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Reactions of Copper(III) Tetraglycine

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The tetraglycine complex of trivalent copper, $Cu^{III}(H_{-3}G_4)^-$, is moderately stable in neutral solution with a redox decomposition half-life of 5.5 h at 25.0 °C. This decomposition is both acid catalyzed and base catalyzed but remains first order in Cu(III) from pH 0.3 (k_{obsd} = 0.0136 s^{-1}) to pH 13.5 (k_{obsd} = 0.0222 s^{-1}). In acid two protonated intermediates, $Cu^{III}(H_{-3}G_4)H$ and $Cu^{III}(H_{-3}G_4)H_2^+$, form rapidly and the latter complex rearranges with a rate constant of 0.3 s^{-1}, producing the $Cu^{III}(H_{-2}G_4)H^+$ species in which one Cu(III)–N(peptide) bond has been broken. These reactions precede redox decomposition. No other substitution reactions are observed for the Cu(III) complex in acid whereas the Cu(II) complex rapidly loses G_4. In base amine deprotonation above pH 12 gives $Cu^{III}(H_{-4}G_4)^{2-}$ prior to redox decomposition. The reaction products are Cu(II), diglycinamide, glycinamide, oxidized fragments of G_4, and 50–83% of the initial G_4. The Cu(III) complex also is photochemically decomposed, leading to triglycinamide and G_4 as products. The product distributions vary with pH as well as with photolysis. The Cu(III) complex is a powerful oxidizing agent in acid, rapidly oxidizing Br^-, Ce(III), and Mn(II) but not water. These substrate oxidations are made possible in part by the sluggish substitution reactions of copper(III) compared to the very labile acid reactions of the copper(II)–peptide complexes.

Introduction

The triply deprotonated tetraglycine complex $Cu^{III}(H_{-3}G_4)^-$ was the first trivalent copper–peptide complex to be characterized in solution.[1,2] Its formation was initially observed during studies of the reactions of oxygen with copper(II) tetraglycine,[1] and subsequently its stoichiometry and reduction potential were determined by $IrCl_6^{2-}$ oxidation.[2] The $E°$ value for this complex (0.63 V) and for a wide range of copper–peptide complexes have been determined by cyclic voltammetry.[3] Since these reports we have routinely generated copper(III) peptides electrochemically by using a flow-through cell.[4-9]

This paper describes some of the properties and reactions of copper(III) tetraglycine. Although $Cu^{III}(H_{-3}G_4)^-$ is moderately stable in neutral solution (with a half-life of 5.5 h at 25.0 °C), its decomposition is both acid catalyzed and base catalyzed. The loss of Cu(III) is preceded by a number of acid–base rearrangements which are reflected in the complicated pH profile of the decomposition rate constant given in Figure 1. The decomposition reaction results in the formation of copper(II) accompanied by some peptide oxidation. If reducing substrates are added, 100% of the tetraglycine can be recovered. These redox reactions are very rapid one-electron processes. An interesting property of the copper(III)–peptide complexes is their ability to perform uphill electron-transfer reactions in acid. This has been shown in their oxidation of $Ir^{III}Cl_6^{3-}$ in dilute acid.[7] In strong acid it is possible to oxidize Br^- to Br_2, Ce(III) to Ce(IV), and Mn(II) to Mn(III). These oxidations are much faster than the rate of loss of tetraglycine from copper(III). The effective potential is pH dependent, and part of the extra driving force for these reactions is the rapid acid dissociation of the copper(II)–peptide complex compared to the very sluggish substitution reactions of the copper(III)–peptide complex. Although the effective potential is high enough for solvent oxidation, none is observed.

Experimental Section

Reagents. Copper(II) perchlorate solutions were prepared from $CuCO_3$ and perchloric acid. After recrystallization, stock solutions were prepared and standardized by EDTA titration with murexide indicator. Tetraglycine (Biosynthetika, Oberdorf, Switzerland) was chromatographically homogeneous and was used without further purification. Sodium hexachloroiridate(IV) was purified by oxidation with chlorine in acid solution and then lyophilized. The resulting solid was assayed for $IrCl_6^{2-}$ spectrophotometrically at 490 nm, where ϵ = 4075 M^{-1} cm^{-1}.[10] Solutions for kinetic analysis were adjusted to 1.0 M total ionic strength by using $NaClO_4$ prepared by reaction of $HClO_4$ with Na_2CO_3.

Preparation of $Cu^{III}(H_{-3}G_4)^-$. Chemical Oxidation. The $Cu^{II}(H_{-3}G_4)^{2-}$ solutions were prepared by dissolving solid tetraglycine, usually in 5% excess, in an appropriate volume of $Cu(ClO_4)_2$ solution and raising the pH to 8–9. If the solution was to be stored for more than 1 h, it was necessary to exclude O_2 in order to prevent autoxidation.[5] The $Cu^{III}(H_{-3}G_4)^-$ solutions were prepared by mixing the above solution with a 2–5% excess of Na_2IrCl_6 solution (pH 3–4) and adjusting the final pH to 7.5–7.8 by the dropwise addition of dilute NaOH. As reported previously[2] the yields of Cu(III) are pH dependent. This solution was then rapidly passed through a neutral AG1-X10 anion-exchange column in the perchlorate form to remove both the $IrCl_6^{3-}$ produced in the reaction and the excess $IrCl_6^{2-}$. Unreacted Cu(II) may be removed by passing the solution down a Chelex 100 cation-exchange column. These solutions were deoxygenated by N_2 bubbling for several minutes. The yields with use of this procedure were never quantitative. By performing all manipulations at 0–5 °C and working in a minimum of light, we obtained yields of $Cu^{III}(H_{-3}G_4)^-$ in excess of 80% based on the starting $Cu^{II}G_4$.

Electrochemical Oxidation. The $Cu^{II}(H_{-3}G_4)^{2-}$ solutions were prepared as above except that the ionic strength was controlled at 0.1 M ($NaClO_4$) and the pH was raised to 10.3–10.5. The solutions were passed through Millipore 100-mμ filters immediately before use. Electrochemical oxidation was performed by using a flow system.[11] The working electrode was maintained at +0.66 to +0.69 V vs. Ag/AgCl. High yields (≥95%) were obtained with a flow rate of 0.6 mL/min for solutions as high as 0.01 M in $Cu^{II}(H_{-3}G_4)^{2-}$. For less concentrated solutions, higher flow rates could be employed without lowering the yield. Cooling both the bulk electrolysis cell and the collection vessel to 0–5 °C is suggested when large volumes are collected to slow Cu(III) decomposition. The exclusion of light is very important for Cu(III) stability.

Measurements. The kinetics of the copper(III) tetraglycine decomposition was measured by mixing the complex with an excess of various buffers to provide a constant pH during the run and then monitoring the loss of absorbance at 365 nm by using a Cary 14 or

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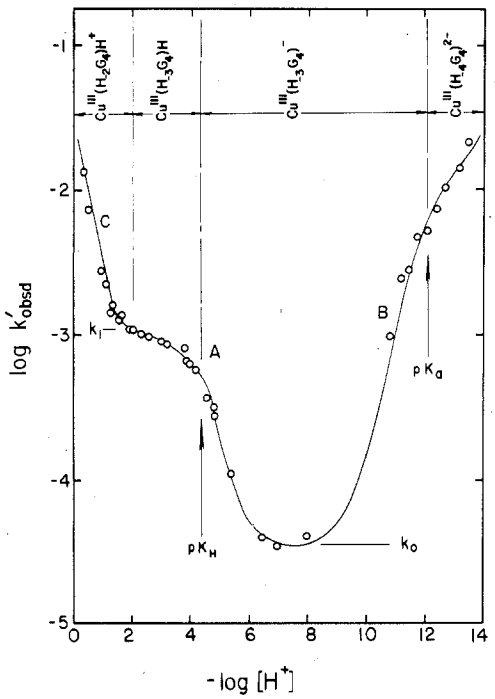

Figure 1. Dependence of the redox decomposition rate constant of copper(III) tetraglycine on acidity. The main reactant prior to the redox step is $Cu^{III}(H_{-3}G_4)^-$ in neutral solution. In the acid-decomposition region (A) $Cu^{III}(H_{-3}G_4)H$ also is present, and below pH 2 $Cu^{III}(H_{-2}G_4)H^+$ forms prior to redox (region C). In the base-decomposition region (B) the amine-deprotonated species $Cu^{III}(H_{-4}G_4)^{2-}$ is present. The equilibrium and rate constants used to calculate the solid line are summarized in Table III.

Cary 16 spectrophotometer. Faster reactions were followed by using a Durrum stopped-flow spectrometer with data obtained by using an on-line digital computer (HP 2115A) interfaced to the instrument.[12] The rate constants are averages of three to five replicates and in general have a precision of 5% of better unless otherwise specified.

Cerium(IV) was assayed spectrophotometrically at 318 nm (ϵ = 5.58 × 10^3 M^{-1} cm^{-1} in 1.0 M H_2CO_4).[13] The molar absorptivity of Br_3^- (ϵ = 3.78 × 10^4 M^{-1} cm^{-1} at 268 nm) was determined by measuring the absorbance of an aliquot of standardized bromine dissolved in 4 M HBr. Our value is similar to the literature value of 3.46 × 10^4 M^{-1} cm^{-1} at 270 nm.[14]

Chromatographic peptide analyses were performed on a Beckman 120B automated amino acid analyzer. A column (0.9 cm × 10 cm) of Beckman PA-35 cation-exchange resin was maintained at 55 °C. Samples were eluted with pH 3.2, 0.2 N sodium citrate buffer, at a flow rate of 70 mL/h. Standard samples containing each of the target substances were run with each set of analyses. Detection of the samples was by the ninhydrin reaction at the amine terminal.

Photochemical experiments were performed, and quantum yields were calculated by measuring the loss of $Cu^{III}(H_{-3}G_4)^-$ at 365 nm after irradiation at the wavelength of interest. Fresh, electrochemically generated $Cu^{III}(H_{-3}G_4)^-$ was swept with N_2 and buffered to pH 5.0 with dilute acetate buffer. Light from a 1 kW Xe lamp was collimated and passed through a monochromator (band-pass ~20 nm) and allowed to impinge upon a sample in a stirred 1.00-cm quartz cell thermostated to 20 or 25 °C. In determining quantum yields, we calibrated the lamp immediately before and after the experiment by the method of Parker and Hatchard.[15]

The solution pH was determined by using an Instrumentation Laboratory Model 245 pH meter with a NaCl-saturated standard calomel electrode. This electrode system was calibrated for ionic strength correction by a pH titration of $HClO_4$ with NaOH; it was observed that $-\log [H^+] = pH + 0.29$ at μ = 1.0 M ($NaClO_4$). Below pH 3 and above pH 11 the $-\log [H^+]$ was defined with standard $HClO_4$ and NaOH, respectively.

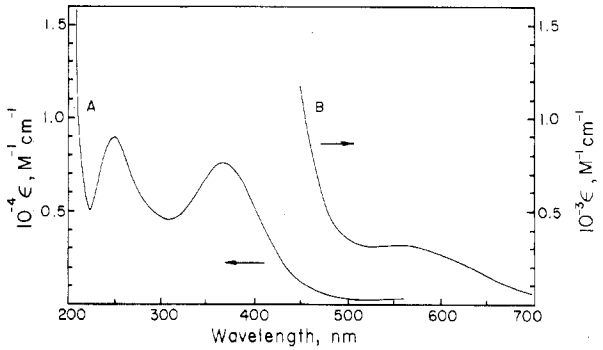

Figure 2. UV–visible spectrum of $Cu^{III}(H_{-3}G_4)^-$ at pH 5.0: A, left-hand ordinate; B, right-hand ordinate.

Results and Discussion

Previous work has verified the presence of the trivalent oxidation state in peptide complexes.[2,3,16] These studies have shown that the oxidized species is the triply deprotonated complex $Cu^{III}(H_{-3}G_4)^-$. This was first observed in the pH dependence of the $IrCl_6^{2-}$ oxidation[2] and further supported by the pH dependence of the $E°$ values in basic media.[11]

The electronic spectrum of $Cu^{III}(H_{-3}G_4)^-$ in solution at pH 5.0 (Figure 2) consists of two intense charge-transfer bands at 250 and 365 nm with molar absorptivities of 9000 and 7200 M^{-1} cm^{-1}, respectively, determined by spectrophotometric titration with ascorbic acid. There is also a weaker band at 550 nm (ϵ = 320 M^{-1} cm^{-1}). Two charge-transfer bands are also observed for other copper(III)–peptide complexes with four nitrogen atom donors, e.g., with the ligands pentaglycine, tetraglycine, and triglycylamide.[3] A similar set of bands are observed for the copper(III)–tripeptide complexes except that these are slightly red-shifted, λ_{max} = 275 and 390 nm. The reaction of $Cu^{II}(OH)_3^-$ with hypochlorite produces a species that is proposed as $Cu_2^{III}O(OH)_7^{3-}$ and also has a similar spectrum.[17] Hence these absorption bands appear to be characteristic of copper(III) complexes with either nitrogen or oxygen donors.

The Redox-Decomposition Reaction

When freshly prepared $Cu^{III}(H_{-3}G_4)^-$ is allowed to age in the absence of reducing agents in aqueous solution, a spontaneous decomposition of the copper(III) results in the oxidation of some of the coordinated tetraglycine and the formation of copper(II). The rate of this self-redox reaction and the product distribution are pH dependent. The observed first-order rate constants are given in Table I, and the kinetic pH profile is shown in Figure 1.

Acid Decomposition. In the pH 3–6 region a single reaction is observed that is first order in copper(III) with a rate constant defined by eq 1, where $[Cu(III)]_T$ refers to all forms of copper(III) tetraglycine. The value of k_{obsd} depends on the buffer concentration as well as the pH. Plots of k_{obsd} vs. the total buffer concentration, $[HB]_T$, were linear with slopes of k^T_{HB} and intercepts of k'_{obsd} as summarized in Table II. The points on the pH profile represent k'_{obsd} as defined in eq 2. The value

$$-d[Cu(III)]_T/dt = k_{obsd}[Cu(III)]_T \quad (1)$$

$$k'_{obsd} = k_{obsd} - k^T_{HB}[HB]_T \quad (2)$$

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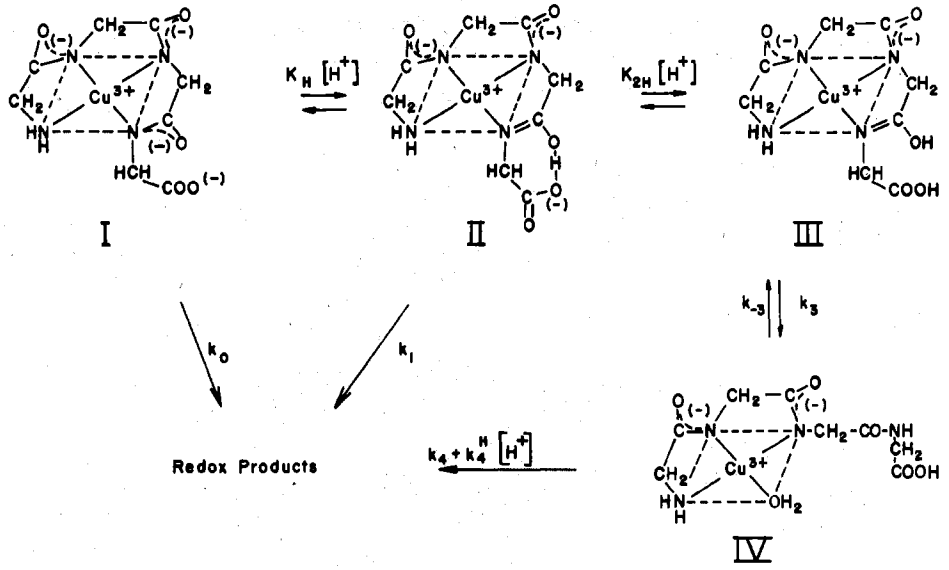

Figure 3. Mechanism for the acid decomposition of copper(III) tetraglycine. Structure I is $Cu^{III}(H_{-3}G_4)^-$, the main species present from pH 4.4 to pH 12. Structure II shows the outside protonated species $Cu^{III}(H_{-3}G_4)H$ where the proton is hydrogen bonded between the carboxylate group and the peptide oxygen. Structure III is proposed for a second protonated species formed rapidly at low pH which decays into $[Cu^{III}(H_{-2}G_4)H]^+$ proposed in structure IV. The products are Cu(II) and oxidized peptides.

Table I. Observed First-Order Rate Constants for the Redox-Decomposition Reaction of Copper(III) Tetraglycine[a]

$-\log [H^+]$	$[HB]_T$, M	buffer	$10^4 k_{obsd}$, s^{-1}
0.30	0.500	$HClO_4$	135.9 ± 0.8
0.60	0.250		72.1 ± 0.6
1.00	0.100		27.5 ± 0.4
1.30	0.050		16.2 ± 0.2
1.60	0.025		12.8 ± 0.2
2.00	0.010		11.0
2.30	0.0050		10.3 ± 0.1
2.60	0.0025		9.7 ± 0.1
3.00	0.0010		9.2 ± 0.2
1.20	0.10	$CHCl_2COOH$	31 ± 4
1.70	0.01		14 ± 4
1.70	0.05		19 ± 2
1.70	0.10		20 ± 3
2.20	0.10		19 ± 3
2.80	0.10	$CH_2ClCOOH$	12 ± 1
3.30	0.01		11 ± 1
3.30	0.10		11.5 ± 0.5
3.30	0.38		12 ± 1
3.80	0.10		10.5 ± 0.4
3.80	0.05	CH_3COOH	8 ± 1
3.90	0.05		6.9 ± 0.5
4.00	0.05		6.5 ± 0.2
4.20	0.05		5.9 ± 0.3
4.80	0.05		3.1 ± 0.3
5.05	0.01		2.9 ± 0.1
5.04	0.10		3.0 ± 0.1
5.02	0.50		4.4 ± 0.3
5.04	1.00		5.1 ± 0.2
5.52	0.10		1.3 ± 0.1
6.00	0.05	phosphate	0.5 ± 0.1[b]
6.02	0.10		0.9 ± 0.1[b]
9.07	0.050	carbonate	5.2 ± 0.4[b]
9.57	0.005		3.4 ± 0.3[b]
9.57	0.050		6.8 ± 0.3[b]
10.07	0.050		11.2 ± 0.2[b]
10.76	0.0010	NaOH	10.3 ± 0.3[b]
11.16	0.0025		25 ± 6[b]
11.46	0.0050		29 ± 2[b]
11.76	0.010		47.8 ± 0.5[b]
12.16	0.025		55 ± 4[b]
12.46	0.050		77 ± 3[b]
12.76	0.100		105 ± 3[b]
13.16	0.250		142 ± 2[b]
13.46	0.500		222 ± 8[b]

[a] $[Cu^{III}(H_{-3}G_4)^-]_0 = 1.5 \times 10^{-5}$ M, $\mu = 1.0$ M ($NaClO_4$), 25.0 °C. [b] Initial rate determination.

Table II. Rate Constants for the Redox Decomposition of $Cu^{III}(H_{-3}G_4)^-$ with Various Buffers[a]

buffer	pH	k^T_{HB},[b] M^{-1} s^{-1}	k'_{obsd}, s^{-1}
dichloroacetic acid	1.00	8.4×10^{-3}	1.3×10^{-3}
chloroacetic acid	2.60	2.8×10^{-4}	9.6×10^{-4}
acetic acid	4.64	2.2×10^{-4}	3.8×10^{-4}
phosphate	6.50	7×10^{-4}	4.0×10^{-5}
carbonate	9.75	7.6×10^{-3}	3.0×10^{-4}
HEPES	7.00	8×10^{-2}	2.3×10^{-4}

[a] 25.0 °C, $\mu = 1.0$ M ($NaClO_4$). [b] The second-order rate constant is first order in Cu(III) and first order in the total buffer concentration at the given acidity.

of k'_{obsd} has a complex $[H^+]$ dependence consistent with a preequilibrium, in which the original complex (structure I in Figure 3) becomes "outside" protonated to form $Cu^{III}(H_{-3}G_4)H$ (structure II). This complex has been protonated at a peptide oxygen and hydrogen bonded to the free carboxylate group, but the Cu(III)–N(peptide) bond has not been broken. Similar "outside" protonations have been observed for nickel(II)- and copper(II)-oligopeptide complexes.[18,19] The proposed mechanism for pH 3–6 is given by eq 3–5. The

$$Cu^{III}(H_{-3}G_4)^- + H^+ \overset{K_H}{\rightleftharpoons} Cu(H_{-3}G_4)H \qquad (3)$$

$$Cu^{III}(H_{-3}G_4)^- \xrightarrow{k_0} \text{redox products} \qquad (4)$$

$$Cu^{III}(H_{-3}G_4)H \xrightarrow{k_1} \text{redox products} \qquad (5)$$

corresponding expression for the observed rate constant is given by eq 6 with $[Cu(III)]_T = [Cu^{III}(H_{-3}G_4)^-] + [Cu^{III}(H_{-3}G_4)H]$.

$$k'_{obsd} = k_0 + \frac{k_1 K_H [H^+]}{1 + K_H [H^+]} \qquad (6)$$

The value of $k_0 = 3.5 \times 10^{-5}$ s^{-1} is defined by the minimum of the kinetic pH profile. The values of k_1 and K_H were determined from a plot of eq 7 from which $K_H = 10^{4.3}$ and $k_1 = 1.1 \times 10^{-3}$ s^{-1} in 1.0 M $NaClO_4$ and were used to generate curve A from pH 2–7 in Figure 1.

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$$\frac{1}{k'_{obsd} - k_0} = \frac{1}{k_1 K_H}\left(\frac{1}{[H^+]}\right) + \frac{1}{k_1} \quad (7)$$

Other copper(III)–peptide complexes show similar acid-decomposition reactions characterized by "outside" protonation. For the ligands G_6, G_5, and G_3a the values of log K_H are 2.6, 2.7,[20] and 3.5,[21] respectively, all at μ = 1.0 M ($NaClO_4$). These complexes are significantly less basic than $Cu^{III}(H_{-3}G_4)^-$. For the complexes $Ni^{II}(H_{-3}G_4)^{2-}$ and $Ni^{II}(H_{-3}G_3a)$, the values of log K_H are 4.1 and 2.4[18] at 0.1 M $NaClO_4$. In the case of $Cu^{II}(H_2GGhis)^-$ two outside protonations are observed with log K_H = 2.3 and 4.2 in 1.0 M $NaClO_4$.[19] Internal hydrogen bonding (structure II) is proposed to explain why the tetraglycine complexes of both Cu(III) and Ni(II) and the first protonation of $Cu^{II}(H_2GGhis)^-$ are between 1 and 2 log units more basic than other metal peptides.

Below pH 3 three reactions are observed for the loss of absorbance at 365 nm. The first is a 5% absorbance drop that is complete within the dead time on the stopped flow (4 ms). The second reaction is first order in [Cu(III)] with an observed rate constant of 0.3 s^{-1} that has no hydrogen ion dependence below pH 1 and accounts for another 15% of the absorbance loss. A much slower reaction accounts for the remaining absorbance change. This final reaction is first order in [Cu(III)] and its rate constant (k'_{obsd}) has a first-order [H^+] dependence below pH 1 as shown by curve C in Figure 1. The initial absorbance of the slowest reaction (extrapolated to zero time) has a pH dependence with an inflection point at pH 1.6. A macrocyclic tetrapeptide complex of copper(III) shows a similar series of reactions in its acid decomposition, and it has been demonstrated that the faster reactions involve conversions to copper(III) intermediates as opposed to reduction of the metal in the final reaction.[22] It is assumed that this is also true for the tetraglycine complex. The proposed mechanism for the reactions below pH 3 is given by eq 8–10. The very

$$Cu^{III}(H_{-3}G_4)H + H^+ \overset{K_{2H}}{\rightleftharpoons} [Cu^{III}(H_{-3}G_4)H_2]^+ \quad (8)$$

$$[Cu^{III}(H_{-3}G_4)H_2]^+ \underset{k_{-3}}{\overset{k_3}{\rightleftharpoons}} [Cu^{III}(H_{-2}G_4)H]^+ \quad (9)$$

$$[Cu^{III}(H_{-2}G_4)H]^+ \xrightarrow{k_4 + k_4^H[H^+]} \text{redox products} \quad (10)$$

rapid but small initial absorbance drop involves a second protonation of the complex without breaking any peptide bonds to copper(III) to form the product of reaction 8. The $[Cu^{III}(H_{-3}G_4)H_2]^+$ species could correspond to structure III, or the proton could go to one of the other peptide oxygens. The formation of a partially unwrapped species (structure IV) is proposed in reaction 9. The value of $K_{2H}k_3/k_{-3}$ is estimated as $10^{1.6}$, and the value of k_3 is 0.3 s^{-1}. It should be noted that eq 9 is not a simple proton-transfer reaction but as shown in Figure 3 it involves the cleavage of a Cu(III)–N(peptide) bond as well. After the reactions in eq 8 and 9, the major species in solution is $[Cu^{III}(H_{-2}G_4)H]^+$, which is consistent with the observation that in 0.4 M $HClO_4$ at 0 °C the absorbance maxima in the UV spectrum shift from 250 to 253 nm and from 365 to 373 nm (i.e., closer to those of a copper(III) tripeptide). This $[Cu^{III}(H_{-2}G_4)H]^+$ species undergoes self-redox with $k_4 \approx 10^{-3}$ s^{-1} and has a H^+ dependence with a second-order rate constant, k_4^H = 0.026 M^{-1} s^{-1}. The proposed overall acid-decomposition mechanism is presented in Figure 3, and the equilibrium and rate constants are summarized in Table III. The rate at which $[Cu^{III}(H_{-2}G_4)H]^+$ undergoes redox decomposition is consistent with our observation of the rate at which $Cu^{III}(H_{-2}G_3)$ decomposes. Acid does not strip the peptide from copper(III) to give the aquo ion, but rather ligand oxidation occurs before loss of much of the coordinated peptide. However, once copper(II) is formed, the peptide is rapidly stripped from the metal by acid.

Table III. Equilibrium and Rate Constants for Copper(III) Tetraglycine Reactions (25.0 °C)

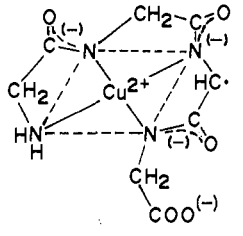
Protonation and Deprotonation Equilibrium Constants	
$Cu^{III}(H_{-3}G_4)^- + H^+ \rightleftarrows Cu^{III}(H_{-3}G_4)H$	log K_H = 4.3
$Cu^{III}(H_{-3}G_4)H + H^+ \rightleftarrows Cu^{III}(H_{-2}G_4)H^+$	log $(K_{2H}K_3)$ = 1.6
$Cu^{III}(H_{-3}G_4)^- \rightleftarrows Cu^{III}(H_{-4}G_4)^{2-} + H^+$	log K_a = −12.1
Reduction Potentials	
$Cu^{III}(H_{-3}G_4)^- + e^- \rightleftarrows Cu^{II}(H_{-3}G_4)^{2-}$	0.63 V
$Cu^{III}(H_{-2}G_4)H^+ + e^- \rightleftarrows Cu^{II}(H_{-2}G_4)H$	0.96 V
Rate Constants	
Rearrangement	
$Cu^{III}(H_{-3}G_4)H_2^+ \rightarrow Cu^{III}(H_{-2}G_4)H^+$	k_3 = 0.3 s^{-1}
Redox	
$Cu^{III}(H_{-3}G_4)^- \rightarrow$ redox products	$k_0 = 3.5 \times 10^{-5}$ s^{-1}
$Cu^{III}(H_{-3}G_4)H^+ \rightarrow$ redox products	$k_1 = 1.1 \times 10^{-3}$ s^{-1}
$Cu^{III}(H_{-2}G_4)H^+ \rightarrow$ redox products	$k_4 \simeq 10^{-3}$ s^{-1}
$Cu^{III}(H_{-2}G_4)H^+ + H^+ \rightarrow$ redox products	k_4^H = 0.026 M^{-1} s^{-1}
$Cu^{III}(H_{-3}G_4)^- + OH^- \rightarrow$ redox products	k_5 = 0.6 M^{-1} s^{-1}
$Cu^{III}(H_{-4}G_4)^{2-} + OH^- \rightarrow$ redox products	k_6 = 0.01 M^{-1} s^{-1}

Table IV. Chromatographic Peptide Analysis of $Cu^{III}(H_{-3}G_4)^-$ Decomposition Products (in the Dark)

pH of decompn	% original G_4 recovered as[a]		
	G_4	G_2a	Ga
2.0	46 (50)	1 (41)	1 (3)
5.0	54 (53)	1 (29)[b]	1 (3)[b]
9.0	60	35	5
10.0	74	17	6
11.0	76	9	6
12.0	80	3	4
13.0	83	5	7
14.0	75	5	15

[a] Numbers in parentheses refer to analysis after hydrolysis of the acid-decomposition samples at pH 10–11. [b] Hydrolysis of dehydropeptide incomplete.

The analysis of the products of the acid decomposition of $Cu^{III}(H_{-3}G_4)^-$ shows that 50% of the tetraglycine is recovered intact (Table IV). The remainder of the ligand is converted to a new product that is stable in acid but hydrolyzes in base to yield glycylglycinamide, a carbonyl species, and smaller amounts of glycine and glycinamide. The initial product has been previously proposed[5] as the dehydropeptide of tetraglycine (HG_4DHP^+) with the formula $^+H_3NCH_2CONH$-$CH_2CON{=}CHCONHCH_2COOH$. It is proposed that a carbon-centered radical (structure V), which results from



V

copper(III) reduction, reacts with another $Cu^{III}(H_{-3}G_4)^-$ to produce the reaction stoichiometry of eq 11.[5] Evidence to

$$2Cu^{III}(H_{-3}G_4)^- \xrightarrow{H^+} 2Cu^{2+} + HG_4DHP^+ + HG_4^+ \quad (11)$$

(20) Kirksey, S. T., Jr. Ph.D. Thesis, Purdue University, 1978.
(21) Neubecker, T. A. Ph.D. Thesis, Purdue University, 1978.
(22) Rybka, J. S.; Margerum, D. W., to be submitted for publication.
(23) Smith, R. M., Martell, A. E., Eds. "Critical Stability Constants"; Plenum Press: New York, 1974; Vol. 1.

Table V. $Cu^{III}G_4$ Decomposition Rates at pH 8.0 and pH 8.3 with $[Cu^{II}G_4]$ Controlled[a]

pH	$[Cu^{II}G_4]$, mM	$10^4 \times k_{obsd}$, s^{-1}	pH	$[Cu^{II}G_4]$, mM	$10^4 \times k_{obsd}$, s^{-1}
7.97	0[b]	0.42	8.29	0[b]	0.54
7.97	0.1	1.32	8.28	0.1	1.85
7.99	0.2	2.22	8.28	0.2	3.13
7.98	0.4	3.49	8.28	0.4	5.09
7.98	0.6	4.72	8.27	0.6	6.12
7.97	0.8	5.62	8.29	0.8	6.84
7.97	1.2	6.71	8.28	1.2	7.99
8.02	2.0	7.55	8.30	2.0	8.88

[a] $[Cu^{III}G_4]_0 = 1 \times 10^{-5}$, THAM buffer, $\mu = 0.1$ M ($NaClO_4$), $T = 25$ °C. [b] 1×10^{-4} M EDTA present to control $[Cu^{II}G_4]$.

support the proposed carbon-centered radical intermediate is that substitution of the methylene hydrogens by methyl groups leads to enhanced kinetic stability. The $Cu^{III}(H_{-2}Aib_3)$ complex (where Aib_3 is the tripeptide of aminoisobutyric acid) is stable toward self-redox for months,[6] while $Cu^{III}(H_{-2}G_3)$ decomposes completely in minutes in acid media.

Basic Decomposition. Above pH 6, the redox decomposition of $Cu^{III}(H_{-3}G_4)^-$ is not first order because catalysis by the reaction products occurs. It has previously been proposed[5] that in neutral solutions $Cu^{II}(H_{-2}G_4)^-$, which is the predominant form of the copper(II) complex at pH 6–8, catalyzes the $Cu^{III}(H_{-3}G_4)^-$ decomposition via eq 12 to form $Cu^{III}(H_{-2}G_4)$.

$$Cu^{III}(H_{-3}G_4)^- + Cu^{II}(H_{-2}G_4)^- \rightleftarrows Cu^{II}(H_{-3}G_4)^{2-} + Cu^{III}(H_{-2}G_4) \quad (12)$$

$$Cu^{III}(H_{-2}G_4) \rightarrow \text{redox products} \quad (13)$$

This complex is expected to have a higher reduction potential than that of the original complex because one less peptide nitrogen is coordinated.[3] It appears to undergo a faster redox decomposition reaction. First-order plots are obtained when measures are taken to hold the concentration of copper(II) tetraglycine constant. In the presence of EDTA the copper(II) is effectively scavenged and the reactions become first order with an observed rate constant of 3.5×10^{-5} s^{-1} from pH 6 to pH 7. This value defines the minimum of the pH profile in Figure 1.

It is also possible to maintain an essentially constant concentration of copper(II) tetraglycine by making it at least a 10-fold excess over $Cu^{III}(H_{-3}G_4)^-$. Under these conditions the reactions are first order in [Cu(III)] and are dependent upon the [Cu(II)], buffer media, and pH. First-order rate constants for the decomposition of 1.0×10^{-5} M $Cu^{III}(H_{-3}G_4)^-$ in tris(hydroxymethyl)aminomethane (THAM) buffer at pH 8.0 and pH 8.3 are given in Table V. The dependence on [Cu(II)] is quite marked and tends toward saturation at higher [Cu(II)]. This dependence has been explained in terms of the reaction sequence of eq 12 and 13.[5] We have noticed that the buffer media also affect the kinetics and this may be due to complexation of copper(II) by the buffer.

At higher pH (9–12), the reactions are still catalyzed by the products in spite of the fact that $Cu^{II}(H_{-3}G_4)^{2-}$ rather than $Cu^{II}(H_{-2}G_4)^-$ is the predominant form of the copper(II) complex. In basic solution, the dehydropeptide of tetraglycine hydrolyzes to form carbonyl products and glycylglycinamide, which is a significant product at pH 9.0 (Table IV), can complex Cu^{2+} to form $Cu(H_{-2}G_2a)OH$, and reacts further in a manner similar to that of eq 12. We have observed that excess $Cu^{II}(H_{-2}G_2a)OH^-$ has a similar effect on the kinetics as that of excess $Cu(H_{-2}G_4)^-$ at lower pH. At higher pH copper(III) tetraglycine undergoes amine deprotonation to form $Cu^{III}(H_{-4}G_4)^{2-}$. The pK_a for eq 14 is 12.1 ± 0.2.[11] The

$$Cu^{III}(H_{-3}G_4)^- \overset{K_a}{\rightleftharpoons} Cu^{III}(H_{-4}G_4)^{2-} + H^+ \quad (14)$$

$Cu^{III}(H_{-4}G_4)^{2-}$ complex is known to react with carbonyl species;[22] however, there is no significant decrease in the recovery of ninhydrin active products (Table IV).

At pH >9 the recovery of tetraglycine increases from ~60% to as much as 83% (Table IV). This change is accompanied by a decrease in the recovery of G_2a and by an increase in the amounts of other fragments including G, G_2, and Ga. The reason for this change in the distribution of the products is that $Cu^{III}(H_{-3}G_4)^-$ can react with Cu(II) complexes of ligand fragments in preference to its own redox reaction.

The base-decomposition reactions that were studied without the addition of EDTA or without excess copper(II) tetraglycine were analyzed by the initial-rate method because of the non-first-order kinetics. The observed first-order rate constants, k_{obsd}, are presented in Table I. These rate constants show a buffer dependence (Table II) and have been corrected to k'_{obsd} by using eq 2. Above pH 9 the value of k'_{obsd} becomes dependent upon $[OH^-]$ as shown in Figure 1. The proposed reaction scheme is given in eq 15–17 with k_0 defined by eq

$$Cu^{III}(H_{-3}G_4)^- + OH^- \xrightarrow{k_5} \text{redox products} \quad (15)$$

$$Cu^{III}(H_{-4}G_4)^{2-} + OH^- \xrightarrow{k_6} \text{redox products} \quad (16)$$

$$k'_{obsd} = \frac{k_0 + k_5[OH^-] + k_6(K_a/K_w)[OH^-]^2}{1 + (K_a/K_w)[OH^-]} \quad (17)$$

4. The values of k_5 and k_6 were determined by iteration to yield the best fit of the data. Curve B in Figure 1 was generated by using eq 18 with the values $k_5 = 0.6$ M^{-1} s^{-1} and

$$k_{obsd} = \frac{k_0 + k_5K_w[H^+]^- + k_6K_aK_w[H^+]^{-2}}{1 + K_a[H^+]^{-1}} \quad (18)$$

$k_6 = 0.010$ M^{-1} s^{-1}. These results indicate that while both $Cu^{III}(H_{-3}G_4)^-$ and $Cu^{III}(H_{-4}G_4)^{2-}$ undergo a base-catalyzed decomposition, the former complex reacts faster.

In fitting these data we noticed that the rate constants, k'_{obsd}, obtained in borate and in carbonate buffer were significantly larger than those obtained in the presence of EDTA or with NaOH at a comparable pH. Apparently the buffer dependence is more complex than that of eq 2. The reactions in HEPES (*N*-(2-hydroxyethyl)piperazine-*N*'-2-ethanesulfonic acid) had a buffer dependence that was two orders of magnitude greater than that of borate or carbonate and also has a much larger value of k'_{obsd} (Table II). The results obtained in HEPES buffer suggest that copper(III) may be reacting with the buffer with a complex dependence.

Photochemical Decomposition. $Cu^{III}(H_{-3}G_4)^-$ in neutral solution is sufficiently photosensitive for room light to cause substantial reaction (radiation with wavelengths less than 600 nm will cause decomposition). The quantum yield determined at 365 nm is 0.06 ± 0.01. At pH 5 the products are the same after irradiation at 365, 450, or 550 nm. Approximately 50% tetraglycine, 30–40% glycylglycylglycinamide, and smaller quantities of glycylglycinamide and glycinamide are recovered. Glycylglycylglycinamide is not produced in appreciable concentrations by the redox decomposition of $Cu^{III}(H_{-3}G_4)^-$ at any pH conditions in the dark. Another copper(III) complex, $Cu^{III}(H_{-2}Aib_3)$, where the ligand is the tripeptide of α-aminoisobutyric acid, undergoes a photocatalyzed decarboxylation to produce acetone and CO_2.[6] We propose a similar decarboxylation leading to G_3a in the case of the photolysis of $Cu^{III}(H_{-3}G_4)^-$.

$Cu^{III}(H_{-3}G_4)^-$ as an Oxidizing Agent. In the presence of many reducing agents the loss of Cu(III) is rapid, occurring much faster than the redox decomposition of tetraglycine. Product analysis after chemical reduction shows 100% recovery of the tetraglycine.

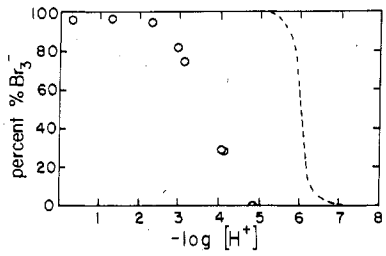


Figure 4. Amount of Br_3^- generated (percent of oxidation possible) when 5.0×10^{-5} M $Cu^{III}(H_{-3}G_4)^-$ is added to 1.0 M Br^- at varying pH. The dashed line is calculated from the effective potential of the Cu(III)–Cu(II) couple which increases in acid due to rapid equilibration of the copper(II) tetraglycine species. The points are the experimental yield of Br_3^-.

Table VI. pH Dependence of the Oxidation of Bromide by $Cu^{III}(H_{-3}G_4)^-$ [a]

−log [H^+]	% Br_3^- [b]	−log [H^+]	% Br_3^- [b]
0.30[c]	96	3.11[d]	74
1.30[c]	96	4.07[d]	28
2.30[c]	94	4.13[d]	28
2.95[d]	82	4.91[d]	0

[a] Conditions: $[Br^-]_T = 1.0$ M, $[Cu(III)]_T = 5 \times 10^{-5}$ M, O_2 excluded. [b] Br_3^- measured at 268 nm ($\epsilon = 3.78 \times 10^4$ M^{-1} cm^{-1}); the percent oxidation is based on $[Cu(III)]_T$. [c] $HClO_4$. [d] 0.1 M acetate buffer.

Because of the unusual nature of this complex and the possibility that it might act as either a 1 or a 2 equiv/mol oxidant in redox reactions, the stoichiometries of its reactions with a variety of reducing agents have been investigated. The reactions of $Cu^{III}(H_{-3}G_4)^-$ with di-*tert*-butyl nitroxide and cysteine, two 1 equiv/mol reductants, show a 1:1 stoichiometry indicating reduction to Cu(II). The reactions with ascorbic acid and hydroquinone, which are 2 equiv/mol reducing agents, give a Cu(III) to reductant ratio of 2:1, further indicating that the product is Cu(II). We have found no evidence for a two-electron reduction of Cu(III) to Cu(I).

The oxidizing power of copper(III) tetraglycine solutions depends upon the acidity. In neutral solution $Cu^{III}(H_{-3}G_4)^-$ has an E° value of 0.63 V (eq 19) and it is not capable of

$$Cu^{III}(H_{-3}G_4)^- + e^- \overset{E^\circ}{\rightleftharpoons} Cu^{II}(H_{-3}G_4)^{2-} \qquad (19)$$

oxidizing $IrCl_6^{3-}$ to $IrCl_6^{2-}$ ($E^\circ = 0.89$ V). However, at pH 4–6 this Ir(III) to Ir(IV) oxidation does occur with $Cu^{III}(H_{-3}G_4)^-$. It has been shown[7] that the rate-determining step is an uphill electron-transfer reaction, unfavorable by 0.36 V, which is driven by the overall reaction with rapid dissociation of $Cu^{II}(H_{-3}G_4)^{2-}$ in acid. Thus, the effective potential, E_{eff}, for the overall reaction in eq 20 becomes greater than 1 V by pH 5 and more than 2 V by pH 0.

$$Cu^{III}(H_{-3}G_4)^- + 4H^+ + e^- \overset{E_{eff}}{\rightleftharpoons} Cu^{2+} + HG_4 \qquad (20)$$

The dashed curve in Figure 4 shows that it is thermodynamically possible for $Cu^{III}(H_{-3}G_4)^-$ to oxidize Br^- to Br_3^- ($E^\circ = 1.05$ V) at pH 5. The data in Table VI and in Figure 4 show that this oxidation does not occur at pH 5 but does take place at lower pH, resulting in a 95% yield of Br_3^- at pH 2. The extent of Br^- oxidation depends upon the ratio of substrate oxidation by the Cu(III) complex to its self-decomposition where G_4 is oxidized. We have already shown in Figure 1 that the self-decomposition reaction is strongly catalyzed by acid. The results with Br^- show that this substrate oxidation is catalyzed by acid to an even greater extent. The $Cu^{III}(H_{-3}G_4)^-$ complex is not completely inert in strong acid, and partial dissociation of G_4 occurs to give $Cu^{III}(H_{-2}G_4)H^+$. The E° for this complex can be calculated to be 0.96 V from the protonation constants in Table III and the corresponding constants for the copper(II) species. This is the potential at pH 0–1 for the one-electron-transfer reaction to give $Cu^{II}(H_{-2}G_4)H$. However, the Cu(III) complex will not only oxidize Br^- (where the one-electron step to produce Br·aq requires 2.06 V) but also oxidize Ce(III) and Mn(II), requiring more than 1.5 V. Table VII gives some of the substrate oxidations observed in acid by using $Cu^{III}(H_{-3}G_3a)$. The one-electron-transfer steps are energetically unfavorable, but the oxidation reactions are possible because of the extremely labile reactions of Cu(II). Acid causes very little peptide loss from the sluggish Cu(III) complexes prior to the electron transfer but strips the peptide rapidly from Cu(II) and drives the overall oxidation.

Table VII. Substrate Oxidation by $Cu^{III}(H_{-3}G_3a)$ in Acid

substrate	product	E°, V	acidity
$Ir^{III}Cl_6^{3-}$	$Ir^{IV}Cl_6^{2-}$	0.89	pH 0–4
Br^-	Br_3^-	1.05	pH 0–2
$Fe(phen)_3^{2+}$	$Fe(phen)_3^{3+}$	1.06	1 M H_2SO_4
Ce(III)	Ce(IV)	1.46	0.5 M H_2SO_4
Mn(II)	Mn(III)	1.54	3 M $HClO_4$

The copper(III) peptides are particularly effective in one-electron oxidations such as Ir(III) to Ir(IV), Fe(II) to Fe(III), Ce(III) to Ce(IV), and Mn(II) to Mn(III). It is interesting that although the effective potentials are sufficient to oxidize H_2O to H_2O_2 or to O_2, we found no evidence for these products in either acid- or base-decomposition reactions. In the substrate oxidations in strong acid any excess copper(III) peptide undergoes rapid internal decomposition rather than oxidation of the solvent.

Conclusions

The $Cu^{III}(H_{-3}G_4)^-$ complex is moderately stable in neutral solution with a half-life of 5.5 h under optimal conditions. In the absence of reducing agents, it undergoes a self-redox-decomposition reaction which is both acid catalyzed and base catalyzed. Product analysis of the acid reaction shows that 50% of the tetraglycine is recovered intact and 50% is oxidized to form a dehydropeptide that hydrolyzes to glycylglycinamide. The copper(III) peptides are substitutionally sluggish as would be expected for a square-planar complex with a d^8 electron configuration. These complexes form protonated species at lower pH and undergo partial peptide dissociation which accelerates their redox-decomposition reaction. In neutral and basic media the reaction is complicated due to catalysis by the redox products. These alternate paths for copper(III) tetraglycine reduction account for the change in the distribution of the reaction products as the recovery of tetraglycine increases to 83% and there is a greater degree of oxidation of the peptide fragments.

While the substitution reactions of $Cu^{III}(H_{-3}G_4)^-$ are sluggish, its redox reactions with one-electron reducing agents are rapid. An interesting aspect of copper(III)–peptide redox chemistry is the ability of the copper(III)–peptide complexes to perform uphill electron-transfer reactions. In acid media the effective value for E° is much greater than that determined by cyclic voltammetry in neutral solution. Copper(III) tetraglycine solutions are able to oxidize cerium(III) in 0.5 M H_2SO_4. Even though the formal potential is enough to oxidize solvent, none is observed, suggesting that kinetically rapid, single-electron reactions are favored over slower multielectron processes.

Acknowledgment. This investigation was supported by Public Health Service Grants GM-12152 and GM-19775 from the National Institute of General Medical Sciences.

Registry No. $Cu^{III}(H_{-3}G_4)^-$, 57692-61-2; $Cu^{III}(H_{-3}G_3a)$, 62801-36-9; Br^-, 24959-67-9; Ce^{3+}, 18923-26-7; Mn^{2+}, 16397-91-4; $Ir^{III}Cl_6^{3-}$, 14648-50-1; $Fe(phen)_3^{2+}$, 14708-99-7.