for copper couples and positive for nickel couples. The values of $dE°/dT$ allow evaluation of the entropy difference between oxidation states, $S°_{II} - S°_{III}$, which are negative for the copper couples and positive for the nickel couples. The values of $S°_{II} - S°_{III}$ correspond to a gain of two water molecules for the reduction of copper(III) peptide complexes and a loss of two water molecules for the reduction of nickel(III) peptide complexes. In both cases the $d^8$ electronic configuration (copper(III) and nickel(II)) behaves as though it has no axial coordination of water, while the $d^9$ (copper(II)) and $d^7$ (nickel(III)) electronic configurations behave as if two water molecules are coordinated axially. This explains why alkyl side chains in the peptide ligand, especially bulky ones which significantly hinder axial coordination, will cause $E°$ to shift to lower values for copper(III,II) and to higher values for nickel(III,II). In the case of copper, this behavior could be important in biological systems. If a copper complex with peptide nitrogen coordination were located in a hydrophobic protein environment where axial coordination would be difficult, the copper(III) state might be much more easily accessible than has been realized. Indeed, this prediction is supported by our results which show that $E°$ values for the $Cu^{III,II}(H_{-3}G_3a)^{0,1-}$ couple decrease as the concentration of water is lowered by addition of nonaqueous solvents. In the total absence of water, the $E°$ value for $Cu^{III,II}(H_{-2}Aib_3)^{0,1-}$ is 0.12 V vs. NHE, which is 0.54 V lower than in aqueous solution.

**Acknowledgment.** This investigation was supported by Public Health Service Grant No. GM-12152 from the National Institute of General Medical Sciences.

**Registry No.** $G_4$, 637-84-3; $V_4$, 64577-64-6; $G_3a$, 35790-47-7; $G_4a$, 35790-48-8; $Aib_3$, 50348-89-5; $A_3$, 5874-90-8; DGEN, 5663-60-5; Ni, 7440-02-0; Cu, 7440-50-8.

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# Proton-Transfer and Nucleophilic Displacement Reactions of the Triply Deprotonated Tetraglycine Complex of Copper(II)

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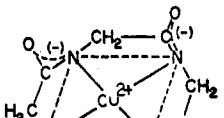
*Received September 18, 1979*

General-acid catalysis in the reaction of $Cu^{II}(H_{-3}G_4)^{2-}$ to form $Cu^{II}(H_{-2}G_4)^-$ indicates direct proton transfer to the terminal deprotonated peptide nitrogen of the triply deprotonated tetraglycine ($G_4$) complex. The rate constants (25.0 °C) increase with acid strength, reaching a value of $1.6 \times 10^8$ $M^{-1}$ $s^{-1}$ for $H_3O^+$. These constants are an order of magnitude greater than for the corresponding reactions with the triglycine complex, $Cu^{II}(H_{-2}G_3)^-$, and the glycylglycylhistidine complex, $Cu^{II}(H_{-2}Ghis)^-$. The $H_2O$ rate constant is 16 $s^{-1}$ for $Cu^{II}(H_{-3}G_4)^{2-}$, compared to a value of 0.12 $s^{-1}$ for the triglycine complex. On the other hand, nucleophilic attack by triethylenetetramine is more than 4 orders of magnitude slower for the tetraglycine complex, with rate constants ($M^{-1}$ $s^{-1}$) for Htrien$^+$ and trien equal to 71 and $4.9 \times 10^2$, respectively. The more protonated forms of trien are also reactive as acids with rate constants ($M^{-1}$ $s^{-1}$) of 62 for $H_2trien^{2+}$ and $3.7 \times 10^2$ for $H_3trien^{3+}$ in their reactions with $Cu^{II}(H_{-3}G_4)^{2-}$.

## Introduction

Copper(II) reacts with tetraglycine ($G_4$) in basic solution to form a complex in which three peptide hydrogens are ionized[1] to give $Cu(H_{-3}G_4)^{2-}$ (structure I). The crystal structure



I

of $Na_2Cu(H_{-3}G_4)\cdot 10H_2O$ has been determined,[2] and all four nitrogen atoms (one amine and three deprotonated peptide nitrogens) are bound to copper in a nearly square-planar arrangement. The carboxylate group is not coordinated. Potentiometric titrations,[1] the infrared spectrum,[3] and the visible spectrum[4] indicate that the same groups are coordinated to the copper(II) in solution. In the present study the kinetics of the reactions of $Cu(H_{-3}G_4)^{2-}$ with acids (eq 1) and with triethylenetetramine (trien) (eq 2) are examined.

$$Cu(H_{-3}G_4)^{2-} + HB \rightarrow Cu(H_{-2}G_4)^- + B^- \quad (1)$$

$$Cu(H_{-3}G_4)^{2-} + trien \rightarrow Cu(trien)^{2+} + G_4^- + 3OH^- \quad (2)$$

Two kinetic pathways have been observed for proton-transfer reactions of metal peptide complexes. The first of these is the outside protonation pathway[5,6] in which rapid protonation of the peptide oxygen occurs. The rapid protonation preequilibrium is followed by breaking of the metal–peptide nitrogen bond as the rate-determining step. The outside protonation pathway is accelerated by hydrogen ion but not by general acids. The second kinetic pathway for proton-transfer reactions of metal peptide complexes is direct, or inside, protonation of the deprotonated peptide nitrogen, which occurs simultaneously with metal–nitrogen bond breaking.[7] This pathway is accelerated by both hydrogen ion and general acids.[6] The outside protonation pathway contributes to the $H_3O^+$ rate constant only when the inside protonation is slow due to slow metal–peptide nitrogen bond rupture[5] or when metal–peptide nitrogen bond rupture is otherwise restricted.[8]

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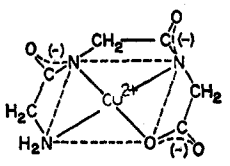
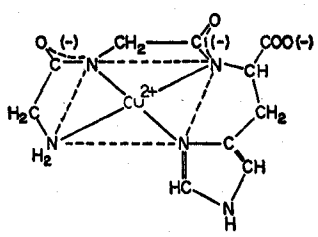
The rate of protonation of $Cu(H_{-3}G_4)^{2-}$ (eq 1) is accelerated by general acids, indicating direct proton transfer to the terminal deprotonated peptide nitrogen. The reactions of $Cu(H_{-3}G_4)^{2-}$ with general acids show a Brønsted dependence characteristic of other metal peptide complexes in which proton transfer to the peptide nitrogen occurs as the metal–nitrogen bond weakens.[7] The subsequent reactions of $Cu(H_{-2}G_4)^{-}$ with acids are slow by comparison to the removal of the third peptide nitrogen from copper(II).

The rates of reaction of acids and solvent with $Cu(H_{-3}G_4)^{2-}$ are much faster than those of the corresponding reactions of either the triglycine complex,[7] $Cu(H_{-2}G_3)^{-}$ (structure II), or the glycylglycylhistidine complex,[8] $Cu(H_{-2}GGhis)^{-}$ (structure III). In contrast to this behavior the relative reactivity of the



II



III

nickel(II) complex of tetraglycine, $Ni(H_{-3}G_4)^{2-}$, with acids is less than that of the triglycine complex, $Ni(H_{-2}G_3)^{-}$. Indeed, the protonation of $Ni(H_{-3}G_4)^{2-}$ occurs almost totally by reaction with $H_3O^+$, and the reaction with general acids is virtually eliminated.[5] The reasons for the above differences as well as other features of the proton-transfer reactions of $Cu(H_{-3}G_4)^{2-}$ are discussed.

The kinetics of the reactions of copper(II) peptide complexes with acids are important not only because there is interest in the proton-transfer mechanisms but also because these reactions are coupled to the electron-transfer reactions of copper(III) peptide complexes. Thus, the rapid acid dissociation of copper(II) peptide complexes makes it possible to study the thermodynamically unfavorable reduction of copper(III) peptide complexes by $IrCl_6^{3-}$ in acidic media.[9] The rapid acid dissociation of the copper(II) complexes pulls the unfavorable redox reaction uphill. Knowing the rate constants for the reaction of $Cu(H_{-3}G_4)^{2-}$ with acids (which are reported in this work), it is possible to evaluate what conditions (pH and concentration of buffer) are required in order to study the electron-transfer reaction between $Cu^{III}(H_{-3}G_4)^{-}$ and $IrCl_6^{3-}$ as well as other reductants. Since $Cu^{III}(H_{-3}G_4)^{-}$ is being used extensively in kinetic studies of redox reactions in this laboratory, knowledge of the kinetics of the proton-transfer reactions of $Cu(H_{-3}G_4)^{2-}$ is important.

A comparison of the kinetics of the reaction of $Cu(H_{-3}G_4)^{2-}$ with trien vs. other copper(II) peptide complexes is helpful in understanding the mechanism of copper(II) transfer from peptide complexes to polydentate ligands. The rate of direct nucleophilic displacement by trien is very much dependent on the peptide bound to copper(II). Thus, $Cu(H_{-3}G_4)^{2-}$ is less reactive by a factor of $10^4$ with unprotonated trien than is $Cu(H_{-2}G_3)^{-}$,[10] but $Cu(H_{-3}G_4)^{2-}$ is $10^3$ times more reactive than is $Cu(H_{-2}GGhis)^{-}$.[8] The reaction of $Cu(H_{-2}GGhis)^{-}$ with trien between pH 6.5 and pH 8.5 has been shown to occur via a proton-assisted mechanism in which proton transfer to a deprotonated peptide nitrogen occurs prior to peptide displacement by $H_2trien^{2+}$.[8] This proton-assisted mechanism was not detected as an effective pathway for the reaction of $Cu(H_{-2}G_3)^{-}$ with trien,[10] nor is it observed for the reaction of $Cu(H_{-3}G_4)^{2-}$ with trien. All trien species (trien, $Htrien^+$, $H_2trien^{2+}$, and $H_3trien^{3+}$) react with $Cu(H_{-3}G_4)^{2-}$ directly without the need for a prior protonation step. Due to the higher susceptibility of $Cu(H_{-3}G_4)^{2-}$ toward acid attack, the relative reactivities of $H_3trien^{3+}$ and $H_2trien^{2+}$ compared to unprotonated trien are much greater for reactions with $Cu(H_{-3}G_4)^{2-}$ than for reactions with $Cu(H_{-2}G_3)^{-}$.

## Experimental Section

**Chemicals.** Tetraglycine was obtained from Biosynthetika and was used without further purification. A 0.353 M stock solution of $Cu(ClO_4)_2$ was prepared from the twice recrystallized salt and standarized by EDTA titration using murexide as an indicator. Triethylenetetramine (trien) was prepared as the free base by reacting the recrystallized disulfate salt with sodium hydroxide. The free base was obtained by vacuum distillation.

**Kinetic Experiments.** Solutions of $1.0 \times 10^{-4}$–$1.5 \times 10^{-3}$ M $Cu(H_{-3}G_4)^{2-}$ were freshly prepared by the reaction of a 2–5% excess of tetraglycine with $Cu(ClO_4)_2$ solution. The violet complex was formed as the pH was adjusted to 10.5 with sodium hydroxide. All solutions were adjusted to 1.0 M total ionic strength with a 5.0 M $NaClO_4$ stock solution.

The kinetics of the reaction of $Cu(H_{-3}G_4)^{2-}$ with acids and with trien were studied under pseudo-first-order conditions. Solutions of acid were always well buffered with tenfold or greater excess of buffer (general acid) over the concentration of $Cu(H_{-3}G_4)^{2-}$. The concentration of trien, which served as a buffer, was also always at least tenfold higher than the $Cu(H_{-3}G_4)^{2-}$ concentration.

Reactions of $Cu(H_{-3}G_4)^{2-}$ with trien were followed at either 260 nm (monitoring the appearance of $Cu(trien)^{2+}$) or 500 nm (monitoring disappearance of $Cu(H_{-3}G_4)^{2-}$). The reactions of $Cu(H_{-3}G_4)^{2-}$ with acids were followed at either 250 or 520 nm (monitoring the disappearance of $Cu(H_{-3}G_4)^{2-}$). The kinetic results were independent of wavelength over the pH range studied. Kinetic data were obtained by using a Durrum stopped-flow spectrophotometer interfaced to a Hewlett-Packard 2115A general-purpose computer.[11] In all cases excellent first-order traces were obtained.

**Measurement of Hydrogen Ion Concentration.** A perchloric acid–sodium hydroxide titration in 1.0 M $NaClO_4$ was used to calibrate the hydrogen ion concentration: $-\log [H^+] = pH + 0.30$. The correction of 0.30 is an empirical one which is affected predominantly by liquid junction potentials in the cell used to measure pH. The pH of solutions were determined with the use of a saturated sodium chloride calomel reference electrode.

## Results

**Reaction of $Cu(H_{-3}G_4)^{2-}$ with Acids.** For the reaction of $Cu(H_{-3}G_4)^{2-}$ with acids to form $Cu(H_{-2}G_4)^{-}$ (eq 1), first-order kinetics were observed according to the rate law in eq 3. From

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

studies of the reaction of $Cu(H_{-2}G_4)^{-}$ with hydroxide the triply deprotonated copper tetraglycine complex has been shown to be a mixture of 88% $Cu(H_{-3}G_4)^{2-}$ and 12% $Cu(H_{-2}G_4)OH^{2-}$.[12] The latter species is expected to react much more rapidly with acids to form $Cu(H_{-2}G_4)^{-}$ since it involves the protonation of a coordinated hydroxide ion. For the reaction with trien at pH 10.8, a rapid absorbance change corresponding to the displacement of $G_4$ from $Cu(H_{-2}G_4)OH^{2-}$ prior to the slower displacement from $Cu(H_{-3}G_4)^{2-}$ was detected. However, no rapid absorbance change corresponding to the protonation of $Cu(H_{-2}G_4)OH^{2-}$ was detected at the wavelengths used to monitor eq 1, presumably due to the similar absorption spectra of $Cu(H_{-2}G_4)^{-}$ and $Cu(H_{-2}G_4)OH^{2-}$.

At pH values of 7.0 or less a second, slower reaction due to the protonation of $Cu(H_{-2}G_4)^{-}$ to form $Cu(H_{-1}G_4)$ was observed.[12] This subsequent reaction was sufficiently slow that it did not interfere with kinetic analysis of the reaction of interest.

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**Table I.** Observed First-Order Rate Constants for the Reaction of $Cu(H_{-3}G_4)^{2-}$ with Acids, HB[a]

| HB | pH | $[HB]_T$, M | $k_{obsd}$, $s^{-1}$ |
|---|---|---|---|
| chloroacetic acid | 7.56[b] | 0.012 | 17.8 ± 0.1 |
| | | 0.036 | 18.6 ± 0.1 |
| | | 0.072 | 20.2 ± 0.1 |
| | | 0.100 | 20.9 ± 0.1 |
| acetic acid | 8.00[c] | 0.010 | 17.0 ± 0.7 |
| | | 0.030 | 18.5 ± 0.5 |
| | | 0.070 | 22.0 ± 0.2 |
| | | 0.100 | 24.4 ± 0.2 |
| $NH_3OH^+$ | 6.22[d] | 0.0020 | 84 ± 1 |
| | | 0.0060 | 98 ± 1 |
| | | 0.010 | 110 ± 1 |
| | | 0.020 | 159 ± 1 |
| | | 0.030 | 197 ± 6 |
| MES[e] | 6.26 | 0.065 | 64.6 ± 0.2 |
| | | 0.109 | 71 ± 1 |
| | | 0.153 | 77 ± 1 |
| | | 0.236 | 86 ± 2 |
| trien[f] | 7.53 | 0.054 | 22.7 ± 0.3 |
| | | 0.090 | 26.7 ± 0.5 |
| | | 0.126 | 30.5 ± 0.8 |
| | 7.15 | 0.010 | 23.0 ± 0.3 |
| | | 0.030 | 26.9 ± 0.3 |
| | | 0.050 | 29.7 ± 0.6 |
| | | 0.070 | 34.1 ± 0.3 |
| | | 0.100 | 39.2 ± 0.4 |
| | | 0.150 | 48.9 ± 0.9 |
| | 6.68 | 0.054 | 45.5 ± 0.2 |
| | | 0.090 | 56.1 ± 0.1 |
| | | 0.126 | 63.9 ± 0.2 |
| | | 0.180 | 75.0 ± 1.0 |
| $H_2en^{2+}$ | 7.00[g] | 0.029 | 27.6 ± 0.2 |
| | | 0.040 | 31.6 ± 0.5 |
| | | 0.058 | 36.8 ± 0.5 |
| | | 0.087 | 44.0 ± 1.0 |
| HEPES[e] | 7.60 | 0.066 | 18.5 ± 0.5 |
| | | 0.092 | 19.0 ± 0.1 |
| | | 0.118 | 19.5 ± 0.1 |
| | | 0.158 | 20.8 ± 0.5 |
| | | 0.197 | 21.3 ± 0.2 |
| $H_3O^+$ [h] | 7.19 | $3.2 \times 10^{-8}$ | 20.9 ± 0.3 |
| | 6.86 | $6.9 \times 10^{-8}$ | 28.4 ± 0.7 |
| | 6.58 | $13.2 \times 10^{-8}$ | 40.1 ± 0.6 |
| | 6.38 | $20.9 \times 10^{-8}$ | 49.6 ± 0.9 |
| | 6.28 | $26.3 \times 10^{-8}$ | 62.2 ± 2.0 |
| | 6.18 | $33.1 \times 10^{-8}$ | 67.0 ± 1.0 |

[a] $\mu$ = 1.0 M ($NaClO_4$); $T$ = 25 °C; $Cu(H_{-3}G_4)^{2-}$ initially at pH 10.5 unbuffered. [b] $[HEPES]_T$ = 0.10 M. [c] $[HEPES]_T$ = 0.050 M. [d] $[MES]_T$ = 0.14 M. [e] The good buffers[22] are MES = 2-morpholinoethanesulfonic acid, PIPES = piperazine-*N*,*N*′-bis(2-ethanesulfonic acid), and HEPES = *N*-(2-hydroxyethyl)piperazine-*N*′-2-ethanesulfonic acid. [f] Triethylenetetramine. [g] Ethylenediamine. [h] $[H(PIPES)^+]$ = 0.012 M; protonated form of buffer held constant as pH was varied.

The value of $k_{obsd}$ depends on the pH as well as the buffer, or general-acid, concentration according to eq 4 where [HB] represents the concentration of general acid. The kinetic results of the reaction of $Cu(H_{-3}G_4)^{2-}$ with acids are given in Table I. The value of $k_H$, $(1.6 \pm 0.1) \times 10^8$ $M^{-1}$ $s^{-1}$, the rate constant for protonation of $Cu(H_{-3}G_4)^{2-}$ by hydrogen ion, was determined from the slope of a linear plot of $k_{obsd}$ vs. $H_3O^+$ concentration (Figure 1). Plots of $k_{obsd}$ vs. total concentration of HB ($[HB]_T = [HB] + [B]$) were also linear, and the slopes were used to evaluate $k_{HB}$ for each general acid according to eq 5. Figure 1 shows the dependence upon acetic acid concentration at pH 8.00. The rate constant $k_{H_2O}$ is the contribution to $k_{obsd}$ which is independent of pH and independent of general-acid concentration. Table II summarizes the rate constants for protonation of $Cu(H_{-3}G_4)^{2-}$.

$$k_{obsd} = k_H[H^+] + k_{HB}[HB] + k_{H_2O} \quad (4)$$

$$k_{HB} = \left(\frac{K_a + [H^+]}{[H^+]}\right)(\text{slope}) \quad (5)$$

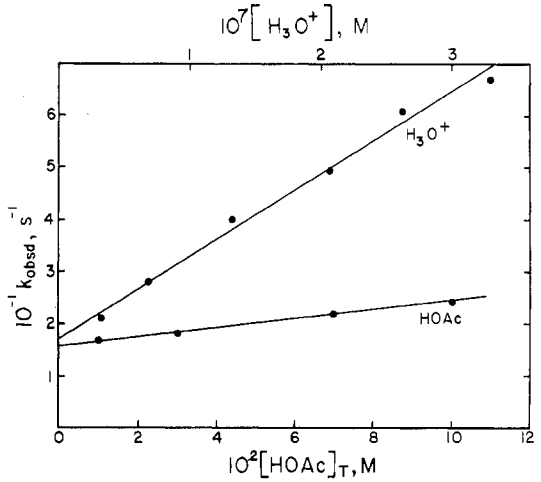


**Figure 1.** Dependence of the rate constants of protonation of $Cu(H_{-3}G_4)^{2-}$ upon $[H_3O^+]$ (PIPES buffer; $[H(PIPES)^+]$ = constant = 0.012 M) and upon [acetic acid] (pH 8.00; [HEPES buffer] = 0.050 M) at 25 °C and 1.0 M $NaClO_4$.

**Table II.** Rate Constants for the Proton-Transfer Reactions of $Cu(H_{-3}G_4)^{2-}$

| HB | $pK_a$ | $k_{HB}$, $M^{-1}$ $s^{-1}$ |
|---|---|---|
| $H_3O^+$ | −1.75 | $(1.6 \pm 0.1) \times 10^8$ |
| chloroacetic acid | 2.86[b] | $(3.6 \pm 0.2) \times 10^6$ |
| acetic acid | 4.64[c] | $(3.8 \pm 0.05) \times 10^5$ |
| $NH_3OH^+$ | 5.98[d] | $(1.8 \pm 0.1) \times 10^4$ |
| MES | 6.27[e] | $(3.7 \pm 0.3) \times 10^2$ |
| $H_3trien^{3+}$ | 7.13[f] | $(3.7 \pm 0.5) \times 10^2$ |
| $H_2en^{2+}$ | 7.48[g] | $(4.7 \pm 0.2) \times 10^2$ |
| HEPES | 7.56[e] | $(9.6 \pm 0.5) \times 10$ |
| $H_2O$[a] | 15.53[a] | $(2.9 \pm 0.2) \times 10^{-1}$ |

[a] $pK_a$(for $H_3O^+$) = −log 55.5. $pK_a$(for $H_2O$) = $pK_w$ + log 55.5. The $k_{HB}$ for $H_2O$ is $k_{H_2O}$(16 ± 1 $s^{-1}$) divided by 55.5 to give the corresponding second-order rate constant. [b] Day, R. A.; Stoughton, R. W. *J. Am. Chem. Soc.* **1950**, *72*, 5662. [c] Feldman, I.; Koval, T. *Inorg. Chem.* **1963**, *2*, 145. [d] Hagisawa, H. *Rikagaku Kenkyusho Iho* **1941**, *20*, 251. [e] Vega, C. A.; Bates, R. G. *Anal. Chem.* **1976**, *48*, 1293. [f] See ref 13. [g] Nasanen, R.; Merilainen, P. *Acta Chem. Scand.* **1964**, *18*, 1337.

**Reaction of $Cu(H_{-3}G_4)^{2-}$ with trien.** The reaction of $Cu(H_{-3}G_4)^{2-}$ with trien to form $Cu(trien)^{2+}$ (eq 2) followed the rate law in eq 3. When the appearance of $Cu(trien)^{2+}$ was monitored at 260 nm, the observed initial absorbances for the reaction were higher than predicted from the absorbances of the reactants. This observation is consistent with an equilibrium between the two forms of triply deprotonated copper tetraglycine, $Cu(H_{-3}G_4)^{2-}$ (88%) and $Cu(H_{-2}G_4)OH^{2-}$ (12%).[12] The latter species apparently reacts rapidly with trien, causing an initial absorbance increase. When the reaction is monitored at 500 nm (disappearance of $Cu(H_{-3}G_4)^{2-}$), the absorbance change due to the initial rapid reaction is too small to be observed.

The dependence of $k_{obsd}$ upon trien concentration was linear and is represented by eq 6 where $k_{trien}$ is dependent on pH and

$$k_{obsd} = k_{trien}[trien]_T + k_H[H^+] + k_{H_2O} \quad (6)$$

$[trien]_T$ is the total concentration of all trien species. The observed first-order rate constants for the trien reactions are presented in Table I (pH <8.0) and Table III (pH >8.0). At pH 10.8, where $k_H[H^+]$ is negligible, the trien dependence plot yields a value of 16 ± 1 $s^{-1}$ for $k_{H_2O}$.

Using the known values of $k_H$ and $k_{H_2O}$ ($1.6 \times 10^8$ $M^{-1}$ $s^{-1}$ and 16 $s^{-1}$, respectively), it is possible to obtain the dependence

**Table III.** First-Order Observed Rate Constants for Nucleophilic Attack by trien on $Cu(H_{-3}G_4)^{2-}$

| pH | $10^3 \times$ [trien]$_T$, M | $k_{obsd}$, $s^{-1}$ | pH | $10^3 \times$ [trien]$_T$, M | $k_{obsd}$, $s^{-1}$ |
|---|---|---|---|---|---|
| 11.0 | 60.0 | 43.7 ± 0.1 | 10.16 | 60.0 | 37.6 ± 0.4 |
| 10.8 | 3.04 | 18.2 ± 0.5 | 9.90 | 30.6 | 25.9 ± 0.7 |
| 10.8 | 9.15 | 21.3 ± 0.2 | 9.84 | 60.0 | 34.0 ± 0.4 |
| 10.8 | 18.3 | 26.1 ± 0.8 | 9.62 | 60.0 | 29.8 ± 0.4 |
| 10.8 | 27.4 | 30 ± 1 | 9.50 | 30.6 | 21 ± 1 |
| 10.8 | 36.6 | 34 ± 1 | 9.48 | 60.0 | 27.9 ± 0.4 |
| 10.8 | 52.0 | 41.6 ± 0.9 | 9.15 | 60.0 | 24.1 ± 0.4 |
| 10.4 | 1.22 | 16.3 ± 0.4 | 9.00 | 60.0 | 22.1 ± 0.5 |
| 10.4 | 10.2 | 19.6 ± 0.8 | 8.90 | 30.6 | 19.6 ± 0.5 |
| 10.4 | 30.6 | 27.1 ± 0.1 | 8.51 | 60.0 | 20.0 ± 0.1 |
| 10.4 | 51.0 | 35 ± 2 | 8.40 | 30.6 | 18.4 ± 0.1 |
| 10.4 | 60.0 | 39.1 ± 0.3 | | | |

**Table IV.** Resolved Rate Constants for the Reaction of $Cu(H_{-3}G_4)^{2-}$ with trien species

| trien species | $k$, $M^{-1}$ $s^{-1}$ | reactivity |
|---|---|---|
| trien | $(4.9 \pm 0.1) \times 10^2$ | nucleophile |
| Htrien$^+$ | $(7.1 \pm 2.7) \times 10$ | nucleophile |
| $H_2$trien$^{2+}$ | $(6.2 \pm 2.3) \times 10$ | coordinating general acid |
| $H_3$trien$^{3+}$ | $(3.7 \pm 0.5) \times 10^2$ | general acid |

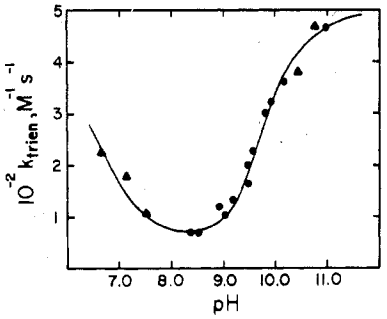


**Figure 2.** pH profile for the rate of reaction of trien with $Cu(H_{-3}G_4)^{2-}$ at 25 °C and 1.0 M $NaClO_4$. The second-order rate constant, $k_{trien}$, is defined by eq 6. Points at pH 10.8, 10.4, 7.53, 7.15, and 6.68 (▲) were determined from plots of $k_{obsd}$ vs. [trien]. The remaining points (●) were calculated from $k_{obsd}$ at single trien concentrations by using eq 6 where $k_H = 1.6 \times 10^8$ $M^{-1}$ $s^{-1}$ and $k_{H_2O} = 16$ $s^{-1}$. The calculated curve was fitted by using eq 7.

of $k_{trien}$ on pH. The values of $k_{trien}$ as a function of pH from 6.5 to 11.0 are shown in Figure 2. The distribution of trien species leads to an expression of $k_{trien}$ given by eq 7, where $K_{HT}$

$$k_{trien} = \{k_T K_{HT} K_{H_2T} K_{H_3T} + k_{HT} K_{H_2T} K_{H_3T}[H^+] + k_{H_2T} K_{H_3T}[H^+]^2 + k_{H_3T}[H^+]^3\} / \{K_{HT} K_{H_2T} K_{H_3T} + K_{H_2T} K_{H_3T}[H^+] + K_{H_3T}[H^+]^2 + [H^+]^3\} \quad (7)$$

($10^{-10.02}$), $K_{H_2T}$ ($10^{-9.39}$), and $K_{H_3T}$ ($10^{-7.00}$) are the acid dissociation constants for Htrien$^+$, $H_2$trien$^{2+}$, and $H_3$trien$^{3+}$, respectively,[13] and $k_T$, $k_{HT}$, $k_{H_2T}$, and $k_{H_3T}$ are the second-order rate constants for reaction of the various trien species with $Cu(H_{-3}G_4)^{2-}$. Below pH 8.0, $k_{trien}$ increases with decreasing pH. In this pH region the expression for $k_{trien}$ reduces to eq 8. From trien dependences at pH 7.53, 7.15, and 6.98 (shown

$$k_{trien} = \frac{k_{H_2T} K_{H_3T} + k_{H_3T}[H^+]}{K_{H_3T} + [H^+]} \quad (8)$$

in Figure 3) the value of $k_{H_3T}$ was resolved and found to be $(3.7 \pm 0.5) \times 10^2$ $M^{-1}$ $s^{-1}$. Above pH 8.0 where the concentration of $H_3$trien$^{3+}$ is small, the value of $k_{trien}$ increases with

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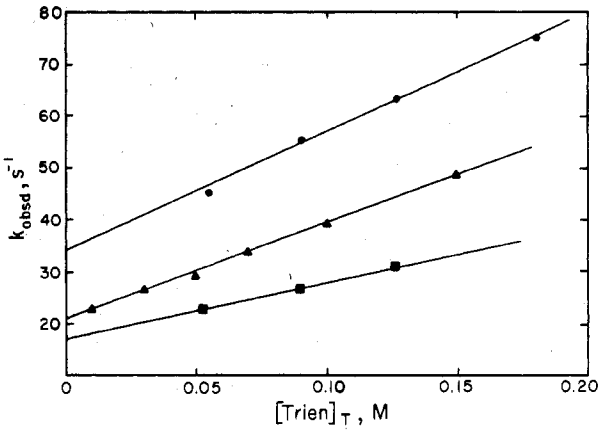


**Figure 3.** Dependence of $k_{obsd}$ for the reaction of trien with $Cu(H_{-3}G_4)^{2-}$ ($T = 25$ °C; $\mu = 1.0$ M ($NaClO_4$)) at pH 6.68 (●), 7.15 (▲), and 7.53 (■).

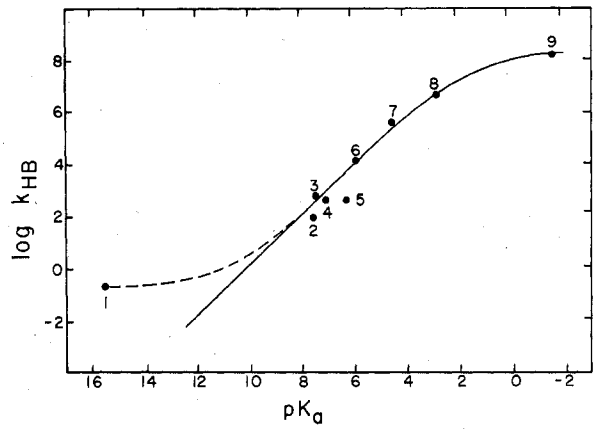


**Figure 4.** Brønsted plot for the reaction of $Cu(H_{-3}G_4)^{2-}$ with acids, HB. The acids are (1) $H_2O$, (2) HEPES, (3) $H_2en^{2+}$, (4) $H_3trien^{3+}$, (5) MES, (6) $NH_3OH^+$, (7) acetic acid, (8) chloroacetic acid, and (9) $H_3O^+$.

increasing pH. Nonlinear-regression analysis was used to fit the experimental values of $k_{trien}$ at pH greater than 8.0 to eq 7, by using the value of $k_{H_3T}$ determined at lower pH as a known constant. The results of this analysis gave rate constants of $(4.9 \pm 0.1) \times 10^2$, $(7.1 \pm 2.7) \times 10$, and $(6.2 \pm 2.3) \times 10$ $M^{-1}$ $s^{-1}$ for $k_T$, $k_{HT}$, and $k_{H_2T}$, respectively. The solid line in Figure 2 is calculated from eq 6 and 7 by using the values of $k_T$, $k_{HT}$, $k_{H_2T}$, and $k_{H_3T}$ given in Table IV.

## Discussion

**Mechanism of Proton Transfer for $Cu(H_{-3}G_4)^{2-}$.** The rate of protonation of $Cu(H_{-3}G_4)^{2-}$ is accelerated by general acids, and the magnitude of second-order rate constants $k_{HB}$ increases with the acid strength of HB. Figure 4 shows the plot of log $k_{HB}$ as a function of p$K_a$ for the general acids used in this study. The slope of the linear portion of this Brønsted plot corresponds to an $\alpha$ coefficient of unity. The shape of the Brønsted plot is very similar to those observed for other metal peptide complexes exhibiting general-acid catalysis including $Cu(H_{-2}G_3)^-$,[7] $Cu(H_{-2}GGhis)^-$,[8] and $Ni(H_{-2}G_3)^-$.[7] The occurrence of general-acid catalysis indicates that proton transfer occurs during the rate-determining step of the reaction. Hence, the reaction of $Cu(H_{-3}G_4)^{2-}$ with acids to form $Cu(H_{-2}G_4)^-$ occurs by direct protonation of the terminal deprotonated peptide nitrogen according to eq 9 and 10.

$$Cu(H_{-3}G_4)^{2-} + HB \xrightarrow{k_{HB}} Cu(H_{-2}G_4)^- + B^- \quad (9)$$

$$Cu(H_{-3}G_4)^{2-} + H_2O \xrightarrow{k_{H_2O}} Cu(H_{-2}G_4)^- + OH^- \quad (10)$$

**Table V.** Rate Constants for the Reaction of Copper(II) Peptide Complexes with $H_3O^+$, $CH_3COOH$, and trien

| peptide complex | rate constant, $M^{-1}$ $s^{-1}$ | | |
|---|---|---|---|
| | $H_3O^+$ | $CH_3COOH$ | trien (T) |
| $Cu(H_{-2}G_3)^-$ | $1.3 \times 10^7$ | $3.4 \times 10^4$ | $1.1 \times 10^7$ (T)<br>$5.1 \times 10^6$ ($HT^+$)<br>$1.2 \times 10^5$ ($H_2T^{2+}$) |
| $Cu(H_{-2}GGhis)^-$ | $1.4 \times 10^7$ | $6.3 \times 10^3$ | 0.5 (T) |
| $Cu(H_{-3}G_4)^{2-}$ | $1.6 \times 10^8$ | $3.8 \times 10^5$ | $4.9 \times 10^2$ (T) |

One of the characteristic properties of proton-transfer reactions of metal peptide complexes is the S-shaped Brønsted curve where the limiting rate constants for strong acids level off well below the diffusion-controlled rate constant and where the $H_2O$ value is larger than expected for its $pK_a$. The large value of $k_{H_2O}$ ($16 \pm 1$ $s^{-1}$) was previously attributed to nucleophilic attack by $H_2O$. However studies in $D_2O$ have shown that the reaction of solvent with $Cu(H_{-3}G_4)^{2-}$ exhibits a normal isotope effect.[14] Therefore the solvent pathway appears to be a proton-transfer step as shown in eq 10. Apparently $H_2O$ is a very effective acid because it can coordinate to the metal in an axial position while simultaneously transferring a proton to the terminal deprotonated peptide nitrogen. The value of $k_{H_2O}$ for $Cu(H_{-3}G_4)^{2-}$ is much larger than the $H_2O$ rate constant for $Cu(H_{-2}G_3)^-$ (0.12 $s^{-1}$), indicating that $Cu(H_{-3}G_4)^{2-}$ is much more susceptible to acid attack than is the triglycine complex.

The rates for general-acid catalysis of $Cu(H_{-3}G_4)^{2-}$ are approximately 10 times larger than those for copper complexes of glycylglycyl-L-histidine, $Cu(H_{-2}GGhis)^-$,[8] and triglycine, $Cu(H_{-2}G_3)^-$,[7] as seen in Table V. The presence of the deprotonated peptide nitrogen in the fourth coordination site in $Cu(H_{-3}G_4)^{2-}$ suggests three explanations for the higher reactivity of $Cu(H_{-3}G_4)^{2-}$ with acids: (1) relatively free readjustment of the metal–peptide nitrogen bond length during proton transfer for $Cu(H_{-3}G_4)^{2-}$, (2) strain in the terminal chelate ring, and (3) trans effect. These three factors are discussed below.

As protonation of a peptide nitrogen proceeds, the readjustment of the copper–nitrogen bond length is restricted in the cases of $Cu(H_{-2}G_3)^-$ and $Cu(H_{-2}GGhis)^-$ by chelate rings formed by the carboxylate and imidazole groups, respectively. This readjustment in the case of the terminal peptide nitrogen in $Cu(H_{-3}G_4)^{2-}$ is relatively unhindered. The strong chelation provided by binding of the imidazole group in $Cu(H_{-2}GGhis)^-$ in the terminal position effectively inhibits bond rupture and precludes the direct protonation pathway unless sufficient concentration of trien is present to displace the imidazole group.[8]

A second possible explanation for the relative rapidity of protonation of $Cu(H_{-3}G_4)^{2-}$ is suggested by examination of the crystal structure of $Na_2Cu(H_{-3}G_4)\cdot 10H_2O$ which shows the metal–peptide bond distance to be longer for the terminal peptide nitrogen than for either of the nonterminal ones.[2] This greater bond length probably reflects the increased strain introduced by the closing of the third five-membered chelate ring. The ring strain can enhance the rate of proton transfer by facilitating the stretching of the metal–nitrogen bond as proton transfer takes place.

A third possibility for rationalizing the greater proton-transfer reactivity of $Cu(H_{-3}G_4)^{2-}$ compared to those of $Cu(H_{-2}G_3)^-$ and $Cu(H_{-2}GGhis)^-$ is that the bonding of copper to the terminal deprotonated peptide nitrogen in $Cu(H_{-3}G_4)^{2-}$ is weakened by the presence of another peptide group coordinated to copper in the trans position.[15] Neither $Cu(H_{-2}G_3)^-$ nor $Cu(H_{-2}GGhis)^-$ has trans peptide nitrogens.

For proton-transfer reactions, hydrogen bonding, steric factors, and solvent reorganization and electronic rearrangements during proton transfer are important in determining the rate of general-acid catalysis.[7] The general shape of the Brønsted plot for $Cu(H_{-3}G_4)^{2-}$ (Figure 4) is remarkably similar to those observed for other copper and nickel peptide complexes.[6,7] The Brønsted plots for proton-transfer reactions of these metal peptide complexes resemble those of normal proton-transfer reactions[16] in that they have slopes of 1.0 which abruptly change to zero at high acid strength. However, unlike those for normal proton-transfer reactions, the limiting rate constant at high acid strength is below the diffusion-controlled value. The results of analysis of the proton-transfer reactions of metal peptide complexes using the Marcus theory[17,18] indicate that the limiting rate constants reflect the work required for reorganization during proton transfer which is independent of the acidity of both the metal complex and general acid used.[7] The work term ($W_R$) is of the order $Ni(H_{-2}G_3)^-$ (10.5 kcal $mol^{-1}$) > $Cu(H_{-2}G_3)^-$ (7.3 kcal $mol^{-1}$) > $Cu(H_{-3}G_4)^{2-}$ (6.3 kcal $mol^{-1}$). The work terms for these complexes are associated with the solvation-assisted bond-weakening during proton transfer and are a function of metal–nitrogen bond strength.[19] The higher work term for $Ni(H_{-2}G_3)^-$ compared to those for the copper complexes reflects the greater metal–nitrogen bond strength and the difficulty of axial solvation of the square-planar nickel complex. The smaller difference between $Cu(H_{-2}G_3)^-$ and $Cu(H_{-3}G_4)^{2-}$ is due to the presence of the peptide nitrogen in the fourth coordination site in $Cu(H_{-3}G_4)^{2-}$ as discussed earlier.

In this work it is seen that for copper(II) complexes, the change from triglycine to tetraglycine causes an enhancement of reactivity with acids. However, for nickel(II) complexes, the same change of ligand causes a drastic reduction in the rate of reaction with general acids.[6,7] This apparent paradox can be explained by the relative importance of ligand field stabilization for nickel(II) peptide complexes compared to that for copper(II) peptide complexes. The deprotonated peptide group is a much stronger ligand field donor than is the carboxylate group. Thus, the ligand field stabilization increases as the ligand is changed from triglycine to tetraglycine. For the nickel(II) peptides, which are square-planar $d^8$ complexes, the increased metal–peptide nitrogen bond strength in $Ni(H_{-3}G_4)^{2-}$ compared to that in $Ni(H_{-2}G_3)^-$ causes the protonation of the former complex to occur via the outside protonation mechanism in which metal–nitrogen bond rupture rather than proton transfer is rate limiting.[5] This is not the case for the copper(II) complexes where the $d^9$ system gives only half of the crystal field stabilization energy of the $d^8$ system. Hence, factors such as the absence of protective carboxylate chelation, trans effect, and strain in the third chelate ring, which make $Cu(H_{-3}G_4)^{2-}$ more reactive than $Cu(H_{-2}G_3)^-$, are overcome in the nickel(II) case by the increased ligand field stabilization of $Ni(H_{-3}G_4)^{2-}$ vs. $Ni(H_{-2}G_3)^-$.

**Mechanism of the Reaction of trien with $Cu(H_{-3}G_4)^{2-}$.** For nucleophilic displacement reactions, the reactivity of trien should decrease with increasing degree of protonation. However for the reactions of $Cu(H_{-3}G_4)^{2-}$, the order of reactivity is trien > Htrien$^+$ ~ $H_2$trien$^{2+}$ < $H_3$trien$^{3+}$ (Table IV). This trend suggests that $H_2$trien$^{2+}$ and $H_3$trien$^{3+}$ are reacting with $Cu(H_{-3}G_4)^{2-}$ as acids. The reactivity of $H_2$trien$^{2+}$, like that of $H_2O$, is greater than predicted from its

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acid strength because it has the ability to coordinate to copper(II) axially while simultaneously transferring a proton. Other coordinating acids such as $HCO_3^-$, $H_2PO_4^-$, and $HC_2O_4^-$ have been shown to behave similarly in their reactions with metal peptide complexes.[5,20,21] The coordinating ability of $H_3trien^{3+}$ is apparently low enough that its reactivity is appropriate for its acid strength and it falls on the Brønsted plot (Figure 4).

The reactions of trien with $Cu(H_{-2}G_3)^-$ and $Cu(H_{-2}GGhis)^-$ have been studied previously, and the rate constant for nucleophilic displacement is very dependent on the peptide ligand. The rate constants for reaction with unprotonated trien are $1.1 \times 10^7$ and $0.50\ M^{-1}\ s^{-1}$, respectively, for $Cu(H_{-2}G_3)^{-10}$ and $Cu(H_{-2}GGhis)^-$.[8] This comparison suggests that in nucleophilic displacement reactions of trien with copper(II) peptide complexes it is important that the nucleophile obtain a foothold in an equatorial position. The rate constant for the nucleophilic displacement reaction of trien with $Cu(H_{-3}G_4)^{2-}$ is $4.9 \times 10^2\ M^{-1}\ s^{-1}$, more than $10^4$ times slower than with $Cu(H_{-2}G_3)^-$ but nearly $10^3$ times faster than with $Cu(H_{-2}GGhis)^-$. This indicates that a deprotonated peptide is much more difficult to displace than is a carboxylate group but considerably less difficult to displace than is the imidazole group.

The trends in reactivity of trien species for $Cu(H_{-3}G_4)^{2-}$ and $Cu(H_{-2}G_3)^-$ are included in Table V. For $Cu(H_{-2}G_3)^-$, the rate constant for $H_2trien^{2+}$ is approximately 100 times less than that of unprotonated trien,[10] while, for $Cu(H_{-3}G_4)^{2-}$, $H_2trien^{2+}$ is only 8 times less reactive than the unprotonated nucleophile. This suggests that $H_2trien^{2+}$ reacts as a nucleophile with $Cu(H_{-2}G_3)^-$ but as a coordinating acid with $Cu(H_{-3}G_4)^{2-}$. This difference is due to the fact that $Cu(H_{-3}G_4)^{2-}$ is more reactive than $Cu(H_{-2}G_3)^-$ toward proton transfer but much less reactive toward nucleophilic displacement.

Further comparison of the reactions of trien with $Cu(H_{-3}G_4)^{2-}$ with $Cu(H_{-2}GGhis)^-$ illustrates that the presence of histidine as the third amino acid residue in a given peptide has a significant effect on the mechanism by which $Cu^{2+}$ is transferred from a peptide complex to trien. The nucleophilic displacement of the peptide ligand from $Cu(H_{-2}GGhis)^-$ is very ineffective unless assisted by protonation forming $Cu(H_{-1}GGhis)^-$ (eq 11, 12).[8] Thus, $H_2trien^{2+}$, the predominant

$$Cu(H_{-2}GGhis)^- + H^+ \underset{}{\overset{k_1}{\rightleftharpoons}} Cu(H_{-1}GGhis) \quad (11)$$

$$Cu(H_{-1}GGhis) + H_2trien^{2+} \xrightarrow{k_2} Cu(trien)^{2+} + GGhis + H^+ \quad (12)$$

trien species in solution at pH values where $Cu(H_{-1}GGhis)$ is formed, can effectively displace $GGhis^-$ from $Cu(H_{-1}GGhis)$, while unprotonated trien is relatively ineffective in displacing $GGhis^-$ from $Cu(H_{-2}GGhis)^-$. Evidence for this proton-assisted nucleophilic displacement mechanism is provided by a trien dependence at pH 6.9 in which a limiting rate constant corresponding to $k_1[H^+]$ (eq 11) is reached at concentrations of nucleophile greater than 0.07 M. General-acid catalysis is observed only at trien concentration greater than 0.07 M, indicating a shift in the rate-determining step from eq 12 to eq 11.[8] In contrast to the case of $Cu(H_{-2}GGhis)^-$, the reaction of $Cu(H_{-3}G_4)^{2-}$ with trien does not reach a limiting rate at high trien concentration (up to 0.180 M) at pH 6.68, 7.15, and 7.53 (Figure 4). Furthermore, general-acid catalysis of the protonation of $Cu(H_{-3}G_4)^{2-}$ is observed even in the absence of trien. These results indicate that the proton-assisted nucleophilic mechanism is not required in the rate-determining step for the displacement of $G_4^-$ from $Cu(H_{-3}G_4)^{2-}$ by trien. For $Cu(H_{-3}G_4)^{2-}$ the reaction with trien from pH 6.7 to pH 7.5 follows the mechanism in eq 13 and 14 (HB represents

$$Cu(H_{-3}G_4)^{2-} + HB \xrightarrow{k_{HB}} Cu(H_{-2}G_4)^- + B^- \quad (13)$$

$$Cu(H_{-2}G_4)^- + H_2trien^{2+} \xrightarrow{fast} Cu(trien)^{2+} + G_4^- \quad (14)$$

$H_3O^+$, $H_2O$, and $H_3trien^{3+}$ or $H_2trien^{2+}$) in which proton transfer is rate determining regardless of trien concentration. The presence of histidine in the third peptide amino acid residue causes the transfer of $Cu^{2+}$ from the peptide to trien to proceed by a pathway not required for complexes in which $Cu^{2+}$ is bound only by the peptide backbone.

## Conclusions

The kinetics of the reaction of $Cu(H_{-3}G_4)^{2-}$ with acids show that proton transfer to the third deprotonated peptide nitrogen (coordinated in the terminal site of copper(II) peptide complex) is more rapid than proton transfer to $Cu(H_{-2}G_3)^-$, $Cu(H_{-2}GGhis)^-$, or $Cu(H_{-2}G_4)^-$. The greater reactivity of $Cu(H_{-3}G_4)^{2-}$ is caused by a combination of three effects: (1) relatively free readjustment of the copper–peptide nitrogen bond length during proton transfer due to the absence of additional chelation protection; (2) increased strain in the third five-membered chelate ring compared to that of the first and second chelate rings; (3) the presence of the deprotonated peptide nitrogen trans to the terminal one. The high reactivity of $Cu(H_{-3}G_4)^{2-}$ with acids is particularly obvious when the attacking acid is $H_2O$. In this case axial coordination by solvent makes the proton transfer much more effective than would be expected on the basis of the acid strength of $H_2O$.

The kinetic study of the reaction of $Cu(H_{-3}G_4)^{2-}$ with trien shows that, unlike proton-transfer reactions, nucleophilic displacement reactions are not particularly rapid when the terminal coordination site is occupied by a deprotonated peptide nitrogen. The reaction of $Cu(H_{-3}G_4)^-$ with unprotonated trien is more than $10^4$ times slower than the corresponding reaction of $Cu(H_{-2}G_3)^-$ but $10^3$ times faster than that of $Cu(H_{-2}GGhis)^-$. Hence the nature of the group in the terminal coordination site of the copper(II) complexes drastically affects the rate of nucleophilic displacement. The greater reactivity of trien with $Cu(H_{-3}G_4)^{2-}$ vs. $Cu(H_{-2}GGhis)^-$ makes the proton-assisted nucleophilic pathway, by which displacement of $GGhis^-$ from $Cu(H_{-2}GGhis)^-$ proceeds, unnecessary. trien species remain quite reactive with $Cu(H_{-3}G_4)^{2-}$ despite increased degree of protonation at lower pH. This is due mainly to the ability of $H_3trien^{3+}$ to behave as an effective acid in its reaction with $Cu(H_{-3}G_4)^{2-}$.

**Acknowledgment.** This investigation was supported by Public Health Service Grant No. GM-12152 from the National Institute of General Medical Sciences.

**Registry No.** I, 57603-18-6; II, 34803-37-7; III, 53554-01-1; chloroacetic acid, 79-11-8; acetic acid, 64-19-7; $NH_3OH^+$, 20712-83-8; MES, 4432-31-9; trien, 112-24-3; $H_2en^{2+}$, 22534-20-9; HEPES, 7365-45-9; $H_3O^+$, 13968-08-6; $H_3trien^{3+}$, 71890-22-7; $Htrien^+$, 71890-10-3; $H_2trien^{2+}$, 64934-07-2.

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