s^{-1} as a lower limit for the intramolecular ET rate constant k_ϵ.

Discussion

Matsubara and Creutz[21] have reviewed the influence of EDTA-like ligands on replacement rates for water molecules also coordinated to various metal ions. They have pointed out that metal ions with partially vacant t_{2g} orbitals (their first class; Cr(III), Ru(III), Os(III)) demonstrate very large (10^5–10^{10}) increases in rate when EDTA and similar ligands replace water, but the metals with filled t_{2g} shells (their second class; Co(III), Rh(III), Ru(II)) demonstrate more modest increases, up to 10^2. For Ti(III), the data presented here show that rates of substitution for H_2O, HEDTA, and EDTA[22] complexes are in the ratios 1/360/10^4. Ti(III), though it has unfilled t_{2g} orbitals and hence falls into Matsubara and Creutz's first class, is not as sensitive to labilization as other cations of this class. The substitution reactions of the other aquo ions of the first class are primarily dissociative, but the reactions of the EDTA complexes have associative character. The substitution reaction of the Ti(III) aquo ion appears to have a large associative component so mechanism does not change on complex formation, perhaps explaining lower rate differences.

The reduction of $Ru(NH_3)_5SCN^{2+}$ by Ti^{3+} is much faster than the corresponding reaction[23] of $Co(NH_3)_5NCS^{2+}$. This indicates that the latter reaction is not substitution limited but that the factors which retard ET reactions of Co(III) also are important.[24]

The rate constant for reaction of $Ru(NH_3)_5NCS^{2+}$ with Ti^{3+}, 840 M^{-1} s^{-1}, is similar to the rate constant for reduction of $Ru(NH_3)_5OOCCH_3^{2+}$ by the same reductant (700 M^{-1} s^{-1}, μ = 1 M).[1] Both of these values are in the range of rate to be expected for substitution of a dipositive ion onto Ti^{3+}. The linear free energy relationship for outer-sphere Ru(III)–Ti(III) ET predicts a rate of 10^2 M^{-1} s^{-1} for an outer-sphere process of similar driving force. Sutin[25] has calculated values in the range of 10^{-4}–10^{-2} M^{-1} for the formation constants of precursor complexes for reactions involving highly charged cations. Other calculations[26] suggest that these estimates may be somewhat low for high ionic strength media. Taking 10^{-2} M^{-1} as a conservative estimate for the stability constant of the outer-sphere (or encounter) complex which would correspond to the Ru(III)–Ti(III) reaction which would fall on the measured linear free energy relationship (at the potential corresponding to the same driving force) leads to an estimate of 100 s^{-1} for the first-order rate constant for outer-sphere Ru(III)–Ti(III) ET. Comparison of this value with the values of k_ϵ previously calculated (500 s^{-1} for Ti^{3+} and 2×10^4 s^{-1} for Ti(HEDTA)) indicates that SCN^- as an inner-sphere bridging ligand greatly facilitates Ru(III)–Ti(III) ET.

In the Ru(III)–Ti(III) reaction, chloride is not an effective bridging ligand although thiocyanate is. In the case of Co(III)–Cr(II) ET both Cl^- and SCN^- are effective bridging ligands.[26] In the case of Ru(III)–Ti(III) reactions both electron-donor and electron-acceptor orbitals are t_{2g} orbitals, and their mutual interaction is greatly facilitated by low-lying antibonding π orbitals on thiocyanate. In the Co(III)–Cr(II) case e_g orbitals are involved, and outer-sphere paths are unfavorable. Coordination of both oxidant and reductant to a common ligand provides a path for reaction, but there is no special advantage for thiocyanate.[27]

Acknowledgment. This research was begun under support by a National Science Foundation Grant, for which we are grateful.

Registry No. $[Ru(NH_3)_5(SCN)](ClO_4)_2$, 38139-15-0; $Ti(H_2O)_6^{3+}$, 17524-20-8; $Ti(HEDTA)(H_2O)$, 75431-40-2.

Supplementary Material Available: Tables of angles of maximum reflectance and assigned Miller indices, half-wave potentials for the Ti(IV)/Ti(III) couple and the $Ti(HEDTA)^{4+/3+}$ couple, and rate constants (8 pages). Ordering information is given on any current masthead page.

(21) T. Matsubara and C. Creutz, *Inorg. Chem.*, **18**, 1956 (1979).
(22) G. A. K. Thompson and A. G. Sykes, *Inorg. Chem.*, **18**, 1731 (1979).
(23) J. Birk, *Inorg. Chem.*, **14**, 1724 (1975).
(24) H. C. Stynes and J. A. Ibers, *Inorg. Chem.*, **10**, 2304 (1971).
(25) (a) M. Chow, C. Creutz, and N. Sutin, *J. Am. Chem. Soc.*, **99**, 5615 (1979). (b) N. Sutin, *Inorg. Biochem.*, **2**, 623 (1973).
(26) J. L. Walsh, J. A. Bauman, and T. J. Meyer, *Inorg. Chem.*, **19**, 2149 (1980).
(27) T. Przystas and N. Sutin, *J. Am. Chem. Soc.*, **95**, 5545 (1973).

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Proton-Transfer Reactions of Copper(II)–Tetraglycine Complexes

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The triply, doubly, and singly deprotonated tetraglycine complexes $Cu^{II}(H_{-3}G_4)^{2-}$, $Cu(H_{-2}G_4)^-$, and $Cu(H_{-1}G_4)$ all show general-acid catalysis in their reactions with acids, indicating that direct protonation of the peptide nitrogen is rate determining. The H_3O^+ rate constants (M^{-1} s^{-1} at 25.0 °C) decrease in a stepwise manner with values of 1.6×10^8, 1.6×10^6, and 2.1×10^5, respectively. The reactions of $Cu(H_{-2}G_4)^-$ with base provide evidence for the $[Cu(H_{-2}G_4)OH]^{2-}$ species, and it is proposed that the hydroxide ion is coordinated in an equatorial position. The $[Cu(H_{-2}G_4)OH]^{2-}$ and the $Cu(H_{-3}G_4)^{2-}$ species, which are in equilibrium, account for 11% and 89% of the triply deprotonated copper(II)–tetraglycine species in solution.

Introduction

Copper(II) reacts with tetraglycine (G_4) in aqueous solution to form the complex CuG_4^+, which may ionize up to three peptide hydrogens depending on the solution pH. Deprotonation of CuG_4^+ occurs stepwise to form $Cu(H_{-1}G_4)$, $Cu(H_{-2}G_4)^-$, and $Cu(H_{-3}G_4)^{2-}$ with successive dissociation constants of $10^{-5.6}$, $10^{-6.96}$, and $10^{-9.14}$ (25 °C, μ = 1.0 M).[1] The crystal structure of $Na_2Cu(H_{-3}G_4)\cdot 10H_2O$ has been determined, and all four nitrogens (one amine and three deprotonated peptide nitrogens) are bound to copper with approximately square-planar geometry.[2] Spectral[3,4] and potentio-

(1) Smith, R. M.; Martell, A. E., Eds., "Critical Stability Constants"; Plenum Press: New York, 1974; Vol. 1.

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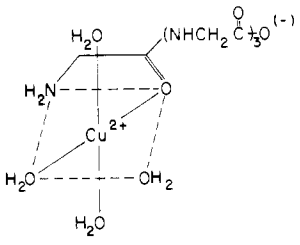
metric[5] studies indicate that the structure of $Cu(H_{-3}G_4)^{2-}$ in solution is essentially the same as in the crystal. No crystal structures have been determined for the species CuG_4^+, $Cu(H_{-1}G_4)$, and $Cu(H_{-2}G_4)^-$. The ESR spectra of the copper(II)–tetraglycine complexes have been examined in solution, and it was concluded that copper(II)–N(peptide) coordination occurs concurrently with peptide deprotonation.[6] Thus the structures of CuG_4^+, $Cu(H_{-1}G_4)$, and $Cu(H_{-2}G_4)^-$ in solution are best represented by structures I, II, and III, respectively.[7]



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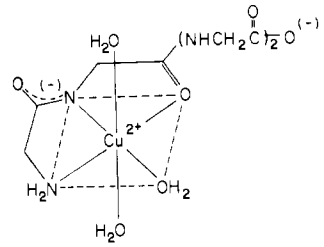

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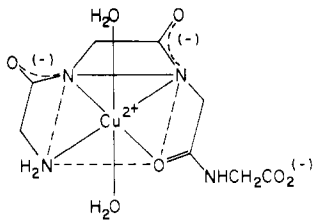


III

Two kinetic pathways have been observed for the proton-transfer reaction of metal–peptide complexes. The first of these is the outside protonation pathway[7-9] in which rapid protonation of a peptide oxygen occurs. This rapid protonation preequilibrium is followed by the rate-determining breaking of the metal–N(peptide) bond. The outside protonation pathway is accelerated by hydrogen ion but not by general acids. The second kinetic pathway for the proton-transfer reactions of metal–peptide complexes occurs by the direct protonation of the deprotonated peptide nitrogen, which occurs simultaneously with metal–N(peptide) bond breaking.[9-12] This pathway is accelerated both by hydrogen ion and by general acids. The outside protonation pathway contributes to the H_3O^+ rate constant only when the inside protonation is slow due to slow metal–N(peptide) bond rupture[7] or when metal–N(peptide) nitrogen bond rupture is otherwise restricted.[9]

In a previous report[13] the kinetics of the reaction of $Cu(H_{-3}G_4)^{2-}$ to form $Cu(H_{-2}G_4)^-$ were examined. The occurrence of general-acid catalysis in this reaction is indicative of direct proton transfer to the terminal deprotonated peptide nitrogen of the triply deprotonated complex. Proton transfer to $Cu(H_{-3}G_4)^{2-}$ was shown to be 1 order of magnitude more rapid than proton transfer to the tripeptide complexes copper(II) triglycine, $Cu(H_{-2}G_3)^-$,[10] and copper(II) glycylglycylhistidine, $Cu(H_{-2}GGhis)^-$.[9] The greater proton-transfer reactivity of $Cu(H_{-3}G_4)^{2-}$ compared with that of the tripeptide complexes was explained in terms of the greater lability of the terminal deprotonated peptide nitrogen.[13]

The rate constant for reaction of $Cu(H_{-3}G_4)^{2-}$ with H_2O, which acts as an acid, is 16 s^{-1}. This rate constant is much larger than would be expected for the acid strength of H_2O. The reason for the high reactivity of solvent with $Cu(H_{-3}G_4)^{2-}$ is that H_2O acts as a coordinating acid, coordinating axially to $Cu(H_{-3}G_4)^{2-}$, while simultaneously transferring a proton to the terminal deprotonated peptide nitrogen. In the present study, the reverse of this H_2O pathway, in which hydroxide coordinates axially to $Cu(H_{-2}G_4)^-$ while simultaneously removing a proton from the terminal peptide nitrogen to form $Cu(H_{-3}G_4)^-$, is observed. A second hydroxide pathway, in which OH^- forms an equatorial adduct before proton transfer occurs, is also observed.

The kinetics of formation of $Cu(H_{-3}G_4)^{2-}$ from $Cu(H_{-2}G_4)^-$ is linked to studies in our laboratory of copper(III)–peptide complexes. The oxidation of triply deprotonated copper(II) complexes to the trivalent oxidation state is thermodynamically more favorable by approximately 0.3 V than the oxidation of doubly deprotonated complexes. Hence, the rate of oxidation of copper(II)–tetrapeptide complexes by moderately strong oxidizing agents in neutral solution, where the doubly deprotonated form predominates, is likely to require the formation of the triply deprotonated form before oxidation can occur. Stopped-flow kinetics of the oxidation of copper(II)–tetrapeptide complexes by $IrCl_6^{2-}$ confirm this.[14]

The kinetics of the reaction converting $Cu(H_{-2}G_4)^-$ to $Cu(H_{-3}G_4)^{2-}$ are followed by pH jump and provide evidence for the existence of a $[Cu(H_{-2}G_4)OH]^{2-}$ species, which accounts for approximately 11% of the triply deprotonated copper(II) tetraglycine at equilibrium. This result is interesting in view of early discussions of the nature of metal–peptide complexes and whether there is coordination by deprotonated peptide nitrogens or by hydroxide ion.[7] In the case of tetraglycine, it appears that there is some of each type of coordination.

Also examined in the present study are the proton-transfer reactions of the doubly and singly deprotonated tetraglycine complexes $Cu(H_{-2}G_4)^-$ and $Cu(H_{-1}G_4)$. The reactions of $Cu(H_{-2}G_4)^-$ to form $Cu(H_{-1}G_4)$ and of $Cu(H_{-1}G_4)$ to form CuG_4^+ are general-acid catalyzed. Hence, the protonation of each of the three copper(II)–tetraglycine complexes $Cu(H_{-3}G_4)^{2-}$, $Cu(H_{-2}G_4)^-$, and $Cu(H_{-1}G_4)$ occurs via direct reaction of a deprotonated peptide nitrogen. The protonations occur in successively slower stepwise reactions with H_3O^+. The rates of proton transfer decrease with decreasing basicity of the copper(II) complexes.

Experimental Section

Chemicals. Tetraglycine was obtained from Biosynthetika and was used without further purification. A stock solution of $Cu(ClO_4)_2$ was prepared from twice-recrystallized salt and standardized by EDTA titration using murexide as an indicator. Buffer solutions were prepared from sodium carbonate, triethylamine, *N*-methylpiperidine, disodium EDTA, and acetic acid, all of which were obtained commercially as reagent grade chemicals.

Kinetics Experiments. Solutions of 8.0×10^{-4}–3.2×10^{-3} M copper(II) tetraglycine were prepared by reaction of 5–10% excess of tetraglycine with $Cu(ClO_4)_2$ solution. The doubly deprotonated complex $Cu(H_{-2}G_4)^-$ was formed as approximately 86% of the total copper(II) at pH 8.0. At this pH the remaining copper(II) consists of 6% $Cu(H_{-3}G_4)^{2-}$ and 8% $Cu(H_{-1}G_4)$. The singly deprotonated complex $Cu(H_{-1}G_4)$ was formed as approximately 70% of the total copper(II) at pH 6.5 where the remaining copper(II) consists of

(2) Freeman, H. C.; Taylor, M. R. *Acta Crystallogr.* **1965**, *18*, 939.
(3) Kim, M. K.; Martell, A. E. *J. Am. Chem. Soc.* **1966**, *88*, 914.
(4) Billo, E. J. *Inorg. Nucl. Chem. Lett.* **1975**, *10*, 613.
(5) Koltun, W. L.; Roth, R. H.; Gurd, F. R. N. *J. Biol. Chem.* **1963**, *238*, 124.
(6) Falk, K. E.; Freeman, H. C.; Jansson, T.; Malmström, B. G.; Vanngard, T. *J. Am. Chem. Soc.* **1967**, *89*, 6071.
(7) Margerum, D. W.; Dukes, G. R. In "Metals in Biological Systems"; Sigel, H.; Ed.; Marcel Dekker: New York, 1974; Vol. 1, Chapter 5.
(8) Paniago, E. B.; Margerum, D. W. *J. Am. Chem. Soc.* **1972**, *94*, 6704.
(9) Wong, L. F.; Copper, J. C.; Margerum, D. W. *J. Am. Chem. Soc.* **1976**, *98*, 7268.
(10) Bannister, C. E.; Margerum, D. W.; Raycheba, J. M. T.; Wong, L. F. *Symp. Faraday Soc.* **1975**, *No. 10*, 78.
(11) Pagenkopf, G. K.; Margerum, D. W. *J. Am. Chem. Soc.* **1968**, *90*, 6963.
(12) Billo, E. J.; Margerum, D. W. *J. Am. Chem. Soc.* **1970**, *92*, 6811.
(13) Youngblood, M. P.; Margerum, D. W. *Inorg. Chem.* **1980**, *19*, 3072.
(14) Bossu, F. P.; Chellappa, K. L.; Margerum, D. W. *J. Am. Chem. Soc.* **1977**, *99*, 7268.

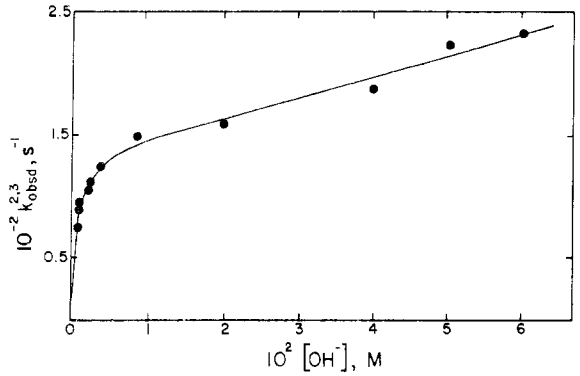


Figure 1. Dependence of the observed first-order rate constant $k_{obsd}^{2,3}$ (see eq 1) on the concentration of hydroxide ion.

approximately 7% $Cu(H_{-2}G_4)^-$ and 23% CuG_4^+. All solutions were adjusted to 1.0 total ionic strength with $NaClO_4$.

The kinetics of the reactions of $Cu(H_{-2}G_4)^-$ with base and acid as well as the kinetics of reaction of $Cu(H_{-1}G_4)$ with acid were studied under pseudo-first-order conditions by mixing portions of the copper(II) peptide with well-buffered solutions of the desired pH. The concentration of buffer was always maintained in at least a 10-fold excess of the concentration of copper(II).

The reaction of $Cu(H_{-2}G_4)^-$ with base was followed at 520 nm, monitoring the appearance of the product $Cu(H_{-3}G_4)^{2-}$. The reaction of $Cu(H_{-2}G_4)^-$ with acid (in the presence of EDTA) was followed at 600 nm, monitoring the disappearance of $Cu(H_{-2}G_4)^-$. The reaction of $Cu(H_{-1}G_4)$ with acid was followed at 635 nm, monitoring the disappearance of $Cu(H_{-1}G_4)$. The wavelength maxima and molar absorptivities for the d–d transitions of the Cu(II)–tetraglycine complexes depend on the number of deprotonated peptide nitrogens present in the complex. The λ_{max} (nm) and ϵ (M^{-1} cm^{-1}; values in parentheses) for the various Cu(II)–tetraglycine species are as follows: CuG_4^+, 730 (39); $Cu(H_{-1}G_4)$, 660 (72); $Cu(H_{-2}G_4)^-$, 590 (101); $Cu(H_{-3}G_4)^{2-}$, 520 (145).[5] Kinetic data were obtained with use of a Durrum stopped-flow spectrophotometer interfaced to a Hewlett-Packard 2115A general purpose computer. In all cases excellent first-order traces were obtained.

In this study the kinetics of three different proton-transfer reactions are reported: (1) the reaction of $Cu(H_{-2}G_4)^-$ with base to form $Cu(H_{-3}G_4)^{2-}$; (2) the reactions of $Cu(H_{-2}G_4)^-$ with acid to form $Cu(H_{-1}G_4)$; (3) the reaction of $Cu(H_{-1}G_4)$ to form CuG_4^+. The observed and resolved rate constants for the three reactions are differentiated from one another by a superscript denoting the number of deprotonated peptide nitrogens in the reactant and product. For example, $k_{obsd}^{2,3}$ refers to the observed rate constant for the reaction of $Cu(H_{-2}G_4)^-$ with base to form $Cu(H_{-3}G_4)^-$.

Measurement of the Hydrogen Ion and Hydroxide Ion Concentrations. A perchloric acid–sodium hydroxide titration in 1.0 M $NaClO_4$ was used to obtain the empirical relationship $-\log [H^+] = pH + 0.30$. The correction of 0.30 is primarily determined by the liquid junction potential in the cell used to measure the pH. The cell employed a saturated sodium chloride calomel reference electrode. When the concentration of hydroxide ion was 0.01 M or less the concentration was calculated from $-\log [H^+]$ and the value of K_w for 1.0 M $NaClO_4$ medium ($10^{-13.80}$).[12]

Results and Discussion

Reaction of $Cu(H_{-2}G_4)^-$ with Base. The reaction of $Cu(H_{-2}G_4)^-$ with base to form $Cu(H_{-3}G_4)^{2-}$ followed the rate law in eq 1. The kinetic results for the reaction are given in Table

$$-\frac{d[Cu(H_{-2}G_4)^{2-}]}{dt} = k_{obsd}^{2,3}[Cu(H_{-2}G_4)^-] \quad (1)$$

I. The observed rate constants showed no dependence on the concentration of triethylamine or *N*-methylpiperidine, but carbonate inhibits the rate of reaction. The hydroxide dependence of $k_{obsd}^{2,3}$ is more pronounced at lower concentrations as shown in Figure 1.

The value of the rate constant k_{OH} for the reaction of hydroxide ion with $Cu(H_{-2}G_4)^-$ to form $Cu(H_{-3}G_4)^{2-}$ (eq 2) can

$$Cu(H_{-2}G_4)^- + OH^- \underset{k_{H_2O}}{\overset{k_{OH}}{\rightleftharpoons}} Cu(H_{-3}G_4)^{2-} + H_2O \quad (2)$$

be calculated from k_{H_2O} (16 s^{-1}),[13] the acid dissociation constant for $Cu(H_{-2}G_4)^-$ ($10^{-9.14}$),[1] and K_w ($10^{-13.80}$).[15] This calculation yields a value of 7.1×10^5 M^{-1} s^{-1} for k_{OH}. Similarly, a calculation of k_B (eq 3), the general base catalysis rate

$$Cu(H_{-2}G_4)^- + B^- \underset{k_{HB}}{\overset{k_B}{\rightleftharpoons}} Cu(H_{-3}G_4)^{2-} + HB \quad (3)$$

constant for a given base, can be made from the equilibrium constant for eq 3 and the value of k_{HB}, which can be estimated from the Brønsted plot established for reactions of HB with $Cu(H_{-3}G_4)^{2-}$.[13] This type of calculation reveals that the value of k_B is never greater than 10 M^{-1} s^{-1} regardless of the base strength of B. Hence, the lack of general-base catalysis by triethylamine and *N*-methylpiperidine is not surprising since the calculated rate constants k_{OH} and k_B indicate that the general-base pathway (eq 3) should be negligible compared to the hydroxide pathway (eq 2). However, the change in hydroxide dependence and the inhibition exhibited by carbonate were not expected and indicate that the deprotonation of $Cu(H_{-2}G_4)^-$ to form $Cu(H_{-3}G_4)^{2-}$ is not a simple direct proton transfer.

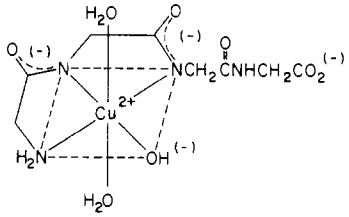
Below 5×10^{-3} M hydroxide, the observed hydroxide dependence (Figure 1) agrees with the calculated value of 7.1×10^5 M^{-1} s^{-1} for k_{OH}. At higher concentrations, however, the dependence on hydroxide decreases. This indicates that the $Cu(H_{-2}G_4)^-$ reactant is converted to a species which is much less reactive with hydroxide. The mechanism given in eq 4–6 is consistent with the observed kinetic results for the

$$Cu(H_{-2}G_4)^- + OH^- \underset{k_{H_2O}}{\overset{k_{OH}}{\rightleftharpoons}} Cu(H_{-3}G_4)^{2-} + H_2O \quad (4)$$

$$Cu(H_{-2}G_4)^- + OH^- \overset{K_5,\ \text{rapid}}{\rightleftharpoons} Cu(H_{-2}G_4)OH^{2-} \quad (5)$$

$$Cu(H_{-2}G_4)OH^{2-} + OH^- \underset{k_{-6}^{OH}}{\overset{k_6^{OH}}{\rightleftharpoons}} Cu(H_{-3}G_4)^{2-} + OH^- + H_2O \quad (6)$$

reaction of $Cu(H_{-2}G_4)^-$ with OH^-. At hydroxide concentrations less than 5×10^{-3} M, $Cu(H_{-2}G_4)^-$ is the predominant copper(II) species and deprotonation of the peptide nitrogen occurs via direct hydroxide attack (eq 4). At hydroxide concentrations greater than 5×10^{-3} M, $Cu(H_{-2}G_4)^-$ is rapidly and fully converted to $Cu(H_{-2}G_4)OH^{2-}$ (eq 5, structure IV).



IV

Deprotonation of $Cu(H_{-2}G_4)OH^{2-}$ by a second hydroxide ion occurs at a slower rate than the deprotonation of $Cu(H_{-2}G_4)^-$, because the coordinated hydroxide ion must be displaced in order to form $Cu(H_{-3}G_4)^{2-}$ (eq 6).

The mechanism in eq 4–6 leads to the expression in eq 7

$$k_{obsd}^{2,3} = \frac{k_{OH}[OH^-] + k_6^{OH}K_5[OH^-]^2}{1 + K_5[OH^-]} + k_{-6}^{OH}[OH^-] + k_{H_2O} \quad (7)$$

for $k_{obsd}^{2,3}$. This expression is derived from reversible first-order

(15) Fischer, R.; Bye, J. *Bull. Soc. Chim. Fr.* **1964**, 2920.

Table I. Observed Rate Constants for the Reaction of $Cu(H_{-2}G_4)^-$ with Base To Form $Cu(H_{-3}G_4)^{2-}$

base	$[base]_T$, M	$10^3[OH^-]$, M	k_{obsd}, s^{-1}
triethylamine	0.057	0.63	74 ± 5
	0.057	0.83	93.5 ± 0.5
	0.057	2.04	104 ± 2
	0.057	3.90	124 ± 4
	0.057	8.71	148 ± 5
	0.096	2.30	114 ± 1
	0.144	2.30	110 ± 2
	0.432	2.30	109 ± 1
1-methylpiperidine	0.041	0.69	88 ± 2
	0.082	0.69	88 ± 2
	0.206	0.69	91 ± 5
sodium carbonate	0.020	0.56	57 ± 1
	0.050	0.56	49.1 ± 0.5
	0.075	0.56	41.7 ± 0.5
	0.100	0.56	39.4 ± 0.3
	0.150	0.56	37.2 ± 0.5
	0.200	0.56	35.8 ± 0.5
sodium hydroxide		20.0	159 ± 3
		40.0	187 ± 19
		50.0	223 ± 15
		60.0	233 ± 9

Table II. Rate Constants and Equilibrium Constants Involved in the Deprotonation of $Cu(H_{-2}G_4)^-$ To Form $Cu(H_{-3}G_4)^{2-}$ [a]

const	value	const	value
k_{OH}, M^{-1} s^{-1}	7.1×10^5	k_6^{OH}, M^{-1} s^{-1}	1.6×10^3
k_{H_2O}, s^{-1}	16	k_{-6}^{OH}, M^{-1} s^{-1}	2×10^2
K_s, M^{-1}	6.4×10^3	K_6 $(=k_6^{OH}/k_{-6}^{OH})$	8

[a] See eq 4–6.

kinetics where $k_{obsd}^{2,3}$ is the sum of the forward and reverse rate constants. The first term on the right-hand side is the forward rate constant, and the last two terms are the reverse rates constants for the interconversion of $Cu(H_{-2}G_4)^-$ and $Cu(H_{-3}G_4)^{2-}$. The data in Table I were fit to eq 7 with use of the calculated value of k_{OH} (7.1×10^5 M^{-1} s^{-1}) and the experimental value of k_{H_2O} (16 s^{-1})[13] as constants. The resolved values of the rate constants and equilibrium constants defined by eq 4–6 are summarized in Table II. The solid curve in Figure 1 was calculated from eq 7 with the use of the constants in Table II.

The value of the equilibrium constant for eq 6, $K_6 = k_6^{OH}/k_{-6}^{OH}$, is 8, indicating that the triply deprotonated copper(II)–tetraglycine complex consists of approximately 89% $Cu(H_{-3}G_4)^{2-}$ and 11% $Cu(H_{-2}G_4)OH^{2-}$ at equilibrium. The reaction of triply deprotonated copper(II) tetraglycine with triethylenetetramine (trien) provides support for this conclusion. When the triply deprotonated complex is mixed with trien, a rapid reaction corresponding to the displacement of the peptide from $Cu(H_{-2}G_4)OH^{2-}$ is observed prior to the slower peptide displacement from $Cu(H_{-3}G_4)^{2-}$.[13] An analogous species distribution has also been proposed to occur in the nickel(II)–diglycinamide, Ni(II)–G_2a, system.[16] In that case, however, the thermodynamically more stable species $Ni(H_{-1}G_2a)OH$ contained one less deprotonated peptide nitrogen than the less stable species $Ni(H_{-2}G_2a)$.

The kinetic inhibition exhibited by carbonate indicates that this coordinating buffer reacts rapidly with $Cu(H_{-2}G_4)^-$ to form an equatorial adduct. The adduct formed, like the $Cu(H_{-2}G_4)OH^{2-}$ adduct, reacts more slowly with hydroxide than does $Cu(H_{-2}G_4)^-$, because the coordinated carbonate ion must be displaced in order to form the $Cu(H_{-3}G_4)^{2-}$ product.

Protonation of $Cu(H_{-2}G_4)^-$ and $Cu(H_{-1}G_4)$. When an unbuffered solution of $Cu(H_{-2}G_4)^-$ at pH 8.0 is mixed with acetate buffer of pH 5.0 or less, two consecutive reactions are observed. These reactions correspond to the stepwise protonations shown in eq 8 and 9. From $-\log [H^+]$ values of 6.6 to 6.1 EDTA acts as a scavenger for $Cu(H_{-1}G_4)$ and makes it possible to study the kinetics of reaction 8 without interference from either the subsequent reaction (eq 9) or from reversibility. The kinetics of reaction 9 were studied by mixing $Cu(H_{-1}G_4)$, initially at pH 6.0, with an acetate buffer at pH less than 4.5.

$$Cu(H_{-2}G_4)^- + H^+ \rightarrow Cu(H_{-1}G_4) \quad (8)$$

$$Cu(H_{-1}G_4) + H^+ \rightarrow CuG_4^+ \quad (9)$$

Reaction of $Cu(H_{-2}G_4)^-$ with Acid in the Presence of EDTA. From $-\log [H^+]$ values of 6.1 to 6.6, the reaction of $Cu(H_{-2}G_4)^-$ with EDTA followed the rate law in eq 10. The

$$\frac{-d[Cu(H_{-2}G_4)^-]}{dt} = k_{obsd}^{2,1}[Cu(H_{-2}G_4)^-] \quad (10)$$

value of $k_{obsd}^{2,1}$ depends on the pH as well as the concentrations of EDTA and acetic acid (eq 11). The observed rate constants

$$k_{obsd}^{2,1} = k_H^{2,1}[H^+] + k_{EDTA}^{2,1}[EDTA]_T + k_{HOAc}^{2,1}[CH_3COOH] + k_{H_2O}^{2,1} \quad (11)$$

Table III. Observed Rate Constants for the Reaction of $Cu(H_{-2}G_4)^-$ with Acids (HB)

HB	$-\log [H^+]$	$[HB]_T$, M	$k_{obsd}^{2,1}$, s^{-1}
EDTA	6.60	0.010	2.74 ± 0.02
		0.020	4.84 ± 0.04
		0.030	6.72 ± 0.04
		0.050	10.6 ± 0.1
	6.33	0.018	4.86 ± 0.03
		0.025	6.66 ± 0.05
		0.038	9.29 ± 0.12
		0.045	10.85 ± 0.02
	6.10	0.010	3.66 ± 0.01
		0.020	6.49 ± 0.03
		0.030	9.20 ± 0.05
		0.050	13.8 ± 0.2
CH_3COOH	6.28	0.016	15.0 ± 0.2[a]
		0.026	16.2 ± 0.2[a]
		0.036	17.5 ± 0.3[a]
		0.047	19.1 ± 0.2[a]
	4.73	0.060	200 ± 30[b]
		0.100	265 ± 13[b]
		0.140	350 ± 30[b]

[a] Determined in the presence of 0.051 M EDTA. [b] Determined with use of a consecutive reaction order analysis.

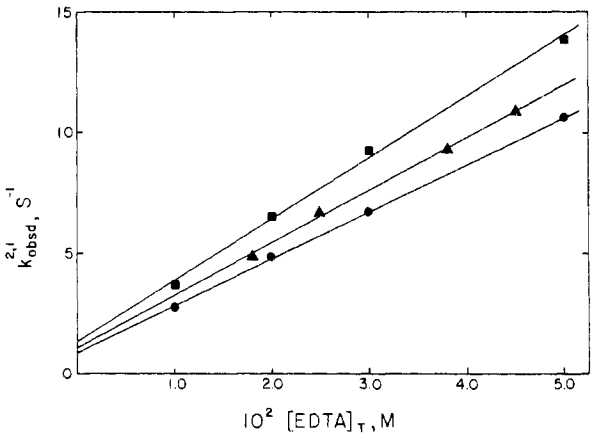


Figure 2. Dependence of the observed first-order rate constant $k_{obsd}^{2,1}$ (see eq 10) on the total concentration of EDTA ($[EDTA]_T = [HEDTA^{3-}] + [H_2EDTA^{2-}]$) at $-\log [H^+] = 6.1$ (■), 6.3 (▲), and 6.6 (●).

(16) Raycheba, J. M. T.; Margerum, D. W. *Inorg. Chem.* **1980**, *19*, 837.

for the proton-transfer reactions of $Cu(H_{-2}G_4)^-$ are given in Table III.

Figure 2 shows the EDTA dependence of $k_{obsd}^{2,1}$ at −log [H^+] values of 6.6, 6.3, and 6.1. The intercepts of these plots were used to evaluate $k_H^{2,1}$ and $k_{H_2O}^{2,1}$. This analysis yields values of $(1.6 \pm 0.4) \times 10^6$ M^{-1} s^{-1} for $k_H^{2,1}$ and 0.4 ± 0.2 s^{-1} for $k_{H_2O}^{2,1}$.

The rate constants for the reaction of $H(EDTA)^{3-}$ and $H_2(EDTA)^{2-}$ with $Cu(H_{-2}G_4)^-$, defined in eq 12 where

$$k_{EDTA}^{2,1} = \frac{k_{H(EDTA)}^{2,1} K_{H_2(EDTA)} + k_{H_2(EDTA)}^{2,1}[H^+]}{K_{H_2(EDTA)} + [H^+]} \quad (12)$$

$K_{H_2(EDTA)}$ is the acid dissociation constant for $H_2(EDTA)^{2-}$ ($10^{-6.28}$ M),[17] were evaluated from the slopes of the EDTA dependences in Figure 2. The resolved values of $k_{H_2(EDTA)}^{2,1}$ and $k_{H(EDTA)}^{2,1}$ are $(3.30 \pm 0.02) \times 10^2$ and $(1.48 \pm 0.03) \times 10^2$ M^{-1} s^{-1}, respectively. Since the diprotonated EDTA is more reactive than the monoprotonated form, $H_2(EDTA)^{2-}$ reacts with $Cu(H_{-2}G_4)^-$ as a general acid. At constant EDTA concentration (0.051 M) and at constant pH (5.98), the value of $k_{obsd}^{2,1}$ increases with the concentration of acetic acid. The rate constant, $k_{HOAc}^{2,1}$ resolved in terms of acetic acid is $(5.9 \pm 0.2) \times 10^3$ M^{-1} s^{-1}. The much larger dependence of $k_{obsd}^{2,1}$ on the total acetic acid concentration at −log [H^+] 4.73 (Table III) demonstrates that acetic acid, rather than the acetate ion, is the reactive species.

Acceleration of the rate of protonation of $Cu(H_{-2}G_4)^-$ by the general acids CH_3COOH and $H_2(EDTA)^{2-}$ indicates that direct proton transfer to the coordinated peptide nitrogen occurs during the rate-determining step. The mechanism for the protonation of $Cu(H_{-2}G_4)^-$ in the presence of EDTA and acetic acid is given in eq 13–15, where HB represents H_3O^+,

$$Cu(H_{-2}G_4)^- + H(EDTA)^{3-} \xrightarrow{k_{H(EDTA)}^{2,1}} Cu(EDTA)^{2-} + OH^- + G_4^- \quad (13)$$

$$Cu(H_{-2}G_4)^- + HB \xrightarrow{k_{HB}^{2,1}} Cu(H_{-1}G_4) + B^- \quad (14)$$

$$Cu(H_{-1}G_4) + H(EDTA)^{3-} \xrightarrow{\text{rapid}} Cu(EDTA)^{2-} + G_4^- \quad (15)$$

$H_2(EDTA)^{2-}$, CH_3COOH, and H_2O. Although $H(EDTA)^{3-}$ is a very weak acid ($pK_a = 10.3$), it is only a factor of 2 less reactive than $H_2(EDTA)^{2-}$. This suggests that $H(EDTA)^{3-}$ reacts with $Cu(H_{-2}G_4)^-$ as a nucleophile rather than as a general acid.

Reaction of $Cu(H_{-1}G_4)$ with Acid. The reaction of $Cu(H_{-1}G_4)$ with acetate buffers at −log [H^+] = 4.7 followed the rate law in eq 16. The observed first-order rate constants for

$$\frac{-d[Cu(H_{-1}G_4)]}{dt} = k_{obsd}^{1,0}[Cu(H_{-1}G_4)] \quad (16)$$

proton transfer to $Cu(H_{-1}G_4)$ are given in Table IV. The value of $k_{obsd}^{1,0}$ depends on the pH as well as the acetic acid concentration as shown in eq 17. Figure 3 shows the de-

$$k_{obsd}^{1,0} = k_H^{1,0}[H^+] + k_{HOAc}^{1,0}[CH_3COOH] + k_{H_2O}^{1,0} \quad (17)$$

pendence of $k_{obsd}^{1,0}$ on the concentration of H_3O^+ and of acetic acid. The resolved rate constants are $(2.1 \pm 0.1) \times 10^5$ and $(6.1 \pm 0.4) \times 10^2$ M^{-1} s^{-1} for $k_H^{1,0}$ and $k_{HOAc}^{1,0}$ respectively. The rate constant $k_{H_2O}^{1,0}$, the contribution to $k_{obsd}^{1,0}$ that is independent of the concentration of both hydrogen ion and acetic acid, was estimated at approximately 0.4 s^{-1}. Gene-

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

Table IV. Observed Rate Constants for the Reaction of $Cu(H_{-1}G_4)$ with Acids (HB)

HB	−log [H^+]	$[HB]_T$, M	k_{obsd}, s^{-1}
CH_3COOH	4.69 ± 0.05	0.060	26.2 ± 0.8
		0.100	42.8 ± 0.5
		0.140	53.7 ± 0.8
		0.200	75.5 ± 1.2
H_3O^+ [a]	4.65	2.24×10^{-5}	42.8 ± 0.5
	4.45	3.55×10^{-5}	44.4 ± 0.6
	4.31	4.90×10^{-5}	47.2 ± 0.6
	4.22	6.03×10^{-5}	47.6 ± 0.8
	3.95	8.91×10^{-5}	55.9 ± 0.5
	3.87	1.35×10^{-4}	65.7 ± 1.1

[a] Acetate buffer was used with the concentration of the acid form held constant at 0.050 (±0.005) M.

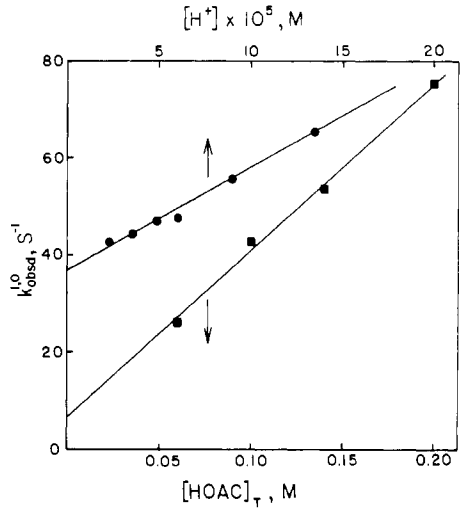


Figure 3. Dependence of the observed first-order rate constant $k_{obsd}^{1,0}$ (see eq 16) on the total concentration of acetic acid (■) at −log [H^+] = 4.68 ± 0.05 and on the concentration of hydrogen ion (●) at [CH_3COOH] = 0.05 M.

ral-acid catalysis by acetic acid indicates that the protonation of $Cu(H_{-1}G_4)$, like that of $Cu(H_{-2}G_4)^-$, occurs via direct proton transfer to the coordinated peptide nitrogen.

Mechanism of the Stepwise Proton-Transfer Reactions of Copper(II) Tetraglycine. Table V gives the resolved rate constants for the reactions of H_3O^+ and acetic acid with $Cu(H_{-3}G_4)^{2-}$, $Cu(H_{-2}G_4)^-$, $Cu(H_{-1}G_4)$, $Cu(H_{-2}G_3)^-$, and $Cu(H_{-1}G_2)$. The larger proton-transfer rate constant for $Cu(H_{-3}G_4)^{2-}$ compared with the other copper(II) complexes in Table V has been explained by three factors: (1) the lack of protective chelation by a carboxylate or a peptide oxygen allowing the unhindered movement of the peptide nitrogen away from copper(II) during proton transfer; (2) strain in the chelate ring containing the third deprotonated peptide nitrogen; (3) a trans effect.[13]

The ring strain and trans effect arguments also explain the greater proton-transfer reactivity of $Cu(H_{-2}G_3)^-$ compared with the reactivity of $Cu(H_{-1}G_2)$. The cumulative ring strain in $Cu(H_{-2}G_3)^-$ is expected to be greater than in $Cu(H_{-1}G_2)$. Also, the proton-transfer site in $Cu(H_{-2}G_3)^-$ is trans to an amine group, while in $Cu(H_{-1}G_2)$ it is trans to a coordinated water molecule, which is a much weaker donor than the amine group. Both of the above factors could cause the rate of proton transfer to $Cu(H_{-2}G_3)^-$ to be greater than to $Cu(H_{-1}G_2)$, by increasing the lability of the peptide nitrogen to which proton transfer occurs. An interesting feature in Table V is that the H_3O^+ rate constant for $Cu(H_{-2}G_4)^-$ is a factor of 8 less than that for $Cu(H_{-2}G_3)^-$ and that the H_3O^+ rate constant for

Table V. Protonation Rate Constants for the Reactions of Copper(II) Complexes of Glycyl Peptides with H_3O^+, CH_3COOH, H_2O, and EDTA

peptide complex	pK_a	rate const, M^{-1} s^{-1}			
		H_3O^+	CH_3COOH	H_2O^a	EDTA
$Cu(H_{-3}G_4)^{2-}$	9.14	1.6×10^8	3.8×10^5	16	
$Cu(H_{-2}G_4)^-$	6.96	1.6×10^6	5.9×10^3	0.4	3.3×10^2 ($H_2(EDTA)^{2-}$), 1.48×10^2 ($H(EDTA)^{3-}$)
$Cu(H_{-1}G_4)$	5.6	2.1×10^5	6.1×10^2	~0.4	
$Cu(H_{-2}G_3)^-$	6.68	1.3×10^7 [b]	3.4×10^4	0.12	3.1×10^3 ($H_2(EDTA)^{2-}$), ~0 ($H(EDTA)^{3-}$)
$Cu(H_{-1}G_2)$	4.06	8.7×10^5 [c]			

[a] Units for H_2O rate constants are s^{-1}. [b] See ref 10. [c] See ref 20.

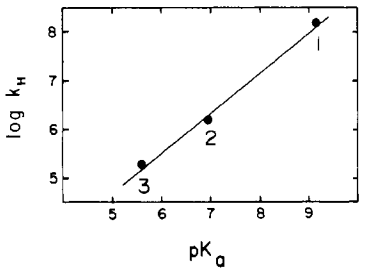


Figure 4. Correlation of the H_3O^+ rate constant for k_H for (1) $Cu(H_{-3}G_4)^{2-}$, (2) $Cu(H_{-2}G_4)^-$, and (3) $Cu(H_{-1}G_4)$ with their respective acid dissociation constants (pK_a).

$Cu(H_{-1}G_4)$ is 4 times less than that for $Cu(H_{-1}G_2)$.

In contrast to its lower proton-transfer reactivity, $Cu(H_{-2}G_4)^-$ reacts with the nucleophile $H(EDTA)^{3-}$ with a rate constant of 148 M^{-1} s^{-1} while no appreciable rate has been detected for the reaction of $Cu(H_{-2}G_3)^-$ and other copper(II)–tripeptide complexes with this species.[18] For the tripeptide complexes, only the unprotonated and diprotonated forms of EDTA appear to be reactive. Nucleophilic displacement reactions of the doubly deprotonated copper(II)–peptide complexes are very dependent on the type of donor group that completes the third chelate ring. For example, the rate constant for reaction of triethylenetetramine with $Cu(H_{-2}G_3)^-$ is 1.1×10^7 M^{-1} s^{-1},[19] while the corresponding rate constant is only 0.50 M^{-1} s^{-1} for $Cu(H_{-2}GGhis)^-$.[9] Since peptide oxygens are weaker coordinating groups than carboxylate oxygen, $H(EDTA)^{3-}$ more readily displaces the peptide from $Cu(H_{-2}G_4)^-$ than from $Cu(H_{-2}G_3)^-$.

The H_3O^+ rate constant for $Cu(H_{-1}G_4)$ is a factor of 4 lower than the corresponding value reported for $Cu(H_{-1}G_2)$,[20] indicating either that the third and fourth glycyl residues in $Cu(H_{-1}G_4)$ in some way make proton transfer to this complex more difficult or that the coordinated carboxylate group in $Cu(H_{-1}G_2)$ makes the proton transfer easier. Coordination to copper(II) by the peptide oxygen and by the carboxylate oxygen in the third and fourth amino acid residues, respectively, is not very likely since no reasonable structure can be assigned to $Cu(H_{-1}G_4)$ in which the oxygen atoms of the last two amino acids of tetraglycine are bound to the metal. Also such an explanation would predict that the pK_a value for CuG_4^+ to form $Cu(H_{-1}G_4)$ would be lower than the pK_a value for CuG_2a^{2+} to form $Cu(H_{-1}G_2a)^+$, while the actual pK_a values are 5.40[21] and 5.00,[22] respectively.

Figure 4 illustrates the relationship between the H_3O^+ rate constants for $Cu(H_{-1}G_4)^-$ and $Cu(H_{-3}G_4)^{2-}$ and the successive acid dissociation constants of the deprotonated peptide nitrogens. The slope of the observed correlation is 0.8. This indicates that those factors which influence the relative basicities of the three forms of the deprotonated copper(II) tetraglycine also influence the relative rates of protonation. Such factors include ring strain, trans effects, the type of donor groups coordinated to the copper(II), and the solvation properties of the metal peptide complexes.

Conclusions

The proton-transfer reactions of copper(II) tetraglycine provide the first example of the kinetic stepwise unwrapping of a peptide from a metal ion. The correlation of log k_H vs. pK_a has a slope of 0.8 and is consistent with the mechanism in which direct proton transfer to the deprotonated peptide nitrogen is rate determining for each of the successive protonations.

In basic solution, the fully deprotonated copper–tetraglycine complex exists in two forms that have different equatorial donor groups. The major species $Cu(H_{-3}G_4)^{2-}$, which accounts for 89% of the copper, has an amine and three deprotonated peptide nitrogen donors in the equatorial plane, while the equatorial donor groups of the minor species $Cu(H_{-2}G_4)OH^{2-}$ are an amine nitrogen, two deprotonated peptide nitrogens, and a hydroxide oxygen.

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Registry No. $Cu(H_{-3}G_4)(H_2O)_2^{2-}$, 76986-67-9; $Cu(H_{-2}G_4)(H_2O)_2^-$, 76986-66-8; $Cu(H_{-1}G_4)(H_2O)_3$, 76986-65-7; $Cu(H_{-2}G_4)(OH)(H_2O)_2^{2-}$, 76986-83-9; carbonate, 3812-32-6; OH^-, 14280-30-9; triethylamine, 121-44-8; 1-methylpiperidine, 626-67-5; H_3O^+, 13968-08-6; CH_3COOH, 64-19-7; $H_2(EDTA)^{2-}$, 12301-02-9; $H(EDTA)^{3-}$, 20737-07-9; OH_2, 7732-18-5.

(18) Hauer, H.; Dukes, G. R.; Margerum, D. W. *J. Am. Chem. Soc.* **1973**, *95*, 3515.
(19) Pagenkopf, G. K.; Margerum, D. W. *J. Am. Chem. Soc.* **1970**, *92*, 2683.
(20) Pasternack, R. F.; Angrum, M.; Gibbs, E. *J. Am. Chem. Soc.* **1970**, *92*, 5878.
(21) Dorigatti, T. F.; Billo, E. J. *J. Inorg. Nucl. Chem.* **1975**, *37*, 1515.
(22) Smith, R. M., Martell, A. E., Eds. "Critical Stability Constants", Plenum Press: New York, 1975; Vol. 2.