that there is 16 ± 3% minor tautomer of guanine in aqueous solution,[16] Chan and coworkers reported that they have obtained ^1H nmr evidence indicating the existence of abnormal G–U base pairing.[17] By increasing the fraction of water in the guanosine–uridine solution dissolved in a DMSO–H_2O mixture, they observed line-width broadening of the GN_1H and UN_3H resonances. However, by increasing the fraction of water in either guanosine or uridine alone, little line-width broadening was observed.[17] This is in contradiction with the observation[32] where the GN_1 or UN_3 line width in guanosine or uridine alone increases in the same order of magnitude as those reported in their study of guanosine–uridine dissolved in a DMSO–H_2O mixture.

Recently Raszka and Kaplan reported ^1H nmr results indicating the interaction of GMP with UMP involved hydrogen bonding[33] and might be taken as evidence of G–U base pairing. It is also important to note that there is no evidence of G–U base pairing in poly G–U in a circular dichroism study.[34] If the lactim tautomer of guanosine were present to ~16% and abnormal G–U base pairing were energetically feasible, the existence of G–U pairing should have been detected in the CD study. In view of this result and the results presented in the previous section that the existence of a minor tautomer of guanine to the extent of ~16% is unlikely, the evidence of G–U base pairing must be taken with caution.

(32) Y. P. Wong, Ph.D. Thesis, University of California, Riverside, 1972.
(33) M. Reszka and N. O. Kaplan, *Proc. Nat. Acad. Sci. U. S.*, **69**, 2025 (1972).
(34) D. M. Gray, I. Tinoco, Jr., and M. J. Chamberlin, *Biopolymers*, **11**, 1235 (1972).

Conclusion

New experimental evidence is presented showing that the GH8 and CH5 line-width broadening phenomena do not appear in purified samples of 2′-GMP and 5′-CMP and that the unusual line-width broadening of these resonances observed in the ^1H nmr spectra can be caused by paramagnetic Cu^{2+} ions. The pD dependence of these line widths in the presence of paramagnetic Cu^{2+} ions can be understood by metal binding or protonation at the GN_7 and CN_3 sites at the appropriate pD. The temperature dependence can be explained by the different rate of chemical exchange between the free nucleotide and the Cu^{2+}–nucleotide complex. We conclude therefore that, while the minor tautomers of guanine and cytosine certainly exist, there is no strong evidence to indicate they are present to the extent of 15 ± 3%. Furthermore, the conclusion reached by the previous authors about the existence of G–U base pairing must be viewed with caution since conflicting ^1H nmr evidence was reported and no G–U base pairing was observed in a recent study of the circular dichroism of poly GU.

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Peptide Steric Effects on the Kinetics of Copper(II)–Tripeptide Reactions

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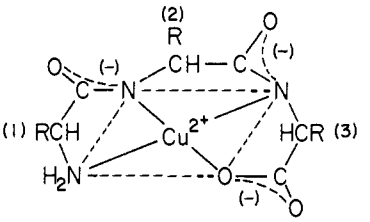
Abstract: Variation of the residues from glycyl to L-leucyl or L-alanyl in copper tripeptide complexes of the type $Cu(H_{-2}\text{ tripeptide})^-$ causes a significant decrease in the rates of nucleophilic attack on these complexes. A L-leucyl residue in the middle position (GLG) exhibits the greatest steric effect for the removal of copper from the tripeptide complex by the reaction of triethylenetetramine (trien). The rate constant for this process is reduced by a factor of 200 compared to the triglycine complex, $Cu(H_{-2}GGG)^-$. A similar effect is observed for the GGL tripeptide while the substitution of L-leucine for glycine at the amine terminal (LGG) is relatively ineffective in reducing the rate of the trien reaction. The reactions of ethylenediamine (en) and $EDTA^{4-}$ with the $Cu(H_{-2}\text{tripeptide})^-$ complexes exhibit similar steric effects and in some instances [$Cu(H_{-1}$tripeptide)en] mixed complexes are observed. The relative rates of the reactions of the different tripeptides with en and trien support a mechanism in which the initial attack is at the carboxylate end with the rate step being the cleavage of the Cu–N(peptide) bond adjacent to the carboxylate terminal. However, in contrast to the nucleophilic reactions, the rate constants for the general acid catalyzed transfer of copper(II) from $Cu(H_{-2}\text{tripeptide})^-$ to $CuEDTA^{2-}$ show relatively little dependence on the structure of the tripeptide, the variation being less than a factor of 3 in all cases. The absence of steric effects for the acid rate constants indicates that for these reactions the rate-determining step is the proton transfer to the deprotonated peptide group rather than cleavage of the Cu–N(peptide) bond.

Previous work has demonstrated the existence of two paths for the replacement of triglycine from copper–triglycine. In general, ligands containing primary or secondary amine groups react rapidly *via* a nucleophilic path[1] while the reactions with tertiary nitrogen containing ligands are slower and are indirect, tending

(1) G. K. Pagenkopf and D. W. Margerum, *J. Amer. Chem. Soc.*, **92**, 2683 (1970).

to be general acid catalyzed.[2] Thus, steric effects due to the attacking ligands are quite important in the reactions of $Cu(H_{-2}GGG)^-$.[3]

The proposed rate-determining step for the nucleophilic path is the rupture of the first Cu–N(peptide) bond.[1] Therefore, steric hindrance due to substituent groups on the tripeptides also may be important to this mechanism. The effect of this type of steric hindrance is tested for nucleophilic reactions in the present work. In structure I, where the R group may be H, CH_3, or



I $Cu(H_{-2}tripeptide)^-$

$CH_2CH(CH_3)_2$, the effects of locating the alkyl groups at positions (1), (2), or (3) are examined for the reactions with trien, en, and EDTA.

Recent work has indicated that a change in the number of Ni–N(peptide) or –N(amide) bonds in a complex can affect the mechanism of acid reaction with nickel(II)–peptide complexes. Thus, protonation reactions of the $Ni(H_{-2}GGG)^-$ complex are subject to general acid catalysis[4] while this is not the case with the $Ni(H_{-3}GGGG)^{2-}$ and $Ni(H_{-3}GGGa)^-$ complexes[5] (GGGa = triglycinamide). Although the reaction path for the protonation of $Cu(H_{-2}GGG)^-$ and $Ni(H_{-2}GGG)^-$ appears to be significantly different from that for the $Ni(H_{-3}GGGG)^{2-}$ and $Ni(H_{-3}GGGa)^-$ reactions, a general mechanism was proposed which accounts for the observed protonation kinetics of these four oligopeptide complexes.[5] The general mechanism is given in eq 1 and 2 (charges are

$$M(H_{-n})L + HX \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} M(H_{-n}L)H + X \quad (1)$$

$$M(H_{-n}L)H \xrightarrow{k_2} M(H_{-n+1}L) \quad (2)$$

omitted, L is the oligopeptide, H_{-n} corresponds to n metal–N(peptide or amide) bonds, and HX is H_3O^+ or some weaker acid). The species written as $M(H_{-n}L)H$ indicates an intermediate protonated complex without cleavage of any metal–N(peptide or amide) bonds which is termed "outside" protonation. The change from general acid catalysis to specific H_3O^+ catalysis is caused by a change in the relative values of k_2 and k_{-1}. If $k_2 \gg k_{-1}[X]$, then $k_{obsd} = k_1[HX]$. This is the condition which fits the general acid catalyzed protonation reactions of $Cu(H_{-2}GGG)^-$ and $Ni(H_{-2}GGG)^-$. The rate-determining step involves the proton transfer from HX to give the reactive $M(H_{-n}L)H$ species which rearranges to $M(H_{-n+1}L)$ in a step which is relatively fast compared to the proton transfer step.

If the relative values of k_2 and k_{-1} were interchanged such that $k_2 \ll k_{-1}[X]$, then $k_{obsd} = k_2K_1[H^+]$, which is equivalent to the specific hydrogen ion catalysis observed for the protonation reactions of $Ni(H_{-3}GGGG)^{2-}$ and $Ni(H_{-3}GGGa)^-$. The important point to note is that the rate step has shifted from the proton transfer step (eq 1) to the cleavage of the metal–N(peptide) bond (eq 2).

If this general mechanism[5] is a valid description of general acid catalysis of the protonation reactions of copper–tripeptide complexes (with eq 1 as the rate-determining step), little or no dependence of the k_{HX} values on the presence of side chain substituents is expected because Cu–N(peptide) bond cleavage occurs after the rate-determining step. However, if the rate step for these reactions involves Cu–N(peptide) bond cleavage, then similar steric effects due to the structure of the tripeptide should be seen for the protonation and the nucleophilic reactions.

Experimental Section

The tripeptides were obtained as chromatographically homogeneous preparations from Schwarz-Mann, Orangeburg, N. Y. (LGG, GLG, and LGL), and the Cyclo Chemical Division of Travenol Laboratories, Los Angeles, Calif. (AAA, AGG, GAG, and GGL). Stock solutions of copper(II) perchlorate were prepared from the twice recrystallized salt and standardized against either EDTA or triethylenetetramine (trien). Solutions of trien were prepared from the twice recrystallized sulfate salt (Baker Analyzed Reagent). Ethylenediamine (en) was used as received (Baker Analyzed Reagent).

The copper(II)–tripeptide solutions were freshly prepared before each series of kinetic runs. Ionic strength was maintained at 0.10 M with $NaClO_4$, except as indicated. Hydrogen ion concentrations were calculated from pH measurements by the relationship $-\log [H^+] = pH - 0.11$,[6] and hydroxide ion concentrations were calculated from $pK_w = 13.78$. Solutions of twice recrystallized sodium tetraborate were used as the buffering agent ($[boron]_T = 0.02\ M$).

The reactions were monitored by following either the disappearance of $Cu(H_{-2}tripeptide)^-$ at 230–240 nm for the polyamine reactions or the appearance of $CuEDTA^{2-}$ at 280 nm for the protonation reactions. Reaction runs were performed using a Durrum-Gibson stopped-flow spectrophotometer with a 2.0-cm cell path. Kinetics data were obtained from treatment of photographs of oscilloscope traces or from direct processing of the photomultiplier output signal *via* interfacing with a Hewlett-Packard 2115A general purpose digital computer.[7] All reactions in this study were run under pseudo-first-order or second-order, equal concentration conditions. Each rate constant is the average of at least four kinetic runs, and the standard deviations were calculated from the deviations of the individual rate constants from the mean.

The oligopeptides, their amine group protonation constants, and the log K_H values (where known) for the copper complexes of interest in this study (where K_H is a protonation constant of the peptide complex, *e.g.*, for $Cu(H_{-2}GAG)^-$, $K_H = [Cu(H_{-1}GAG)]/[Cu(H_{-2}GAG)^-][H^+]$) are given in Table I. Also listed in Table I are the protonation constants of the other ligands and the stability constants of their copper complexes.

Results

Proton Transfer and EDTA Reactions of $Cu(H_{-2}tripeptide)^-$ Complexes. The rate constants for proton transfer were determined by observing the rate as a function of the concentration of one acid while the concentrations of the other acids remained constant, which is the same method which was used to study the

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

(3) The abbreviations used for the tripeptides are derived from the first letter of each amino acid residue. Thus, glycyl-L-alanylglycine is GAG and glycylglycyl-L-leucine is GGL. The abbreviation $Cu(H_{-2}GGG)^-$ represents the copper(II) complex of triglycine which has ionized two peptide protons.

(4) E. J. Billo and D. W. Margerum, *J. Amer. Chem. Soc.*, **92**, 6811 (1970).

(5) E. B. Paniago and D. W. Margerum, *ibid.*, **94**, 6704 (1972).

(6) R. G. Bates, "Determination of pH," Wiley, New York, N. Y., 1964, p 92.

(7) B. G. Willis, J. A. Bittikofer, H. L. Pardue, and D. W. Margerum, *Anal. Chem.*, **42**, 1340 (1970).

Table I

Oligopeptide	Log $K_H(H_2NR)$	μ, M	Ref
A. Protonation Constants of Oligopeptides Used in This Study, 25.0°			
AGG	8.05	0.16 (KCl)	*a*
GAG	8.08	0.16 (KCl)	*a*
AAA	8.08	0.16 (KCl)	*a*
LGG	7.9	~0.02	*b*
GLG	8.10	0.10 ($NaClO_4$)	*c*
GGL	7.90	0.10 ($NaClO_4$)	*c*
B. Protonation Constants of Other Ligands, 25.0°			
$HEDTA^{3-}$	6.16	0.1 (KNO_3)	*d*
$EDTA^{4-}$	10.26	0.1 (KNO_3)	*d*
H_2trien^{2+}	6.56	0.10 ($NaClO_4$)	*e*
$Htrien^{+}$	9.09	0.10 ($NaClO_4$)	*e*
trien	9.81	0.10 ($NaClO_4$)	*e*
Hen^{+}	7.05	0.10 ($NaClO_4$)	*f*
en	9.83	0.10 ($NaClO_4$)	*f*
C. Log K_H Values of the Copper Complexes, 25.0°			
$Cu(H_{-2}AGG)^{-}$	−6.84	0.16 (KCl)	*a*
$Cu(H_{-2}GAG)^{-}$	−6.62	0.16 (KCl)	*a*
$Cu(H_{-2}AAA)^{-}$	−6.63	0.16 (KCl)	*a*
$Cu(H_{-2}GLG)^{-}$	−6.43	0.10 ($NaClO_4$)	*c*
$Cu(H_{-2}GGL)^{-}$	−7.21	0.10 ($NaClO_4$)	*c*
$Cutrien^{2+}$	20.1	0.1	*g, h*
$CuEDTA^{2-}$	18.8	0.1 (KNO_3)	*g, h*
$Cu(en)_2^{2+}$	19.36	0.10 ($NaClO_4$)	*f, h*

[a] G. F. Bryce and F. R. N. Gurd, *J. Biol. Chem.*, **241**, 1439 (1966). [b] S. P. Datta, R. Leberman, and B. R. Rabin, *Nature (London)*, **183**, 745 (1959). [c] This work. [d] G. Schwarzenbach and H. Ackerman, *Helv. Chim. Acta*, **30**, 1798 (1947), 20°. [e] These constants are from data at 20° corrected to 25°: G. Schwarzenbach, *Helv. Chim. Acta*, **33**, 974 (1950); H. B. Jonassen, R. B. LeBlane, A. W. Meiboom, and R. M. Rogan, *J. Amer. Chem. Soc.*, **72**, 2430 (1950). [f] H. Hauer, E. J. Billo, and D. W. Margerum, *J. Amer. Chem. Soc.*, **93**, 4173 (1971). [g] L. G. Sillen and A. E. Martell, Ed., "Stability Constants," The Chemical Society, London, 1964. [h] The values listed here are log K_1 constants rather than protonation constants.

reactions of $Cu(H_{-2}GGG)^{-}$.[2] Experiments were run at several different values of pH to ensure correct species assignment. The observed rate constants, which are first order in $Cu(H_{-2}tripeptide)^{-}$, are given in Table II for each of the tripeptides used.

Under the conditions used in this study the reactions also exhibited a dependence in the total EDTA concentration (eq 3). Plots of k_{obsd} against total EDTA

$$k_{obsd} = k_d + k_{H_3O}[H_3O^+] + k_{HX}[H_3BO_3] + k_a[EDTA]_T \quad (3)$$

concentration yield values of k_a which may contain contributions from H_2EDTA^{2-}, $HEDTA^{3-}$, and $EDTA^{4-}$. In order to determine the contributions from each of these species, a linear regression analysis was performed on eq 4. The values obtained for k_{HY}

$$k_a = k_{H_2Y}\frac{[H_2EDTA^{2-}]}{[EDTA]_T} + k_{HY}\frac{[HEDTA^{3-}]}{[EDTA]_T} + k_Y\frac{[EDTA^{4-}]}{[EDTA]_T} \quad (4)$$

were close to zero for each of the tripeptides, varying from −0.5 to 2.0 M^{-1} sec^{-1}, indicating that $HEDTA^{3-}$ is very much less reactive than either H_2EDTA^{2-} or $EDTA^{4-}$.

In earlier work a rate constant was reported for the direct reaction of $HEDTA^{3-}$ with $Cu(H_{-2}GGG)^{-}$ (k = 15 M^{-1} sec^{-1}).[8] The apparent lack of reactivity of $HEDTA^{3-}$ found with the substituted tripeptide complexes led us to reexamine the earlier $Cu(H_{-2}GGG)^{-}$ data. It was found that the contribution from $EDTA^{4-}$, which we had measured at high pH, had been inadvertently neglected in calculating the contribution of k_{HY} at lower pH and that the observed rate constants could be completely accounted for by the contributions of H_2EDTA^{2-} and $EDTA^{4-}$. Thus, the k_{HY} value for the reaction of EDTA with $Cu(H_{-2}GGG)^{-}$ is also very close to zero.

That $HEDTA^{3-}$ is both a poor nucleophile and poor general acid may be explained on the basis of the protonation constants for the various EDTA species. The predicted[2] rate constant for general acid catalysis by $HEDTA^{3-}$ (with a pK_a of 10.26) is 0.1 M^{-1} sec^{-1}. Therefore, $HEDTA^{3-}$ is too weak an acid for it to contribute significantly to the reaction rate. On the other hand, $HEDTA^{3-}$ is also a weaker base than $EDTA^{4-}$ by a factor of about 10^4 (see Table I). Thus, $HEDTA^{3-}$ is expected to be a much poorer nucleophile than $EDTA^{4-}$.

The following method was used to refine the values of k_{H_2Y} and k_Y assuming from the treatment of eq 4 that k_{HY} = 0. Values of k_b and k_c (eq 5, 6, 7) were obtained from the slopes of plots of k_{obsd} *vs.* $EDTA^{4-}$ and H_2EDTA^{2-}, respectively, at each pH listed in Table II

$$k_{obsd} \propto k_b[EDTA^{4-}] = k_{H_2Y}[H_2EDTA^{2-}] + k_Y[EDTA^{4-}] \quad (5)$$

so that

$$k_b = k_{H_2Y}\frac{[H_2EDTA^{2-}]}{[EDTA^{4-}]} + k_Y \quad (6)$$

and in a similar manner because $k_{obsd} \propto k_c[H_2EDTA^{2-}]$

$$k_c = k_Y\frac{[EDTA^{4-}]}{[H_2EDTA^{2-}]} + k_{H_2Y} \quad (7)$$

The values of k_{H_2Y} and k_Y given in Table III were obtained from the slopes of plots of eq 6 and 7 for each tripeptide. (The intercepts of the plots of eq 6 and 7 agree very well with the slopes of eq 7 and 6, respectively.)

The values of k_{H_3O} and k_d were determined by subtracting the contributions from H_2EDTA^{2-}, $EDTA^{4-}$, and H_3BO_3 (k_{HX} used for H_3BO_3 is 2 M^{-1} sec^{-1} determined with $Cu(H_{-2}GGG)^{-}$)[2] from the k_{obsd} values (Table II) and plotting the resultant k'_{obsd} values against H_3O^+. The slope and intercept yield values of k_{H_3O} and k_d which also are given in Table III.

In earlier work[8] a rate constant of 600 M^{-1} sec^{-1} was determined for the reaction of $EDTA^{4-}$ with $Cu(H_{-2}GGG)^{-}$ at pH 11.6. Using data in the pH range 8–9, and a value of log K_H = 10.26 for $EDTA^{4-}$, k_Y is found to be 200 M^{-1} sec^{-1} (Table III). The discrepancy in the k_Y values might be due to a hydroxide effect at high pH or, more likely, to the value assigned to the protonation constant of $EDTA^{4-}$. If log K_H for $EDTA^{4-}$ were ~10.5 the discrepancy would be removed. Carini and Martell reported a value of log K_H = 11.01 (20°, $\mu \rightarrow 0$).[9] A value of log K_H = 10.46

(8) G. R. Dukes, G. K. Pagenkopf, and D. W. Margerum, *Inorg. Chem.*, 10, 2419 (1971).

(9) F. F. Carini and A. E. Martell, *J. Amer. Chem. Soc.*, 76, 2153 (1954).

Table II. Dependence of k_{obsd} on pH and EDTA Concentration for the Reaction of EDTA with Tripeptide Complexes of Copper(II)[a]

pH	10^3[EDTA]$_T$, M	k_{obsd}, sec^{-1}	pH	10^3[EDTA]$_T$, M	k_{obsd}, sec^{-1}
		L-Alanylglycylglycine			
8.04	2.06	0.277 (±0.009)	9.03	2.06	0.100 (±0.003)
8.06	6.18	0.419 (±0.003)	9.06	6.19	0.138 (±0.003)
8.05	10.3	0.572 (±0.009)	9.04	10.3	0.169 (±0.004)
8.07	15.4	0.726 (±0.007)	9.07	14.4	0.211 (±0.003)
8.28	2.06	0.194 (±0.005)			
8.29	6.18	0.284 (±0.002)			
8.30	10.3	0.367 (±0.004)			
8.31	15.4	0.465 (±0.004)			
		Glycyl-L-alanylglycine			
8.05	2.06	0.219 (±0.001)	8.82	5.17	0.077 (±0.001)
8.06	6.18	0.324 (±0.005)	8.81	7.76	0.091 (±0.001)
8.05	10.3	0.442 (±0.004)	8.83	10.3	0.102 (±0.002)
8.07	15.4	0.552 (±0.005)	8.81	12.9	0.122 (±0.002)
8.28	2.06	0.141 (±0.001)			
8.29	6.18	0.208 (±0.004)			
8.30	10.3	0.269 (±0.002)			
8.31	15.4	0.343 (±0.003)			
		L-Alanyl-L-alanyl-L-alanine			
8.07	2.06	0.130 (±0.004)	8.68	2.06	0.044 (±0.002)
8.08	6.19	0.188 (±0.003)	8.68	6.19	0.059 (±0.001)
8.07	10.3	0.252 (±0.007)	8.68	10.3	0.075 (±0.001)
8.10	14.4	0.293 (±0.007)	8.69	14.4	0.0916 (±0.0003)
8.08	18.6	0.358 (±0.003)	8.70	18.6	0.109 (±0.003)
8.24	2.06	0.085 (±0.002)	9.26	0.201	0.0135 (±0.001)
8.26	6.18	0.121 (±0.003)	9.29	6.18	0.0192 (±0.0008)
8.29	10.3	0.154 (±0.001)	9.29	12.4	0.0295 (±0.0007)
8.27	15.4	0.182 (±0.003)	9.30	16.5	0.0336 (±0.0009)
8.46	5.17	0.0743 (±0.0007)	9.31	20.6	0.0376 (±0.0008)
8.47	7.76	0.0861 (±0.0003)			
8.50	10.3	0.099 (±0.001)			
8.48	12.9	0.118 (±0.004)			
		Glycylglycyl-L-leucine			
8.21	2.06	0.121 (±0.002)	8.86	5.17	0.072 (±0.002)
8.26	6.18	0.197 (±0.003)	8.85	7.76	0.0880 (±0.0009)
8.27	10.3	0.268 (±0.002)	8.86	10.3	0.108 (±0.003)
8.27	15.4	0.353 (±0.009)	8.85	12.9	0.126 (±0.002)
8.49	5.17	0.122 (±0.004)	9.03	2.06	0.0420 (±0.0008)
8.50	7.76	0.155 (±0.003)	9.06	6.19	0.0645 (±0.0003)
8.52	10.3	0.181 (±0.004)	9.03	10.3	0.0884 (±0.0010)
8.49	12.9	0.220 (±0.003)	9.07	14.4	0.109 (±0.002)
		L-Leucylglycylglycine			
8.09	4.12	0.307 (±0.011)	8.82	7.76	0.146 (±0.007)
8.09	8.24	0.440 (±0.006)	8.84	10.3	0.162 (±0.003)
8.09	12.4	0.533 (±0.008)	8.82	12.9	0.185 (±0.004)
8.13	16.5	0.640 (±0.009)			
8.47	5.17	0.187 (±0.002)			
8.48	7.76	0.218 (±0.002)			
8.50	10.3	0.243 (±0.005)			
8.58	15.4	0.285 (±0.002)			
		L-Leucylglycyl-L-leucine			
8.04	2.06	0.176 (±0.004)	8.85	5.17	0.063 (±0.003)
8.04	6.18	0.322 (±0.003)	8.85	7.76	0.082 (±0.002)
8.06	10.3	0.438 (±0.008)	8.86	10.3	0.0953 (±0.0003)
8.07	15.4	0.59 (±0.02)	8.84	12.9	0.113 (±0.001)
8.20	2.06	0.116 (±0.001)	9.02	2.06	0.040 (±0.001)
8.25	6.18	0.196 (±0.004)	9.05	6.19	0.060 (±0.001)
8.23	10.3	0.268 (±0.002)	9.03	10.3	0.083 (±0.001)
8.28	15.4	0.355 (±0.006)	9.06	14.4	0.103 (±0.002)
8.48	5.17	0.115 (±0.001)			
8.50	7.76	0.145 (±0.001)			
8.51	10.3	0.171 (±0.002)			
8.49	12.9	0.211 (±0.005)			

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

(25°, μ = 0.32 (CsCl)) was reported in 1965,[10] and Anderegg reported a value of 10.44 (20°, μ = 0.1 ($(CH_3)_4NCl$)) in 1967 for this constant.[11] In this study we were more interested in the relative values of the rate constants for the various tripeptide complexes

(10) J. Botts, A. Chashin, and H. L. Young, *Biochemistry*, 4, 1788 (1965).

(11) G. Anderegg, *Helv. Chim. Acta*, 50, 2333 (1967).

Table III. Resolved Rate Constants for the Reactions of Cu(H$_{-2}$tripeptide)$^-$ Complexes with EDTA

Tripeptide	$10^{-3}k_{H_2Y}$[a]	k_Y[a]	$10^{-6}k_{H_3O}$[a]	10^2k_d[b]
AGG	2.37 ± 0.04	165 ± 1	11.4 ± 0.6	5.0 ± 0.5
GAG	1.74 ± 0.03	41 ± 1	11.8 ± 0.6	0.6 ± 0.4
AAA	1.1 ± 0.1	4 ± 2	5.1 ± 0.5	−0.3 ± 0.3
LGG	2.06 ± 0.01	90 ± 10	12 ± 1	5.0 ± 1
GGL	1.98 ± 0.09	80 ± 10	8.3 ± 0.8	0.4 ± 0.6
LGL	2.14 ± 0.08	60 ± 10	7.8 ± 0.7	−0.4 ± 0.5
GGG	3.1[c]	200[d]	4.9[c]	12[c]

μ = 0.10 ($NaClO_4$), 25.0 ± 0.1°

[a] M^{-1} sec^{-1}. [b] Sec^{-1}. [c] Reference 2. [d] The k_{H_2Y} and k_Y values determined in this work utilized a value of log K_H = 10.26 for $EDTA^{4-}$.

than the absolute values. Since the protonation constants for EDTA have not been determined under the conditions used in this study, the rate constants listed in Table III should be treated as relative rather than absolute values. (The limited pH range used (for all cases except GGG) tends to keep the standard deviations low for the k_{H_2Y} and k_Y constants in Table III. There also is an interrelationship between k_{H_3O} and k_{H_2Y} and between k_d and k_Y such that as k_{H_2Y} and k_Y become larger k_{H_3O} and k_d decrease. Thus, if the k_{H_2Y} values for the other tripeptides were to more closely approach the value of 3.1×10^3 M^{-1} sec^{-1} determined earlier for Cu(H$_{-2}$GGG)$^-$ then their k_{H_3O} values would also be closer to the k_{H_3O} value found for Cu(H$_{-2}$GGG)$^-$. Nevertheless the relative values for the tripeptide rate constants are valid and the slight uncertainty in regard to their absolute values does not affect the conclusions to be drawn.)

The Reactions of Copper(II)–Tripeptide Complexes with Trien. The reactions of the Cu(H$_{-2}$tripeptide)$^-$ complexes with triethylenetetramine (eq 8 where $trien_T$ = H_2trien^{2+} + $Htrien^+$ + trien) gave no evidence of intermediate species. In all cases, the reactions are first order in both reactants as shown by the experimentally observed second-order rate constants given in Table IV. The rate constants increase with pH, indicating that trien is reacting in a nucleophilic manner. (If the protonated trien species were acting as acids the pH dependence would be opposite to that observed.)

$$Cu(H_{-2}tripeptide)^- + trien_T \longrightarrow Cu(trien)^{2+} + tripeptide^- \quad (8)$$

The largest effect on the trien rate constants occurs when leucine is either the carboxyl terminal or middle residue in the tripeptide (GLG, GGL, and LGL). When leucine is the amine terminal residue the rate constant diminishes only by a factor of 3 or 4 compared to triglycine. A similar factor is observed in the relative rates of reaction of the LGL and GGL complexes with trien.

The wider pH range over which the reactions of Cu(H$_{-2}$GLG)$^-$, Cu(H$_{-2}$LGL)$^-$, and Cu(H$_{-2}$GAG)$^-$ with trien were studied allowed the resolution of the rate constants for each of the trien species. Contributions of the various trien species were assessed by either a weighted regression analysis of the data[12] or alternatively the solution of the simultaneous equations arising from a set of observed rate constants at different distributions of trien species at various pH values. The resolved rate constants are given in Table V.

(12) D. W. Margerum and J. D. Carr, *J. Amer. Chem. Soc.*, 88, 1639 (1966).

Table IV. Observed Rate Constants for the Reaction of Trien with Cu(H$_{-2}$tripeptide)$^-$ Complexes

−Log [H$^+$]	10^5-[Cu(tripep)]$_T$, M	10^4[trien]$_T$, M	$10^{-4}k_{obsd}$, M^{-1} sec^{-1}
		Cu(H$_{-2}$LGL)$^-$	
6.99	5.81	10.2	0.076 ± 0.001
7.48	5.81	10.2	0.054 ± 0.001
7.92	5.81	10.2	0.091 ± 0.001
8.18	4.32	10.2	0.14 ± 0.01
8.19	5.04	5.04	0.16 ± 0.01
8.21	5.04	1.02	0.19 ± 0.01
8.21	4.32	5.1	0.19 ± 0.01
8.23	2.52	1.02	0.20 ± 0.01
8.32	2.52	0.51	0.28 ± 0.02
8.40	3.88	10.2	0.23 ± 0.01
8.41	3.88	5.1	0.24 ± 0.01
8.42	5.81	10.2	0.25 ± 0.01
8.82	5.81	10.2	0.50 ± 0.02
8.97	4.32	5.1	0.72 ± 0.02
8.97	4.32	10.2	0.72 ± 0.01
		Cu(H$_{-2}$GLG)$^-$	
8.06	4.32	10.2	0.32 ± 0.01
8.17	4.32	10.2	0.42 ± 0.02
8.18	4.32	5.10	0.43 ± 0.02
8.20	3.88	15.2	0.40 ± 0.02
8.21	3.88	5.10	0.43 ± 0.01
8.21	3.88	10.2	0.42 ± 0.01
8.22	7.67	10.2	0.38 ± 0.02
8.22	7.67	15.2	0.40 ± 0.02
8.63	4.32	10.2	0.93 ± 0.04
8.97	4.32	5.10	2.00 ± 0.02
8.97	4.32	10.2	1.80 ± 0.03
9.11	4.32	5.10	2.10 ± 0.04
9.63	4.32	5.10	3.69 ± 0.06
		Cu(H$_{-2}$GAG)$^-$	
8.14	4.32	10.2	1.10 ± 0.03
8.20	2.16	0.216	1.4
8.20	4.32	5.10	1.4
8.66	4.32	10.2	3.14 ± 0.05
8.97	2.16	0.216	6.2
8.97	4.32	5.10	6.0
9.16	4.32	5.10	6.39 ± 0.08
9.64	4.32	5.10	9.7 ± 0.2
		Cu(H$_{-2}$GGL)$^-$	
8.20	2.16	0.217	0.54
8.20	2.16	5.10	0.52
8.97	2.16	0.217	3.4 ± 0.4
8.97	2.16	5.10	2.7
		Cu(H$_{-2}$LGG)$^-$	
8.20	2.16	0.217	99 ± 3
8.97	2.16	0.217	27 ± 1
		Cu(H$_{-2}$GGG)$^-$	
8.20	2.16	0.217	440 ± 80
8.97	2.16	0.217	88 ± 1

Table V. Resolved Rate Constants for the Reactions of Trien Species with Cu(H$_{-2}$tripeptide)$^-$ Complexes (μ = 0.1 M $NaClO_4$, 25°)

Tripeptide	10^4k_T, M^{-1} sec^{-1}	10^4k_{HT}, M^{-1} sec^{-1}	$10^4k_{H_2T}$, M^{-1} sec^{-1}
GGG[2]	1100 ± 400	510 ± 50	12 ± 3
GAG	13	10	0.3
GLG	6 ± 1	3.4 ± 0.3	0.01 ± 0.04
LGL	3.6 ± 1.4	1.1 ± 0.1	0.03 ± 0.01

The Reactions of Copper(II)–Tripeptide Complexes with Ethylenediamine. A detailed reaction mechanism for the en reaction with Cu(H$_{-2}$GGG)$^-$ has been pre-

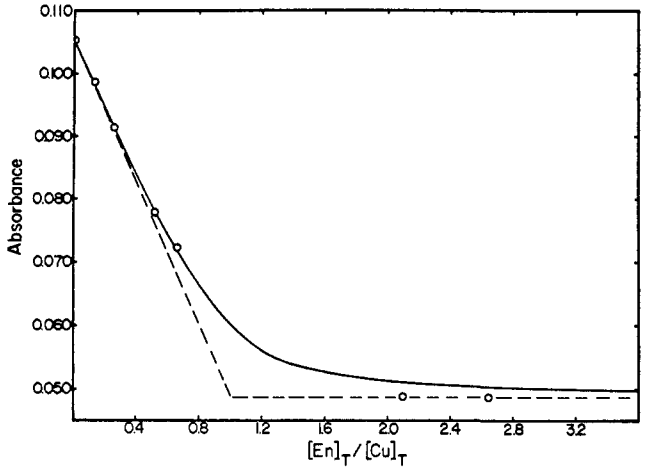


Figure 1. Kinetic mole ratio plot for the formation of $Cu(H_{-1}LGG)en$: $[Cu(H_{-2}LGG)^-]_{initial} = 3.24 \times 10^{-4}$ M, $-\log [H^+] = 8.26$, $\mu = 0.10$ M $NaClO_4$, 25°, $\lambda = 550$ nm.

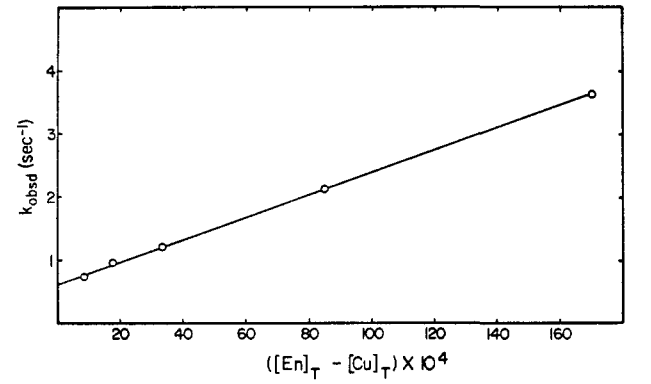


Figure 2. Observed first-order rate constant for the conversion of $Cu(H_{-1}LGG)en$ to $Cu(en)_2^{2+}$ as a function of excess ethylenediamine concentration: $-\log [H^+] = 9.19$, $\mu = 0.10$ M $NaClO_4$, 25°, $\lambda = 230$ nm.

sented in earlier work.[13] The replacement proceeds in two steps as given by eq 9 and 10

$$Cu(H_{-2}GGG)^- + en_T \rightleftharpoons Cu(H_{-1}GGG)en \quad (9)$$

$$Cu(H_{-1}GGG)en + en_T \longrightarrow Cu(en)_2^{2+} + GGG^- \quad (10)$$

where $en_T = en + Hen^+$. The stability of the intermediate, $Cu(H_{-1}GGG)en$, was determined by a kinetic mole ratio plot and by potentiometric titration. The rate expressions for the two steps are given in eq 11 and 12. The same mechanism is observed with Cu-

$$\frac{d[Cu(H_{-1}GGG)en]}{dt} = k_{en}^{Cu(H_{-2}GGG)^-}[Cu(H_{-2}GGG)^-][en] \quad (11)$$

$$\frac{d[Cu(en)_2^{2+}]}{dt} = (k_d^{Cu(H_{-1}GGG)en} + k_{en}^{Cu(H_{-1}GGG)en}[en])[Cu(H_{-1}GGG)en] \quad (12)$$

$(H_{-2}LGG)^-$ but not for $Cu(H_{-2}GLG)^-$ or $Cu(H_{-2}GAG)^-$. When leucine is the middle residue in the tripeptide, the $Cu(H_{-1}GLG)en$ complex is not found and when an alanine residue is in this position $Cu(H_{-1}GAG)en$ is difficult to observe.

(13) H. Hauer, E. J. Billo, and D. W. Margerum, *J. Amer. Chem. Soc.*, 93, 4173 (1971).

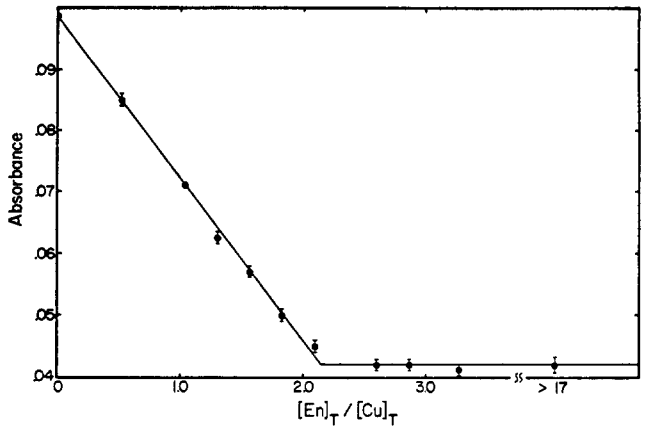


Figure 3. Kinetic mole ratio plot indicating the lack of an intermediate in the reaction of $Cu(H_{-2}GLG)^{2-}$ with ethylenediamine: $[Cu(H_{-2}GLG)^{2-}]_{initial} = 3.24 \times 10^{-4}$ M, $-\log [H^+] = 9.26$, $\mu = 0.10$ M $NaClO_4$, 25°, $\lambda = 550$ nm.

L-Leucylglycylglycine. Two reactions were observed at 230 nm when an excess of en was mixed with $Cu(H_{-2}GLG)^-$ and both reaction rates depended on the en concentration.

The first reaction was studied at 550 nm and $-\log [H^+] = 8.36$. A kinetic mole ratio plot (described in ref 13) shows in Figure 1 that the first reaction product has a 1:1 ratio of en to copper. The 1:1 intermediate is assumed to be similar to that formed with $Cu(H_{-2}GGG)^-$, namely $Cu(H_{-1}LGG)en$, and has an apparent stability constant ($K = [Cu(H_{-1}LGG)en]/[Cu(H_{-2}LGG)^-][Hen^+]$) of 6.3 ($\pm 0.9$) $\times 10^4$ M^{-1} ($\log K = 4.8$) which is in agreement with the value reported for the $Cu(H_{-1}GGG)en$ mixed complex. Second-order rate constants were determined for each of the mole ratios given in Figure 1 which corresponded to a rate constant of 3.8 (± 0.1) $\times 10^6$ M^{-1} sec^{-1} for the reaction of unprotonated en with $Cu(H_{-2}LGG)^-$. Earlier work with $Cu(H_{-2}GGG)^-$ established the unprotonated form of en as the reactant and the rate constant given above for the $Cu(H_{-2}LGG)^-$ complex is in good agreement with the value of 4.8×10^6 M^{-1} sec^{-1} found for the $Cu(H_{-2}GGG)^-$ reaction.[13]

The kinetics of the addition of a second en to $Cu(H_{-1}LGG)en$ were observed at 235 nm using excess en at pH 8.2–9.3. The reactions were pseudo first order in en concentration and the observed rate constants are plotted in Figure 2 against the excess of en over that needed to form $Cu(H_{-1}LGG)en$. The reaction stoichiometry and the rate expression are identical with those found for $Cu(H_{-1}GGG)en$ (eq 10 and 12, respectively) but the rate constants are significantly smaller ($k_{en}^{Cu(H_{-1}GGG)en} = 4.47 \times 10^3$ M^{-1} sec^{-1} and $k_{en}^{Cu(H_{-1}LGG)en} = 9.5$ (± 0.2) $\times 10^2$ M^{-1} sec^{-1}, $k_d^{Cu(H_{-1}GGG)en} = 1.29$ sec^{-1}, and $k_d^{Cu(H_{-1}LGG)en} = 0.62 \pm 0.01$ sec^{-1}).

Glycyl-L-leucylglycine. In contrast to the reactions of $Cu(H_{-2}LGG)^-$ and $Cu(H_{-2}GGG)^-$, the $Cu(H_{-2}GLG)^-$ complex exhibits only one step in the reaction with en. The reactions were first order in $Cu(H_{-2}GLG)^-$ and total en concentrations when en was in stoichiometric or greater concentrations. A kinetic mole ratio plot (Figure 3) gives no evidence for a $Cu(H_{-1}GLG)en$ species. Potentiometric titration of an equilibrium mixture of equimolar Cu^{2+}, GLG, and en

also gave no evidence of a Cu(H_{-1}GLG)en species. The experimentally determined rate expression is given in eq 13. From a weighted regression analysis of the

$$\frac{-d[Cu(H_{-2}GLG)^-]}{dt} = \frac{d[Cu(en)_2^{2+}]}{dt} = (k_{en}^{Cu(H_{-2}GLG)^-}[en] + k_{Hen}^{Cu(H_{-2}GLG)^-}[Hen^+])[Cu(H_{-2}GLG)^-] \quad (13)$$

data given in Table VI, the value of $k_{en}^{Cu(H_{-2}GLG)^-}$ is

Table VI. Observed Rate Constants for the Reaction of $Cu(H_{-2}GLG)^-$ with en (μ = 0.10 ($NaClO_4$), 25.0°)

Second-Order Conditions[a]		
−Log [H^+]	$10^4[en]_T$, M	$10^{-4}k_{obsd}$, M^{-1} sec^{-1}
9.26	1.70	1.9 ± 0.1
9.25	3.40	1.6 ± 0.1
9.26	4.25	1.4 ± 0.1
9.26	5.10	1.7 ± 0.1
9.28	5.95	1.6 ± 0.1
9.26	8.50	1.8 ± 0.1
9.27	9.35	1.9 ± 0.1
9.21	10.6	1.8 ± 0.1
9.13	85.0	1.7 ± 0.1

Pseudo-First-Order Conditions			
−Log [H^+]	10^5Cu-(H_{-2}GLG)$^-$, M	$10^4[en]_T$, M	k_{obsd}, sec^{-1}
8.19	2.16	4.47	1.2 ± 0.1
8.23	2.16	8.94	2.1 ± 0.1
8.25	2.16	4.47	1.2 ± 0.1
8.27	2.16	8.94	2.5 ± 0.1
8.39	4.32	10.3	3.3 ± 0.1
8.94	4.32	10.3	9.5 ± 0.5
8.99	4.32	10.3	10 ± 1
9.35	4.32	20.6	19.4 ± 0.7

[a] $[Cu]_T = 3.24 \times 10^{-4}$ M.

Complexes. A summary of the rate constants obtained in this work for the reaction of polyamines with copper–tripeptide complexes is given in Table VII. The variation in these rate constants reveals quite substantial steric effects due to the tripeptide structure. These differences in substitution rates cannot be attributed to limited access to the axial coordination sites of copper. This is precluded by the planarity of the peptide backbone which forces the substituent groups away from the axial positions, leaving them open for attack.

The ratios of the rate constants for the reaction of trien with $Cu(H_{-2}GGG)^-$ to the reactions of trien with the other tripeptide complexes are about the same at pH 8.2 and 8.97 (Table VII) for each tripeptide complex and they show that substitution for the middle residue of the tripeptide has the most profound effect. The rate constant for the reaction with $Cu(H_{-2}GLG)^-$ is a factor of >200 smaller than the rate constant for the trien reaction with $Cu(H_{-2}GGG)^-$. Substitution of leucine for glycine in the carboxylate terminal residue is only slightly less effective in slowing the rate of the nucleophilic trien attack, the rate constant for the reaction with $Cu(H_{-2}GGL)^-$ being about 150 times smaller than that for the $Cu(H_{-2}GGG)^-$ reaction. By contrast, substitution for the amine terminal residue of the tripeptide is relatively ineffective, the rate constant for the $Cu(H_{-2}LGG)^-$ reaction being only a factor of about 4 less than that for $Cu(H_{-2}GGG)^-$.

It has been shown earlier that steric effects due to the attacking ligand structure may be attributed to the difficulty in achieving nitrogen coordination to the square planar position vacated by the carboxylate group.[1] The same effect is observed in this work when substitution occurs at the carboxylate terminal of the tripeptides. In addition, substitution for the middle residue which causes no steric hindrance to coordination at the carboxylate positions also has a profound steric effect on these reactions. It seems reasonable to ascribe this effect to steric difficulty in opening the chelate ring terminated by the copper–N(peptide) bond.

Table VII. Summary of the Kinetics of Polyamine Reactions with Copper–Tripeptide Complexes (μ = 0.10 ($NaClO_4$), 25.0°)

Tripeptide	Resolved trien constants[a] $10^{-4}k_T$	$10^{-4}k_{HT}$	k_{H_2T}	$10^{-4}k_{trien}^{CuH_{-2}L^-}$, M^{-1} sec^{-1} −Log [H^+] = 8.2	−Log [H^+] = 8.97	$10^{-4}k_{en}^{CuH_{-2}L^-}$, M^{-1} sec^{-1}	10^{-4}. $k_{en}^{Cu(H_{-1}L)en}$, M^{-1} sec^{-1}	$k_d^{Cu(H_{-1}L)en}$, sec^{-1}
GGG	1100[b]	512[b]	12[b]	88	440	480[c]	0.447[c]	1.29[c]
LGG				27	99	380	0.095	0.62
GLG	6	3.4	0.01	0.43	1.9	7.6		
GAG	13	10	0.3	1.4	6.1	12		
GGL				0.53	3.0			
LGL	3.6	1.1	0.03	0.19	0.72			

[a] M^{-1} sec^{-1}. [b] Reference 1. [c] Reference 13.

7.6 (±0.3) × 10^4 M^{-1} sec^{-1} and $k_{Hen}^{Cu(H_{-2}GLG)^-}$ = 9 (±2) × 10^2 M^{-1} sec^{-1}.

Glycyl-L-alanylglycine. The reaction of $Cu(H_{-2}GAG)^-$ with en does not exhibit simple kinetics at stoichiometric conditions. Although a kinetic mole ratio plot gives no evidence for an intermediate, the kinetic traces reveal the existence of two reactions. This implies the formation of an intermediate of low stability with comparable rates for the formation and for the subsequent reaction with a second en molecule. Under pseudo-first-order conditions, the initial part of the trace exhibits first-order behavior and the rate constant for en addition to $Cu(H_{-2}GAG)^-$ is 1.2 (±0.2) × 10^5 M^{-1} sec^{-1}.

Discussion

Reactions of Polyamines with Copper(II)–Tripeptide Complexes. The observation of the intermediates $Cu(H_{-1}GGG)$en and $Cu(H_{-1}LGG)$en with nearly identical stability constants and rates of formation indicates that the polyamine attack is directed initially to the carboxylate end of the tripeptide with the rate step being the cleavage of the Cu–N(peptide) bond adjacent to the carboxylate groups. Replacement of the copper–carboxylate bond with that of an amine gives a species with four coplanar nitrogens and should be a favored reaction.

The rate expression for the reaction of excess en with

$Cu(H_{-2}GLG)^-$ (eq 13) indicates that the first en adds much more slowly than the second which is in contrast to the stepwise reactions of en with $Cu(H_{-2}LGG)^-$ and with $Cu(H_{-2}GGG)^-$. This may be explained by the significant effect of substitution for the middle residue on the rates, as is the case with the trien reactions discussed above. An additional factor causing the absence of an observable addition of a second en to form $Cu(en)_2^{2+}$ may be due to the fact that model studies show that the α carbon of the isobutyl group on the L-leucine residue causes steric crowding in the $Cu(H_{-1}GLG)$en mixed complex. The strain caused by this crowding may accelerate the further dissociation of the copper–tripeptide complex.

Inspection of the k_Y values for the rate of the $EDTA^{4-}$ reaction with the $Cu(H_{-2}tripeptide)^-$ complexes given in Table III reveals a significant dependence of k_Y on the presence of alanyl residues. The difference of about 50 between the rates of the $Cu(H_{-2}GGG)^-$ and $Cu(H_{-2}AAA)^-$ reactions also tends to confirm the same type of steric effects found for other nucleophilic reactions.

The origin of the steric effects exhibited by the copper–tripeptide complexes may be related to the range of allowable conformations for the free tripeptides or unbound segments thereof. Calculations of permissible conformations of free dipeptides indicate a marked decrease in allowable structures for those molecules with side chain substituents. Dipeptides are limited to 52% of the total area of the steric map, a map generated from permissible angles of rotation about the C–C and N–C bonds of the peptide backbone. The addition of the methyl group of L-alanine, *e.g.*, glycyl-L-alanine, reduces the allowed area to 16% while the allowable area is further reduced to 11% by the presence of an L-leucyl group. For dipeptides in which the branching occurs closer to the peptide backbone the reduction in the allowed area is even more dramatic, decreasing to only 4.6% for glycyl-L-valine.[14] Thus, the side chain substituents on the tripeptides may hinder the rotations about the C–C and N–C bonds of the peptide backbone which are required to open the chelate ring during the rate step in these nucleophilic reactions.

Proton Transfer Reactions of Copper(II)–Tripeptide Complexes. From the discussion of the steric effects of substituted tripeptides on nucleophilic reactions given above, the k_2 rate constant of the general mechanism for proton transfer reactions (eq 2) should also be subject to steric effects with an alkyl group in position 2 of structure I—because of the required ring opening reaction. It would be possible for the value of k_2 to decrease to the point where it is influencing the observed kinetics. If this were to occur, the reactions would no longer be general acid catalyzed and would not exhibit a first-order dependence in the H_2EDTA^{2-} concentration. However, a first-order dependence in H_2EDTA^{2-} was observed in all cases in this work, and the variations of less than a factor of 2 in the values of k_{H_3O} and k_{H_2Y} given in Table III for the various tripeptide complexes indicate that the rate step is the proton transfer instead of Cu–N(peptide) bond cleavage. Thus, these data support the concept of a proton transfer rate-limiting step in the general mechanism for all the copper–tripeptide reactions with acids.

It is interesting to note that with the same ligand (EDTA), the absence of steric influence on the proton-transfer reactions due to tripeptide structure may be directly contrasted to the substantial steric influence on the nucleophilic reactions.

The variations in the values of the acid-independent rate constant, k_d, in Table III indicate that this rate constant may refer to a reaction in which water acts as a nucleophile. This proposal is supported by the fact that k_d values for $Cu(H_{-2}GGG)^-$, $Cu(H_{-2}AGG)^-$, and $Cu(H_{-2}LGG)^-$ are far too large, based on the pK_a of water, to be attributed to proton transfer from water as a noncoordinated general acid. Water is known to coordinate to a fifth position in the crystal structure of $NaCu(H_{-2}GGG)\cdot H_2O$,[15] but the reaction path represented by k_d would include the dissociation of both the carboxylate group and the adjacent peptide group from copper prior to reaction with EDTA. Alkyl groups in position 1 of structure I have relatively little effect on the k_d value while a methyl group in position 2 or an isobutyl group in position 3 reduces the value of k_d by at least an order of magnitude.

In conclusion, variation of the amino acid residues in the tripeptide complexes of copper(II) has little effect on reactions which are controlled by proton-transfer rates but this variation has a significant effect on the rate of nucleophilic substitution reactions. This is observed in the reaction rates of trien, en, $EDTA^{4-}$, and H_2O. The effect is attributed to steric hindrance of alkyl groups at positions 2 and 3 (structure I). In position 3 the alkyl groups make it more difficult to replace the tripeptide carboxylate bond to copper by the nucleophile, while in position 2 the alkyl groups are believed to slow the rate of opening of the chelate ring which occurs in the cleavage of the copper–N(peptide) bond. Other amino acid residues can be anticipated to have similar steric effects in the reactions of metal–peptide complexes.

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