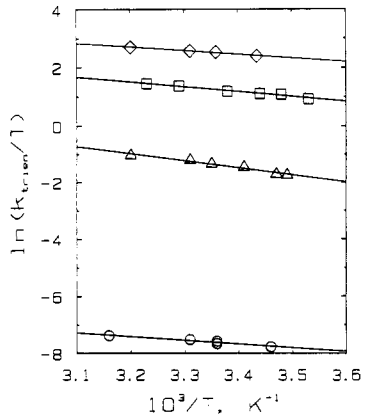


Figure 2. Temperature dependence of k_{trien}: $Cu(H_{-2}GAA)^-$ (◇), $Cu(H_{-2}A_3)^-$ (□), $Cu(H_{-2}GAibG)^-$ (△) and $Cu(H_{-2}Aib_3)^-$ (○).

Table V. Activation Parameters for k_{trien}[a]

tripeptide	$\Delta H^{\ddagger}$, kcal mol^{-1}	$\Delta S^{\ddagger}$, cal K^{-1} mol^{-1}
GAA	2.3 ± 0.1	−35 ± 1
AAA	2.6 ± 0.2	−36 ± 2
GAibG	4.5 ± 0.2	−35 ± 2
Aib_3	3.1 ± 0.2	−52 ± 5

[a] Based on $k_{trien} = kT/he^{-\Delta H^{\ddagger}/RT}e^{\Delta S^{\ddagger}/R}$.

number of methyl groups present in the ith amino acid residue from the amine terminus of the tripeptide (I). A linear least-squares analysis of the data in Table IV gives resolved values of $\beta_0 = 6.7 \pm 0.3$, $\beta_1 = -0.20 \pm 0.26$, $\beta_2 = -2.2 \pm 0.2$, and $\beta_3 = -1.6 \pm 0.2$ with a coefficient of multiple determination, R^2, of 0.961. The calculated values of k_{trien} are plotted against the observed values in Figure 1. The correlation shows that methyl groups in the first residue have little or no effect on the k_{trien} values. Each methyl group in the second residue decreases the rate constant by a factor of ≃160 and each methyl group in the third residue decreases the k_{trien} value by a factor of ≃40. The effects are additive rather than synergistic.

Activation Parameters. Studies of the temperature dependence of the trien rate constants at pH 11 were carried out for the GAA, A_3, GAibG, and Aib_3 complexes. Figure 2 gives the plots used to obtain the activation parameters (Table V). The activation enthalpies are small ($\Delta H^{\ddagger}_{obsd}$ = 2.3 to 4.5 kcal mol^{-1}), while the activation entropies are unusually negative ($\Delta S^{\ddagger}_{obsd}$ = −35 to −52 cal K^{-1} mol^{-1}). Since some coordination of trien and displacement of the tripeptide occurs prior to the rate-determining step, the $\Delta H^{\ddagger}_{obsd}$ and $\Delta S^{\ddagger}_{obsd}$ values are a combination of thermodynamic and kinetic values. The large negative entropies of activation indicate highly ordered transition states relative to the reactants. Factors that will affect the entropy changes from reactants to the transition state include the following: (1) the decrease in ΔS^0 due to the association of trien and $Cu(H_{-2}L)^-$; (2) loss or gain of water molecules (ΔS^0 = +7 cal K^{-1} mol^{-1} per released H_2O)[18-20] from the solvated reactants; (3) entropy decrease due to chelation (ΔS^0 = −8 cal K^{-1} mol^{-1} per chelate ring formed);[21] (4) the entropy decrease required to position trien around the sterically hindered tripeptide in order to achieve bond making and bond breaking. The last factor appears to be by far the largest.

Triethylenetetramine in water will exist as $trien(H_2O)_4$, with four water molecules strongly hydrogen bonded to the nitrogens.[18] The (tripeptido)cuprate(II) complex is present as $Cu(H_{-2}L)(H_2O)_2^-$ with two axially coordinated water molecules.[19] Tridentate coordination of trien to two copper axial positions and to the equatorial position initially occupied by the carboxylate group would require the release of five water molecules (two from

(15) Bannister, C. E.; Margerum, D. W.; Raycheba, J. M. T.; Wong, L. F. *Symp. Faraday Soc.* **1975**, *10*, 78–88.
(16) Margerum, D. W. *Pure Appl. Chem.* **1983**, *55*, 23–34.
(17) Bannister, C. E.; Raycheba, J. M. T.; Margerum, D. W. *Inorg. Chem.* **1982**, *21*, 1106–1112.
(18) Hinz, F. P.; Margerum, D. W. *Inorg. Chem.* **1974**, *13*, 2941–2949.
(19) Youngblood, M. P.; Margerum, D. W. *Inorg. Chem.* **1980**, *19*, 3068–3072.
(20) Koval, C. A.; Margerum, D. W. *Inorg. Chem.* **1981**, *20*, 2311–2318.
(21) Myers, R. T. *Inorg. Chem.* **1978**, *17*, 952–958.

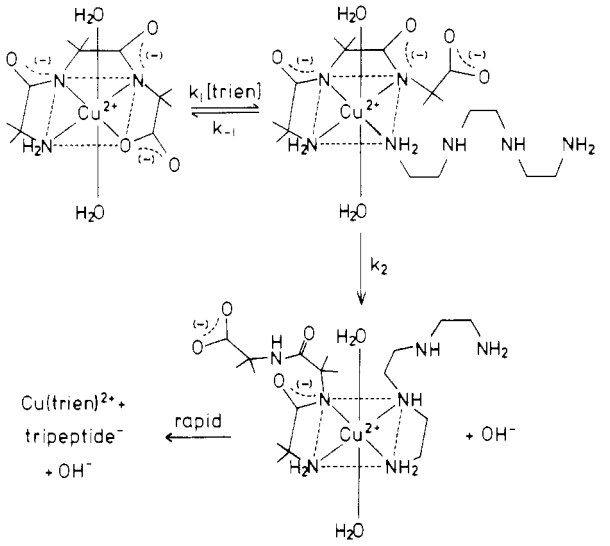


Figure 3. Proposed mechanism for the trien reaction with $Cu(H_{-2}L)^-$, where k_2 is the rate-determining step and $k_{trien} = k_1k_2/k_{-1}$.

Cu(II) and three from trien) with the ΔS^0 increase of 35 cal K^{-1} mol^{-1} offset by the net increase of one additional chelate ring (ΔS^0 = −8 cal K^{-1} mol^{-1}). Since $\Delta S^{\ddagger}_{obsd}$ values are very negative, it seems more probable that there is a much smaller degree of trien coordination to copper prior to the rate-determining step.

Proposed Mechanism. The large effects of methyl groups in the second and third residues indicate that the mechanism must involve both the second and the third chelate rings of the peptide complex. The carboxylate group is easier to displace than the more strongly bound amine or deprotonated-N(peptide) groups. When hydroxide ion occupies the carboxylate site the reaction with trien is suppressed.[4] The large steric effects of the third residue can be accounted for by a mechanism wherein trien coordinates initially to an equatorial position. Methyl groups in the third residue sterically hinder this coordination and make it difficult for trien to obtain the foothold it needs to displace the rest of the peptide from copper.

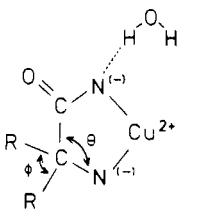
In the pH range where $Cu(H_{-2}L)(OH)^{2-}$ does not form, there is no OH^- suppression of the reaction rate with trien.[4] This indicates that the rate-determining step is before or during the displacement of an N(peptide) group. If the reaction in eq 7 took

$$Cu(H_{-2}L)trien + H_2O \rightleftarrows Cu(H_{-1}L)trien + OH^- \quad (7)$$

place prior to the rate-determining step, then the rate would be proportional to $[OH^-]^{-1}$, which is not the case. Nonetheless, methyl groups in the second residue have the greatest impact on the substitution rate. It is clear that the second chelate ring of the tripeptide needs to open during the trien substitution process and is involved in the rate-determining step (but not before it).

The proposed mechanism is shown in Figure 3, where trien first coordinates by replacing a coordinated carboxylate group. Methyl groups in the third residue sterically hinder equatorial coordination by a trien nitrogen and reduce the k_1/k_{-1} values for the formation of the mixed-ligand complex. (Each methyl group reduces k_1/k_{-1} by a factor of 40.) The rate-determining step (k_2) requires the second chelate ring of the tripeptide to open and the first chelate ring of trien to form. Each methyl group in the second peptide residue reduces the magnitude of k_2 by a factor of 160. The resulting k_{trien} rate constant is a composite value that equals k_1k_2/k_{-1}.

Structure II shows how methyl groups in the second peptide residue make the ring-opening step more difficult. As the Cu–N bond is stretched, the N–C–C bond angle (θ) must expand. This



II

becomes more difficult as the two R groups change from H to CH_3, because the R–C–R bond angle (ϕ) is harder to compress. Incoming chelation by trien in the k_2 step (not shown in II) causes a large degree of Cu–N bond stretch that is accompanied by proton transfer from water to the deprotonated-N(peptide) group. The proposed transition state involves all three processes (i.e. peptide ring opening, solvent protonation of the peptide nitrogen, and chelate formation of the second trien nitrogen). After the transition state, the first two nitrogens of trien are chelated in an equatorial position (Figure 3). There is a large degree of steric repulsion between the rest of the trien molecule and the tripeptide residues that are adjacent to it. The two multidentate ligands are literally trying to occupy the same space on one side of the $Cu(H_{-1}L)$trien complex. As a result, the remaining chelate ring of the first tripeptide residue opens relatively rapidly. Since this occurs after the rate-determining step, the methyl groups in the first residue have little or no effect on the reaction rate.

The magnitude of the negative $\Delta S^{\ddagger}$ values is surprising. In the proposed transition state the number of water molecules lost from the solvated trien is offset by those added to the tripeptide (water will solvate the free carboxylate group and a water molecule is associated with Cu–N(peptide) bond cleavage). The entropy lost in bringing the $Cu(H_{-2}L)^-$ and trien molecules together is cancelled by the entropy gain in opening the carboxylate chelate ring. The net result is that most of the negative $\Delta S^{\ddagger}$ results from the high degree of orientation required in the transition state. The two multidentate ligands must lose many degrees of freedom in order to attain the transition state, where a trien chelate forms as the second peptide residue breaks away from copper(II). Steric hindrance between the noncoordinated portions of the multidentate ligands greatly restricts the possible conformation of each segment and causes $\Delta S^{\ddagger}$ to be very negative.

Conclusions

The general mechanism proposed for the trien-dependent path (Figure 3), where k_2 is the rate-determining step, appears to be valid for all the copper(II)–tripeptide complexes despite their enormous range of reactivity. The k_d rate term represents a solvent path that corresponds to a partial unwrapping of the tripeptide from copper (i.e. the carboxylate group and adjacent N(peptide) group) followed by rapid reaction with trien. The ratio of k_{trien}/k_d decreases from 9.2×10^7 M^{-1} for $Cu(H_{-2}G_3)^-$ to 5.8×10^2 M^{-1} for $Cu(H_{-2}Aib_3)^-$ as methyl groups hinder the trien path.

Trien is a flexible linear polyamine that has minimal steric requirements in its coordination to copper. Larger steric effects and slower displacement reactions can be anticipated for other multidentate ligands.

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Supplementary Material Available: A table of rate constants for the temperature dependence of trien reactions with copper(II) complexes of GAA, AAA, GAibG, and Aib_3 (4 pages). Ordering information is given on any current masthead page.