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Trivalent Copper Catalysis of the Autoxidation of Copper(II) Tetraglycine

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Oxygen reacts with copper(II) tetraglycine (G_4) solutions at pH 7–9 to give $Cu^{III}(H_{-3}G_4)^-$ (where H_{-3} refers to three deprotonated peptide nitrogens coordinated to copper) as an intermediate leading to the oxidation of the peptide. A dehydropeptide which hydrolyzes to diglycinamide and glyoxylglycine is the major product of the peptide oxidation. Copper(III) tetraglycine is the catalyst for the autoxidation, and the rate of decomposition of $Cu^{III}(H_{-3}G_4)^-$ controls the rate of O_2 uptake. This process leads to an autocatalytic increase in Cu(III) concentration. In the proposed mechanism the autoxidation reactions are initiated by traces ($<10^{-7}$ M) of $Cu^{III}(H_{-3}G_4)^-$ which form by the disproportionation of $Cu^{II}(H_{-2}G_4)^-$ into Cu(I) and Cu(III) complexes. It is proposed that the Cu(III) complex decomposes to give a species which reacts rapidly with O_2 and eventually generates more Cu(III) complex as well as the oxidized peptide. Copper is an effective catalyst in this autoxidation because of its multiple oxidation states and because Cu(III) complexes serve as a reservoir of oxidizing power.

Introduction

Copper(II) and tetraglycine (G_4^-) form complexes with a stepwise loss of peptide hydrogen ions occurring at pK_a values of 5.52, 6.78, and 9.16 to give $Cu^{II}(H_{-1}G_4)$, $Cu^{II}(H_{-2}G_4)^-$, and $Cu^{II}(H_{-3}G_4)^{2-}$, respectively.[1] The crystal structure of $Na_2Cu^{II}(H_{-3}G_4)\cdot 10H_2O$ shows that the amine nitrogen and three deprotonated-peptide nitrogens are bound to copper in a nearly square-planar arrangement and that the carboxylate group is not coordinated.[2] The $Cu^{II}(H_{-3}G_4)^{2-}$ complex is relatively labile, reacting very rapidly with acids and somewhat more slowly with solvent and nucleophiles.[3] The triply deprotonated form is easily oxidized to $Cu^{III}(H_{-3}G_4)^-$ using $IrCl_6^{2-}$ or electrochemical techniques.[4,5] Its electrode potential ($Cu^{III} + e^- \rightarrow Cu^{II}$) is 0.63 V (vs. NHE). The $Cu^{III}(H_{-3}G_4)^-$ complex is slow to undergo in-plane substitution reactions but is very rapid in its electron-transfer reactions.[6]

Copper(II) ion activates the reaction of molecular oxygen with tetraglycine wherein the peptide is oxidized to diglycinamide (G_2a) and glyoxylglycine.[7] The reaction with O_2 is spontaneous in neutral solution and is autocatalytic. Copper(III)–peptide complexes are observed as intermediates during the autoxidation. Nickel(II) tetraglycine undergoes similar reactions with O_2,[8] forming $Ni^{III}(H_{-3}G_4)^-$ as an intermediate,[9] but with the nickel complex the G_4 is oxidized to triglycinamide, carbon dioxide, and formaldehyde. In the present work copper(III) peptide intermediates are shown to be the catalysts for the autoxidation. The rate of O_2 uptake is controlled by the rate of decomposition of $Cu^{III}(H_{-3}G_4)^-$. Subsequent reactions lead to the formation of more $Cu^{III}(H_{-3}G_4)^-$.

There have been a number of studies of the autoxidation of cobalt(II) di- and tripeptide complexes.[10-17] In these systems oxygenation produces μ-peroxo binuclear Co(III) complexes. Although this oxygenation can be reversible, the binuclear complexes decompose irreversibly to mononuclear Co(III) species.[13,16] The oxidation of ascorbic acid by oxygen in the cobalt(II)–diglycine system[18] and of glycyltryptophan by oxygen in the cobalt(II)–glycyltryptophan system[11] has been described as catalytic. In the present system the copper(III) peptide serves as a pool of reactive oxidizing power and generates an intermediate which is very reactive with O_2. This intermediate could be a carbon-centered free radical ($Cu^{II}R\cdot$) which reacts with O_2 to form a peroxy radical ($Cu^{II}RO_2\cdot$). Similar R· and $RO_2\cdot$ species (but without metal ions) are proposed as chain centers in the autoxidation of *N*-alkylamides to give hydroperoxides.[19-21] The *N*-alkylamide reactions are slow and require elevated temperatures. The copper-catalyzed autoxidation of tetraglycine in the present system is much more rapid because Cu(III) assists the formation of $Cu^{II}R\cdot$.

An alternative mechanism is possible in which the copper(III) peptide generates a copper(I) dehydropeptide as an intermediate which reacts with O_2 to form a copper(III) peroxide. In this mechanism the reactive intermediates are copper(I) and copper(III) peroxide rather than radicals centered at the α-carbon atom. The reaction products are the same and the detailed rate expressions fit both mechanisms.

It is interesting to note that copper(II) is also known to catalyze the oxidative degradation of protein and poly-α-amino acids by H_2O_2[22] and the autoxidation of petroleum products.[23] There are also a number of copper-containing enzymes whose function, so far as known, involves catalysis of O_2 oxidation of various substrates.[24]

Experimental Section

Reagents. Tetraglycine was obtained from Biosynthetika (Oberdorf, Switzerland). Copper(II) perchlorate was prepared from reagent grade copper carbonate and perchloric acid. Stock solutions were standardized by EDTA/murexide titration. Solutions of copper(II) tetraglycine were prepared with a 5–10% weight excess of ligand to suppress precipitation of copper hydroxide.

An electrochemical flow cell, described previously,[25] was used to generate $Cu^{III}(H_{-3}G_4)^-$ for the Cu^{III} decomposition and oxygen-uptake studies. A solution of $Cu^{II}(H_{-3}G_4)^{2-}$ initially at pH 10.4–10.6 in 0.10 M $NaClO_4$ was passed through the working electrode (maintained at +0.66 V vs. Ag/AgCl) at 0.6–1.2 mL/min. The pH of the eluent was between 6 and 8.

Kinetic Experiments. Most kinetic measurements were thermostated at 25.0 ± 0.1 °C. A few reactions were carried out at 37 °C. Ionic strength was maintained essentially constant with 0.10 M $NaClO_4$ supporting electrolyte. All pH measurements were performed on a Radiometer Model 26 pH meter. Oxygen uptake was followed with a Yellow Springs Model 53 polarographic O_2 monitor.

The formation and decomposition of Cu(III) were followed using a Cary Model 16 spectrophotometer. The molar absorptivity of $Cu^{III}(H_{-3}G_4)^-$ was found to be 7400 M^{-1} cm^{-1} at 365 nm. For the initiation experiments, solutions of $Cu^{II}G_4$ were prepared at pH 8.60 in 0.010 M H_3BO_3 buffer (0.1 M $NaClO_4$) and allowed to equilibrate for 2–12 h under nitrogen. Solutions were then passed through 100-mμ Millipore filters and again allowed to equilibrate under N_2 for about 12 h. Reactions were initiated by flowing solutions through a double two-jet tangential mixer into a 5.00-cm observation cell. The initial absorbance was measured by mixing the $Cu^{II}G_4$ solution with a N_2-saturated 0.10 M $NaClO_4$ solution. For the autoxidation studies $Cu^{II}G_4$ solutions were mixed with O_2-saturated 0.10 M $NaClO_4$ solutions.

Chromatographic Peptide Analysis. The products of ligand oxidation were determined chromatographically on a Beckman Model 120B amino acid analyzer. Samples were passed down a 15-cm column of Beckman PA 35 or Hamilton H70 cation-exchange resin and eluted with pH 3.2 citrate buffer (0.2 M Na^+, 0.067 M citrate). The instrument was calibrated as to elution time and detector sensitivity with standard solutions of glycine and di-, tri-, and tetraglycine as well as peptide amide fragments of tetraglycine. This method detects only those oxidation products which contain amine nitrogens.

Analysis for Carbonyl. The autoxidation products were analyzed for carbonyl content by the method of Anbar, Levitzki, and Berger.[26] A 1-mL sample was mixed with 10 mL of 5% trichloroacetic acid

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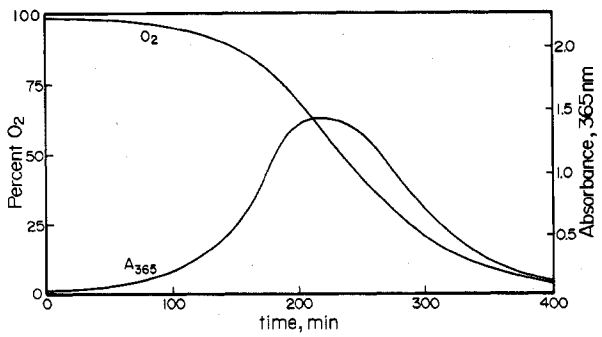


Figure 1. Progress of autoxidation of copper(II) tetraglycine at 25 °C is monitored by O_2 uptake and Cu(III) formation and decay. A solution of 2.5×10^{-3} M $[Cu^{II}G_4]_T$ at pH 7.50 in 0.018 M 2,6-lutidine buffer is initially saturated with O_2 (1 atm) and the sample is divided. The O_2 concentration is followed polarographically and the concentration of $Cu^{III}(H_{-3}G_4)^-$ is monitored from the absorbance at 365 nm in a 1-cm cell.

Table I. Effect of Copper(III) Tetraglycine on the Induction Time for O_2 Uptake by Copper(II) Tetraglycine

$[Cu^{III}(H_{-3}G_4)^-]$,[a] M	t_{ind},[b] min	$[Cu^{III}(H_{-3}G_4)^-]$,[a] M	t_{ind},[b] min
0	187	2.5×10^{-4}	12
2.5×10^{-5}	48	5.0×10^{-4}	8
1.25×10^{-4}	18		

[a] Electrochemically prepared $Cu^{III}(H_{-3}G_4)^-$ added to 5.0×10^{-3} M $[Cu^{II}G_4]_T$ at pH 8.2. [b] Time required to consume 5% saturation of O_2 at 25.0 °C.

and 1 mL of 0.1% 2,4-dinitrophenylhydrazine in 2 M HCl. After 15 min, 20 mL of ethyl acetate was added, and the phases were mixed for 20 s. Then 3.0 mL of 10% Na_2CO_3 was added and the phases were again mixed. After phase separation, 5.0 mL of the aqueous phase was added to 4.0 mL of 2 M NaOH, and absorbance was measured at 440 nm after an additional 15 min.

Results

Initiation and Inhibition of Autoxidation. When a solution of copper(II) tetraglycine, pH 7–9, is saturated with O_2, there is little O_2 uptake observed initially. However, after an induction period (Figure 1) the O_2 uptake rate increases autocatalytically until the depletion of O_2 causes the rate to decrease again. Below pH 7 and above pH 10 no measurable amounts of O_2 are consumed over a period of 3–4 h at 25 °C.

The induction time (which we will define as the time required for the O_2 concentration to decrease by 5%) becomes smaller as the concentration of $[Cu^{II}G_4]_T$ increases, where $[Cu^{II}G_4]_T = [Cu^{II}(H_{-3}G_4)^{2-}] + [Cu^{II}(H_{-2}G_4)^-] + [Cu^{II}(H_{-1}G_4)]$, and as the temperature increases. The induction time is shortened by the addition of small quantities of oxidants such as H_2O_2 and $K_2S_2O_8$. Small amounts of electrochemically prepared $Cu^{III}(H_{-3}G_4)^-$ greatly shorten the induction time (Table I), and larger amounts cause the maximum rate of O_2 uptake to be established immediately.

As reported previously,[7] room light inhibits the reaction, greatly lengthening the induction time for O_2 uptake. Hence the present reactions are carried out in the dark. However, once autoxidation becomes rapid, the system becomes relatively insensitive to room light or even to brief exposures of intense light. Longer exposure (~500 s) to a 75-W tungsten iodide lamp causes photodecomposition of $Cu^{III}(H_{-3}G_4)^-$ and its loss quenches the O_2 reaction until another induction period elapses. [The O_2 monitor is very sensitive to temperature, and intense light can cause a spurious response even with a thermostated compartment. This effect probably caused what appeared to be a rapid on/off photoinhibition reported earlier.[7] Additional photochemical studies are needed, but it is clear that Cu(III) species are photochemically active.]

The O_2 uptake reaction is stopped by the addition of reducing agents (SO_3^{2-}, ascorbic acid, hydroquinone, I^-, SCN^-, or di-*tert*-butyl nitroxide). However, Br^- has little effect.

DPPH (1,1-diphenyl-2-picrylhydrazyl) is a stable nitrogen free radical only slightly soluble in water and is frequently used as a radical scavenger. The effect of trace concentrations of DPPH (2×10^{-7}–1.5×10^{-6} M) on the reaction of $[Cu^{II}G_4]_T$ with O_2 is studied at 37 °C. The length of the induction time is in direct proportion to the amount of DPPH added. However, after the induction period, the maximum rate of O_2 uptake is not altered by the DPPH. The induction period at various levels of DPPH is independent of the O_2 concentration and is independent of pH between 7.5 and 9.0. However, the induction period is shortened as the $[Cu^{II}G_4]_T$ concentration is increased. When $[Cu^{II}G_4]_T$ is 3.4×10^{-3} M, pH 8.0, 37 °C, the rate of loss of DPPH (measured from the difference in induction periods) is 1.1×10^{-9} M s^{-1}.

The autoxidation also can be followed by measuring the absorbance increase at 365 nm, which is due to the formation of Cu(III) and is observable within a few minutes after mixing $[Cu^{II}G_4]_T$ with O_2. This exponential absorbance increase is a more sensitive measure of the progress of the autoxidation than is O_2 uptake. During the apparent induction period observed with the O_2 monitor, there is a continuous increase in the concentration of Cu(III) species.

Identity of the Reactants and Intermediates. For the pH range over which the autoxidation occurs the main copper(II) tetraglycine species is $Cu^{II}(H_{-2}G_4)^-$ with smaller concentrations of $Cu^{II}(H_{-3}G_4)^{2-}$ and $Cu^{II}(H_{-1}G_4)$ present. These species rapidly attain equilibrium.[3] The possibility of an O_2 complex of $Cu^{II}(H_{-2}G_4)^-$ was considered, but we could not find evidence for measurable amounts of such a species. Two methods of detection were used. Difference spectra for N_2-saturated vs. O_2-saturated solutions of $Cu^{II}(H_{-2}G_4)^-$ failed to show absorbance changes which might be assigned to an O_2 complex, although the slow growth of a Cu(III) complex was detected. The O_2 monitor failed to show any immediate O_2 uptake and failed to show any O_2 release upon acidification of freshly mixed solutions of $Cu^{II}(H_{-2}G_4)^-$ and O_2. The latter results indicate that if a complex of $[Cu(H_{-2}G_4)\cdot O_2]^-$ exists, it has a stability constant less than 10 M^{-1} (based on the sensitivity of the O_2 monitor).

Appreciable concentrations of a Cu(III) complex are formed slowly when solutions of $Cu^{II}(H_{-2}G_4)^-$ (10^{-3} M or higher) are saturated with O_2. Evidence for the existence of trivalent copper complexes and a description of their properties are given in other work.[4,5,7] The complex formed in this autoxidation process is identified as $Cu^{III}(H_{-3}G_4)^-$ based on its redox properties, its spectrum, and the kinetics of its decomposition. The Cu(III) species formed by the O_2 reactions decomposes at the same rate, with the same pH dependence, and the same $Cu^{II}(H_{-2}G_4)^-$ dependence (described later) as $Cu^{III}(H_{-3}G_4)^-$ solutions prepared electrochemically.

After the completion of the oxygenation and the decay of the $Cu^{III}(H_{-3}G_4)^-$ complex, a new species is observed in the peptide analyzer. The elution time for this species is similar to that for G_4, but after mild hydrolysis it is converted mainly to glycylglycinamide (GGa) and to an equivalent amount of a carbonyl compound. The intermediate is believed to be a dehydropeptide (G_4DHP) which hydrolyzes according to eq

$$NH_2CH_2CONHCH_2CON{=}CHCONHCH_2COO^- \xrightarrow{H_2O} NH_2CH_2CONHCH_2CONH_2 + HCOCONHCH_2COO^- \quad (1)$$

1 to give GGa and glyoxylglycine. A species with the same elution time, which also hydrolyzes to give GGa and carbonyl, is the sole oxidized ligand product found in the acid decomposition of $Cu^{III}(H_{-3}G_4)^-$.[27] We believe this intermediate

Table II. Stoichiometry of Autoxidation of Copper(II) Tetraglycine[a]

amt of O_2 consumed $\times 10^4$, M	amt of G_4 consumed $\times 10^4$, M	amt of G_2a produced $\times 10^4$, M	$\Delta[G_4]/\Delta[O_2]$	$\Delta[G_2a]/\Delta[O_2]$
2.45	5.90	3.25	2.4	1.3
4.83	9.20	5.88	1.9	1.2
7.38	13.8	9.20	1.9	1.3

[a] 2.5×10^{-3} M $[Cu^{II}G_4]_T$ at pH 8.6 allowed to take up O_2 from saturated solution; reaction quenched by sweeping with N_2.

to be the same in both cases. A similar type of reaction was observed in the decomposition of $Ni^{III}(H_{-3}G_4)^-$ except that the oxidation occurred at a carboxylate terminal giving as the initial products CO_2 and a *N*-(hydroxymethyl)amide, $H_2NCH_2CONHCH_2CONHCH_2CONHCH_2OH$, which hydrolyzed to G_3a and HCHO.[9] Neither CO_2 nor HCHO was found in the Cu–G_4 oxidation products.

Products and Stoichiometry of the Autoxidation. After the oxygenation is complete (and the $Cu^{III}(H_{-3}G_4)^-$ species is decomposed), the solutions no longer have oxidizing ability. Tests for the presence of hydrogen peroxide among the oxidation products were negative. The fluorometric test used[28] is capable of detecting hydrogen peroxide at extremely low levels. Therefore all the oxygen consumed during the reaction is reduced to water and the oxidized ligand products.

The ultimate products of the autoxidation after acidification are Cu(II), GGa, and glyoxylglycine. Smaller quantities of other fragments including glycylglycine and glycinamide are found. These are the same products observed at pH 7–9 for the decomposition (in the absence of O_2) of electrochemically prepared $Cu^{III}(H_{-3}G_4)^-$.[27] The observation that oxidation of the ligand occurs predominantly at the third peptide residue from the amine terminal is also consistent with results using $IrCl_6^{2-}$ oxidation of copper(II) peptide complexes.[26,29]

The stoichiometry of the reaction was determined by measuring the O_2 uptake and then quenching the reaction by sweeping the solution with N_2, when 20, 40, and 60% of the O_2 initially present had been consumed. These samples were analyzed for their carbonyl products and for their ninhydrin-active peptide products. The results in Table II show that the ratio of G_4 lost to O_2 consumed is approximately 2:1 and that GGa accounts for 55–67% of the G_4 consumed. Most of the remaining ninhydrin activity is recovered as G_2, G, and Ga, although a small decrease from the initial level of ninhydrin-active material is observed. The overall reaction stoichiometry observed is expressed in eq 2 where x represents

$$2G_4 + O_2 \rightarrow 1.3GGa + 1.3(HCOCONHCH_2COO^-) + x \quad (2)$$

a mixture of other products resulting from the oxidation of G_4.

Kinetic Measurements. The processes of Cu(III) formation, Cu(III) decomposition, and O_2 consumption all occur simultaneously during the autoxidation. In analyzing the kinetics of the system we have, insofar as possible, studied these processes independently.

Decomposition of $Cu^{III}(H_{-3}G_4)^-$ in the Absence of O_2. The decomposition of electrochemically generated $Cu^{III}(H_{-3}G_4)^-$ in the absence of O_2 between pH 7 and 9 gives a recovery of approximately 50% G_4 and gives 33% GGa and other oxidized ligand products as in eq 2. The loss of $Cu^{III}(H_{-3}G_4)^-$ is first order in $Cu^{III}(H_{-3}G_4)^-$ when the concentration of copper(II) tetraglycine is held constant. Since copper(II) tetraglycine is one of the products of the reaction (at pH 7–9), its concentration may be maintained essentially constant either by carrying out the reaction in the presence of a large excess of copper(II) tetraglycine or by scavenging the copper(II) tetraglycine as it forms. Data for the decomposition of 1×10^{-5} M $Cu^{III}(H_{-3}G_4)^-$ in the presence of copper(II) tetraglycine are plotted in Figure 2. As the $[Cu^{II}G_4]_T$ concentration increases above 10^{-4} M its effect reaches a limiting value and the observed rate constant for $Cu^{III}(H_{-3}G_4)^-$ decomposition becomes independent of the $[Cu^{II}G_4]_T$. The intercept in Figure 2 was measured in the presence of 10^{-4} M EDTA as a scavenger for $[Cu^{II}G_4]_T$ produced during the decomposition. At this concentration EDTA does not affect the decomposition rate itself and the $Cu^{II}EDTA^{2-}$ formed does not catalyze the reaction. Hence excellent first-order plots are obtained with a rate constant of 4×10^{-5} s^{-1} in borate buffer at pH 8.6.

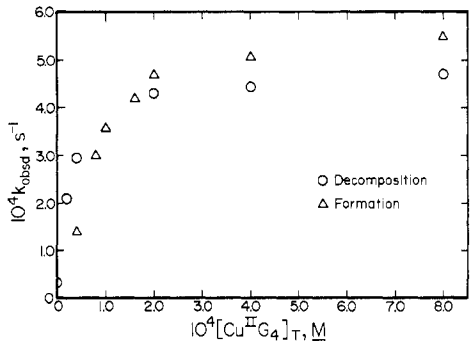


Figure 2. Dependence of the first-order rate constants (k_{obsd}) for $Cu^{III}(H_{-3}G_4)^-$ formation (in the presence of O_2) and for $Cu^{III}(H_{-3}G_4)^-$ decomposition (in the absence of O_2) on the concentration of $[Cu^{II}G_4]_T$. Conditions: pH 8.60 in 0.01 M $[H_3BO_3]_T$, 0.10 M $NaClO_4$, 25.0 °C; formation at 1.2×10^{-3} M O_2; decomposition with initial $[Cu^{III}(H_{-3}G_4)^-]$ of 1.0×10^{-5} M and no O_2.

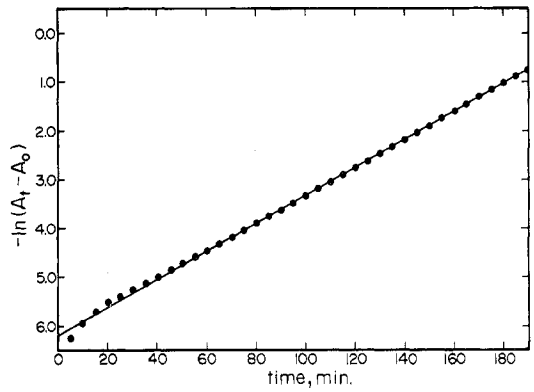


Figure 3. Autocatalytic formation of $Cu^{III}(H_{-3}G_4)^-$ where the reaction is first order in product. A_0 is the initial absorbance (365 nm) and A_t is the absorbance at time t after mixing with dissolved O_2. Conditions: pH 8.60 in 0.01 M $[H_3BO_3]_T$, 0.10 M $NaClO_4$, 25.0 °C, 5×10^{-4} M $[Cu^{II}G_4]_T$, 6×10^{-4} M O_2.

Kinetics of $Cu^{III}(H_{-3}G_4)$ Formation in the Autoxidation. The formation of $Cu^{III}(H_{-3}G_4)^-$ was observed by following the 365-nm absorbance (A_t) of an O_2-saturated solution of copper(II) tetraglycine undergoing autoxidation. When these data were plotted as $\ln(A_t - A_0)$ vs. time, where A_0 is the absorbance before O_2 is added, plots were obtained with long linear segments as shown in Figure 3. This plot corresponds to the rate expression (3) where k_{obsd} is a first-order rate

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

constant *but the reaction is first order in product* and hence is autocatalytic. Early in the reaction the difference between A_t and A_0 is very small and the same type of scatter is observed as found near the end of first-order reactions (where A_t and A_∞ approach each other). The integrated rate expression derived from eq 3 requires some finite concentration of $Cu^{III}(H_{-3}G_4)^-$ at the beginning of the reaction. The intercept

Table III. Rate Constants for Autocatalytic $Cu^{III}(H_{-3}G_4)^-$ Formation at 25.0 °C and 0.10 M $NaClO_4$

run	pH	buffer medium	$[Cu^{II}G_4]_T$, M	$[O_2]_0$, M	k_{obsd}, s^{-1}
1	7.0	unbuffered	5.0×10^{-4}	1.2×10^{-3}	5.5×10^{-4}
2	8.0	unbuffered	2.0×10^{-3}	1.2×10^{-3}	5.6×10^{-4}
3	8.0	unbuffered	2.0×10^{-4}	1.2×10^{-3}	5.1×10^{-4}
4	8.6	borate	8.2×10^{-4}	1.2×10^{-3}	5.5×10^{-4}
5	8.6	borate	4.1×10^{-4}	1.2×10^{-3}	5.1×10^{-4}
6	8.6	borate	2.1×10^{-4}	1.2×10^{-3}	4.7×10^{-4}
7	8.6	borate	1.6×10^{-4}	1.2×10^{-3}	4.2×10^{-4}
8	8.6	borate	1.0×10^{-4}	1.2×10^{-3}	3.6×10^{-4}
9	8.6	borate	8.2×10^{-5}	1.2×10^{-3}	3.0×10^{-4}
10	8.6	borate	4.1×10^{-5}	1.2×10^{-3}	1.4×10^{-4}
11	8.6	borate	2.1×10^{-4}	1.2×10^{-3}	4.5×10^{-4}
12	8.6	borate	2.1×10^{-4}	6.0×10^{-4}	4.1×10^{-4}
13	8.6	borate	2.1×10^{-4}	2.4×10^{-4}	3.3×10^{-4}
14	8.6	borate	2.1×10^{-4}	1.2×10^{-4}	2.5×10^{-4}

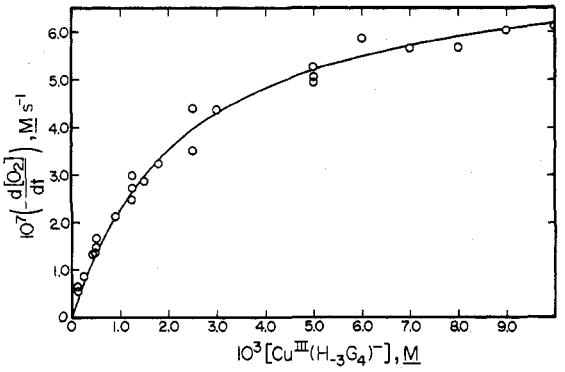


Figure 4. Dependence of the initial rate of O_2 uptake on $[Cu^{III}(H_{-3}G_4)^-]$. Solid line calculated from eq 7 with $k_1 = 3.6 \times 10^{-4}$ s^{-1} and $k_3/k_2 = 0.5$. Conditions: pH 7.5 in 0.018 M 2,6-lutidine buffer, 0.10 M $NaClO_4$, 25.0 °C, 2.5×10^{-3} M $[Cu^{II}G_4]_T$, 1.1×10^{-3} M $[O_2]_i$.

in Figure 3 corresponds to a value of 5×10^{-8} M $Cu^{III}(H_{-3}G_4)^-$.

Values for the autocatalytic rate constant (k_{obsd}) under a variety of conditions are given in Table III. The first four values show that k_{obsd} does not change significantly with pH, the presence or absence of borate buffer, and the initial $[Cu^{II}G_4]_T$ concentration, as long as Cu(II) is greater than 2×10^{-4} M. Runs 4–10 show that as the $[Cu^{II}G_4]_T$ concentration is reduced below 2×10^{-4} M, the k_{obsd} values decrease (see also Figure 2). Runs 11–14 show that k_{obsd} decreases somewhat as the initial O_2 concentration is lowered.

Kinetics of O_2 Uptake. Studying the kinetics of oxygen uptake during autoxidation of $Cu^{II}G_4$ posed difficulties because several hours may elapse before appreciable rates of O_2 uptake are observed. The addition of traces of electrochemically prepared $Cu^{III}(H_{-3}G_4)^-$ causes O_2 consumption to proceed at measurable rates without an induction period. It was thus possible to circumvent the irreproducibility of working with a system of uncertain composition by measuring the initial rate of O_2 uptake of synthetic mixtures of $Cu^{II}G_4$ and electrochemically prepared $Cu^{III}(H_{-3}G_4)^-$. It is observed that at higher $[Cu^{II}G_4]_T$ concentrations the rate of O_2 uptake is essentially independent of Cu(II) concentration, but below 10^{-3} M $[Cu^{II}G_4]_T$ the O_2 uptake rate is lessened. Figures 4 and 5 show the dependence of the initial rate upon $Cu^{III}(H_{-3}G_4)^-$ concentration and upon O_2 concentration, respectively. In both plots there is a trend from first order toward zero order as the concentration of the reactant being varied rises. It is also clear from Figure 5 that the concentration at which the O_2 dependence saturates increases with increasing concentration of $Cu^{III}(H_{-3}G_4)^-$.

Mechanism of $Cu^{III}(H_{-3}G_4)^-$ Decomposition and of O_2 Uptake. Electrochemically prepared $Cu^{III}(H_{-3}G_4)^-$ takes up O_2 as it decomposes at high pH. The concentration dependences of O_2 uptake in Figures 4 and 5 suggest a similar process. Equations 4, 5, and 6 can explain the kinetics where

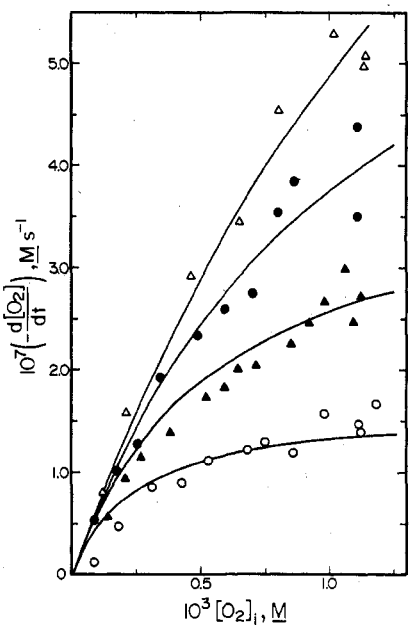


Figure 5. Dependence of the initial rate of O_2 uptake on the O_2 concentration. Solid lines calculated from eq 7 with $k_1 = 3.6 \times 10^{-4}$ s^{-1} and $k_3/k_2 = 0.5$. Conditions: pH 7.5 in 0.018 M lutidine buffer, 0.10 M $NaClO_4$, 25.0 °C, 2.5×10^{-3} M $[Cu^{II}G_4]_T$. $[Cu^{III}(H_{-3}G_4)^-]_i$, M: ○, 0.5×10^{-3}; ▲, 1.25×10^{-3}; ●, 2.5×10^{-3}; △, 5.0×10^{-3}.

$$Cu^{III}(H_{-3}G_4)^- \xrightarrow{k_1} R + H^+ \quad (4)$$

$$R + O_2 \xrightarrow{k_2} RO_2 \quad (5)$$

$$R + Cu^{III}(H_{-3}G_4)^- \xrightarrow{k_3} Cu^{II}(H_{-1}G_4DHP) + Cu^{II}(H_{-3}G_4)^{2-} \quad (6)$$

R is a reactive intermediate (either a carbon-centered free radical or a Cu(I) complex), RO_2 is either a peroxy radical or a copper(III) peroxide, and G_4DHP refers to the dehydropeptide given in eq 1. Treatment of R as a steady-state intermediate gives eq 7.

$$-\frac{d[O_2]}{dt} = \frac{k_1k_2[Cu^{III}(H_{-3}G_4)^-][O_2]}{k_2[O_2] + k_3[Cu^{III}(H_{-3}G_4)^-]} \quad (7)$$

This rate expression is in qualitative agreement with the initial rate data in Figures 4 and 5. As a test of the quantitative agreement, these data were fit to eq 7 by a nonlinear regression analysis, which gives $k_1 = 3.6 \times 10^{-4}$ s^{-1} and $k_3/k_2 = 0.5$. The quality of the fit was reasonable as may be seen from the solid curves in Figures 4 and 5.

Equations 4 and 5 represent the O_2 uptake process while eq 4 and 6 represent the decomposition of $Cu^{III}(H_{-3}G_4)^-$. If $k_1 << k_2[O_2]$ and if $k_1 << k_3[Cu^{III}(H_{-3}G_4)^-]$, then the rates of $Cu^{III}(H_{-3}G_4)^-$ decomposition and of O_2 uptake will be related. Decomposition of $Cu^{III}(H_{-3}G_4)^-$ will have a rate constant equal to $2k_1$ in the absence of O_2 while the rate of O_2 uptake is given by eq 7.

A further quantitative test of the agreement between experiment and the proposed mechanism is obtained by comparing values of k_1 measured by Cu^{III} decomposition to those calculated from initial rates of O_2 uptake using eq 7 and the value of k_3/k_2 obtained from the nonlinear fit. As seen in Figure 6 there is agreement between the values of k_1 obtained from O_2 uptake and from $Cu^{III}(H_{-3}G_4)$ decomposition at different initial $[Cu^{II}G_4]_T$ concentrations. Both processes have

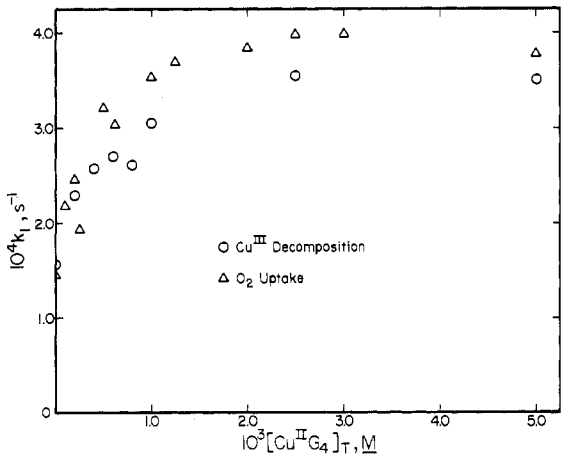


Figure 6. Values of k_1 as a function of $[Cu^{II}G_4]_T$ obtained from the initial rate of decomposition of $Cu^{III}(H_{-3}G_4)^-$ (O) and from O_2 uptake (Δ). Conditions: pH 7.5 in 0.018 M 2,6-lutidine buffer, 0.10 M $NaClO_4$, 25.0 °C. $Cu^{III}(H_{-3}G_4)^-$ data under N_2. O_2 uptake data with 1.2×10^{-3} M O_2.

Table IV. Determination of the Rate Constant for $Cu^{III}(H_{-3}G_4)^-$ Decomposition at 25.0 °C and 0.1 M $NaClO_4$

evaluated from	10^4k_1, s^{-1}	$[Cu^{II}G_4]_T$, M	buffer medium
initial rate of O_2 uptake	3.6	2.5×10^{-3}	2,6-lutidine, pH 7.5
initial rate of Cu^{III} decompn	3.5	$(2.5\text{-}5.0) \times 10^{-3}$	2,6-lutidine, pH 7.5
autocatalytic Cu^{III} formn	2.8	2.0×10^{-3}	unbuffered, pH 8.0
autocatalytic Cu^{III} formn	2.8	5.0×10^{-4}	unbuffered, pH 7.0
autocatalytic Cu^{III} formn	2.8	8.0×10^{-4}	borate, pH 8.6
first-order Cu^{III} decompn	2.4	8.0×10^{-4}	borate, pH 8.6

approximately the same limiting rate at high $[Cu^{II}G_4]_T$ concentrations and the same dependence upon $[Cu^{II}G_4]_T$ concentrations. These data show that the k_1 value is dependent on the $[Cu^{II}G_4]_T$ concentration.

Proposed Mechanism of $Cu^{III}(H_{-3}G_4)^-$ Formation. The $Cu^{III}(H_{-3}G_4)^-$ decomposition and O_2 uptake data are consistent with the steps given in eq 4–6. The autocatalytic formation of $Cu^{III}(H_{-3}G_4)^-$ requires further explanation. The reactive intermediate RO_2 is a strong oxidizing agent which can generate additional Cu(III) as well as the oxidized ligand (dehydropeptide) as shown in eq 8. If $k_2[O_2] >> k_3$-

$$RO_2 + 3Cu^{II}(H_{-3}G_4)^{2-} \xrightarrow{\text{rapid steps}} Cu^{II}(H_{-1}G_4DHP) + 3Cu^{III}(H_{-3}G_4)^- \quad (8)$$

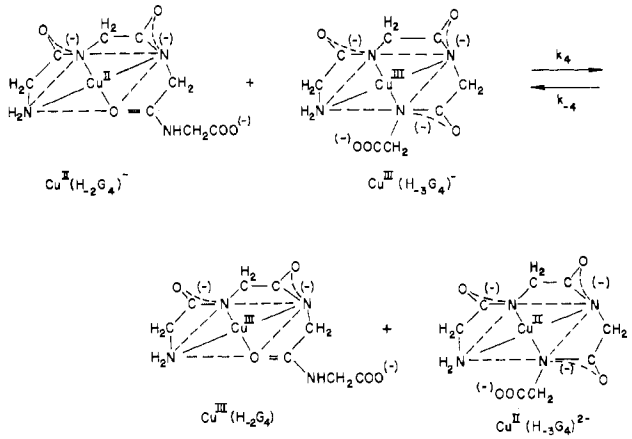
$[Cu^{III}(H_{-3}G_4)^-]$, then the contribution from eq 6 will be small and the sum of eq 4, 5, 7, and 8 gives eq 9 which accounts for the autocatalytic production of $Cu^{III}(H_{-3}G_4)^-$. Equation 9

$$Cu^{III}(H_{-3}G_4)^- + 3Cu^{II}(H_{-3}G_4)^{2-} + O_2 + 4H^+ \rightarrow 3Cu^{III}(H_{-3}G_4)^- + Cu^{II}(H_{-1}G_4DHP) + 2H_2O \quad (9)$$

shows that in the presence of O_2 the decomposition of one $Cu^{III}(H_{-3}G_4)^-$ results in a net gain of two $Cu^{III}(H_{-3}G_4)^-$. This accounts for the autocatalytic plots such as Figure 3. If eq 4 is the rate-determining step, the entire process is first order in $Cu^{III}(H_{-3}G_4)^-$ and independent of the concentration of other reactants in accord with eq 3.

Table IV summarizes values of k_1 determined by three distinct methods: (1) O_2 uptake rates, (2) $Cu^{III}(H_{-3}G_4)^-$ decomposition rates in the absence of O_2, and (3) $Cu^{III}(H_{-3}G_4)^-$ autocatalytic formation in the presence of O_2. Data

Scheme I



obtained under the same conditions (pH, $[Cu^{II}G_4]_T$, and the buffer used) give the same k_1 values.

Copper(II) Tetraglycine Catalysis. The autocatalytic Cu^{III} formation exhibits a $[Cu^{II}G_4]_T$ dependence which saturates at high $[Cu^{II}G_4]_T$ concentrations (Figure 2 and Table III) as previously seen for O_2 uptake and $Cu^{III}(H_{-3}G_4)^-$ decomposition rate constants in Figure 6. Both first-order $Cu^{III}(H_{-3}G_4)^-$ decomposition and autocatalytic $Cu^{III}(H_{-3}G_4)^-$ formation exhibit a catalysis by Cu(II) which saturates at 2×10^{-4} M $[Cu^{II}G_4]_T$ (Figure 2). The limiting rates for both processes are nearly equal; however, the agreement is not perfect.

The major Cu(II) complex over the pH range for the autoxidation is $Cu^{II}(H_{-2}G_4)^-$. It is proposed that the catalysis by Cu(II) involves the oxidation of this species by $Cu^{III}(H_{-3}G_4)^-$ (eq 10). As the structures in Scheme I indicate,

$$Cu^{II}(H_{-2}G_4)^- + Cu^{III}(H_{-3}G_4)^- \underset{k_{-4}}{\overset{k_4}{\rightleftharpoons}} Cu^{III}(H_{-2}G_4) + Cu^{II}(H_{-3}G_4)^{2-} \quad (10)$$

eq 10 is not a proton-transfer reaction (which would require substitution reactions with sluggish Cu(III) complexes) but is an uphill electron-transfer reaction (on the basis of the variation of $E°$ values with ligand donors,[5] eq 10 would have a $\Delta E°$ value of about −0.3 V). Other reactions of Cu^{III} peptides have very rapid electron-transfer rate constants[6] and this pathway could be important if $Cu^{III}(H_{-2}G_4)$ decomposes rapidly (eq 11). The higher oxidizing power of the Cu^{III}-

$$Cu^{III}(H_{-2}G_4) + OH^- \xrightarrow{k_5} R + H_2O \quad (11)$$

$(H_{-2}G_4)$ species compared to $Cu^{III}(H_{-3}G_4)^-$ should favor more rapid ligand oxidation. A similar effect is observed in the greater instability of copper(III) tripeptide complexes compared to copper(III) tetrapeptide complexes.[5] Treatment of $Cu^{III}(H_{-2}G_4)$ as a steady-state intermediate in eq 10 and 11 gives the expanded expression for k_1 in eq 12, where $k_1°$ is

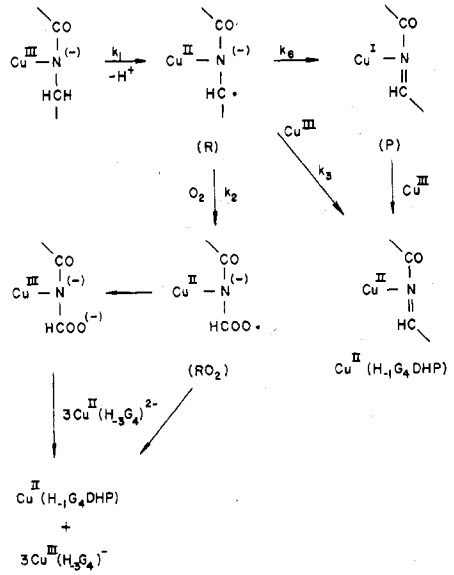
$$k_1 = k_1° + \frac{k_4k_5[OH^-][Cu^{II}(H_{-2}G_4)^-]}{k_{-4}[Cu^{II}(H_{-3}G_4)^{2-}] + k_5[OH^-]} \quad (12)$$

the value of k_1 as the concentration of $Cu^{II}(H_{-2}G_4)^-$ approaches zero (i.e., a Cu(II)-independent pathway as shown in the intercept of Figure 2). Letting $[Cu^{II}(H_{-3}G_4)^{2-}] = (K_3/K_w)[Cu^{II}(H_{-2}G_4)^-][OH^-]$, where K_3 is the ionization constant for the first peptide nitrogen, and substituting into eq 12 gives

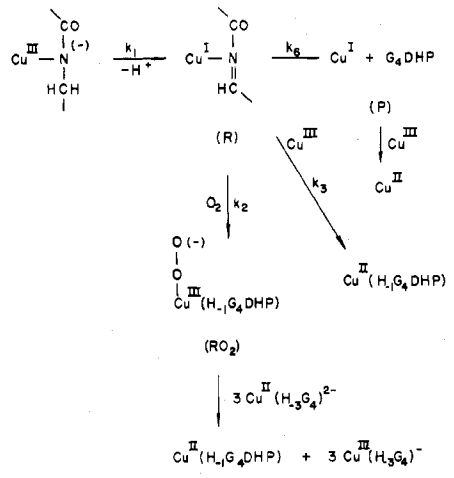
$$k_1 = k_1° + \frac{k_4k_5[Cu^{II}(H_{-2}G_4)^-]}{(K_3/K_w)k_{-4}[Cu^{II}(H_{-2}G_4)^-] + k_5} \quad (13)$$

This corresponds to the observed behavior in which the k_1 value is pH independent and the copper(II) catalysis saturates at

Scheme II



Scheme III



high concentrations ($k_1 = k_1° + (k_4/k_{-4})k_5$). From the data in Table III the ratio $k_5/k_{-4} \approx 4$. The fact that the ligand oxidation occurs predominantly at the third glycyl residue (rather than at the fourth glycyl residue as is the case with nickel) is consistent with the decomposition of $Cu^{III}(H_{-2}G_4)$, in which only three nitrogens (an amine group and two deprotonated peptides) are coordinated. Equation 11 corresponds to the loss of a methylene proton from the third glycyl residue and the reduction of the Cu(III) center as shown in Schemes II and III.

Buffer Medium Effects. The kinetics of $Cu^{III}(H_{-3}G_4)^-$ formation and decomposition are dependent upon the buffer medium. Data for borate buffer are comparable to those for unbuffered solutions. By comparison, in 2,6-lutidine buffer at pH 7.5 the rates of O_2 uptake and of Cu(III) decomposition (Figure 6) require higher $[Cu^{II}G_4]_T$ concentrations to attain the limiting values. This effect is much more pronounced in Tris buffer. The rate of decomposition of $Cu^{III}(H_{-3}G_4)^-$ at low $[Cu^{II}G_4]_T$ concentrations is diminished. The autoxidation reaction does not occur at all in 0.010 M Tris.

An Additional O_2 Dependence. At lower O_2 concentrations the data in Table III show an O_2 dependence even when $k_2[O_2] >> k_3[Cu^{III}(H_{-3}G_4)^-]$. Hence this O_2 dependence cannot be due to the competition between the rates in eq 5 and 6. A parallel path in competition with eq 5 is needed. This competitive path cannot be the reverse of eq 4 since this would cause the decomposition of $Cu^{III}(H_{-3}G_4)^-$ to deviate from a first-order dependence. A reaction is proposed (eq 14) in which

$$R \xrightarrow{k_6} P \qquad (14)$$

R decays to a product P which is less reactive with O_2 but may still react rapidly with Cu(III). The nature of the species R, RO_2, and P depends upon the choice between a radical intermediate pathway and a copper(I) dehydropeptide pathway.

Radical Intermediate Pathway. The ionization of a methylene proton from the third glycyl residue of a Cu(III) complex could produce a Cu(II) complex with a carbon-centered radical as shown in Scheme II. This radical (R) could react with O_2 (k_2 step) to give a peroxy radical (RO_2) which would decay in a series of steps to give more $Cu^{III}(H_{-3}G_4)^-$ and the dehydropeptide complex, $Cu^{II}(H_{-1}G_4DHP)$. Higher concentrations of $Cu^{III}(H_{-3}G_4)^-$ would convert the radical to the dehydropeptide (k_3 step) in competition with the O_2 reaction. Low concentrations of both O_2 and $Cu^{III}(H_{-3}G_4)^-$ could cause an internal conversion (k_6 step) to the copper(I) dehydropeptide complex. The RO_2 complex undergoes a series of electron transfers and decomposition steps to regenerate $Cu^{III}(H_{-3}G_4)^-$ and to give the copper(II) dehydropeptide complex. In this mechanism the radical R must be sufficiently long-lived to have the O_2 and Cu(III) steps compete with the rate of internal electron transfer to give the copper(I) dehydropeptide.

The data in Table III indicate that $k_6 \approx k_2[O_2]$ when the O_2 concentration is 10^{-4} M. Since k_2 cannot be larger than 7×10^9 M^{-1} s^{-1} (the diffusion-controlled rate constant), this sets a maximum possible value of 7×10^5 s^{-1} for k_6. Rate constants for internal electron transfer between Co(III) and other metal centers in binuclear complexes[30] can be many orders of magnitude smaller than this. However, the k_6 rate constant in Scheme II is for the transfer of an electron from a high-energy free-radical species to a nearby copper(II). This process might be expected to be very fast, and the limitiing value for k_6 appears to be one difficulty with the radical intermediate mechanism.

Copper(I) and Copper(III) Peroxide Intermediate Pathway. Scheme III gives an alternate mechanism based on a very short-lived Cu^{II}–radical species which decays to a copper(I) dehydropeptide species as a direct result of the k_1 step. This could be considered as an internal two-electron transfer. In this mechanism the chain center R is $Cu^{I}(H_{-1}G_4DHP)$ and the Cu(I) complex must react rapidly with O_2 to give a copper(III) peroxide complex as the RO_2 intermediate. This mechanism as well as that in Scheme II is subject to catalysis by $Cu^{II}(H_{-2}G_4)^-$. The resulting rate expressions are the same, but in Scheme III the reaction center is at copper rather than at carbon.

Most rate constants for the reaction of O_2 with Cu(I) complexes are in the range of 10^2–10^4 M^{-1} s^{-1}.[31] However, these constants are for reactions believed to be one-electron transfers to give $Cu^{II}(O_2^-)$ rather than the proposed two-electron transfer to give $Cu^{III}(O_2^{2-})$. The k_2 rate constant must be nearly the same as the k_3 rate constant to fit the observed behavior. Rate constants for the reaction of bis(2,9-dimethyl-1,10-phenanthroline)copper(I) with copper(III)–peptide complexes are as large as 10^6 M^{-1} s^{-1}.[32] If k_3 is this large, then k_2 must also be large and this requirement may be a difficulty with this mechanism. On the other hand, the k_6 step is a dissociation process (or perhaps a partial unwrapping of the peptide from Cu(I)) which could be slow enough to compete with the k_2 and k_3 steps. Hence there are pros and cons for both mechanisms.

Initiation Reaction. The proposed mechanisms for the autoxidation provide a rationale for the production of significant amounts of $Cu^{III}(H_{-3}G_4)^-$ from an initially very small

(10^{-7}–10^{-8} M) concentration of Cu^{III} or other oxidizing agent.

In the initiation experiments, solutions of $[Cu^{II}G_4]_T$ were swept with N_2 and stoppered overnight. Since the $Cu^{II}G_4$-catalyzed decomposition of $Cu^{III}(H_{-3}G_4)^-$ has $k_{obsd} = 2k_1$ ($\approx 5.0 \times 10^{-4}$ s^{-1}), any small quantities of $Cu^{III}(H_{-3}G_4)^-$ formed during solution preparation from oxidizing impurities would be completely decomposed before the solution was used. This excludes initiation by oxidizing impurities.

The direct reaction of O_2 with $Cu^{II}(H_{-3}G_4)^{2-}$ to give $Cu^{III}(H_{-3}G_4)^-$ and O_2^- is an unlikely initial step because the equilibrium levels of Cu^{III} and O_2^- would be only 10^{-13} M. This can be estimated from the electrode potential for the reduction of O_2[33] and of $Cu^{III}(H_{-3}G_4)^-$.[5] It is possible for two- or four-electron reactions with O_2 to provide sufficient Cu^{III}, but if these reactions could occur rapidly then there would be no need for the autocatalytic pathway which is observed.

Furthermore, the DPPH experiments showed that O_2 did not affect the difference in induction times caused by the addition of this radical scavenger. Hence, it appears that the initiation reaction does not involve O_2.

We propose that the initiation mechanism involves the disproportionation of Cu(II) to give Cu(I) and Cu(III). A very interesting paper by Österberg[34] has shown that the copper triglycine system forms a mixed Cu(I) and Cu(II) complex with a favorable reduction potential ($E^\circ = +0.34$ V at pH 7 for eq 15). If G_4 reacted in the same manner as G_3 then the

$$Cu_2^{II}H_{-4}(G_3)_2^{2-} + H^+ + e^- \rightarrow Cu^ICu^{II}H_{-3}(G_3)_2^{2-} \quad (15)$$

half-reaction in eq 16 ($E^\circ = -0.63$ V)[5] could be coupled with

$$Cu^{II}(H_{-3}G_4)^{2-} \rightarrow Cu^{III}(H_{-3}G_4)^- + e^- \quad (16)$$

a reaction of the type given in eq 15. Writing these reactions in terms of a $Cu^{II}(H_{-2}G_4)^{2-}$ monomer leads to eq 17 which

$$3Cu^{II}(H_{-2}G_4)^- \rightleftarrows Cu^ICu^{II}H_{-3}(G_4)_2^{2-} + Cu^{III}(H_{-3}G_4)^- \quad (17)$$

would have an equilibrium constant of $10^{-6.8}$ M^{-1}. This predicts that a concentration of 10^{-3} M $Cu^{II}(H_{-2}G_4)^{2-}$ would produce 1.3×10^{-8} M $Cu^{III}(H_{-3}G_4)^-$, which is not far from the experimental value of 10^{-7} M Cu(III). However, a log–log plot of the initial Cu(III) found (varying from 10^{-8} to $10^{-6.5}$ M as measured from the intercepts of the autocatalytic plots) vs. the concentration of $[Cu^{II}G_4]_T$ (from 10^{-4} to $10^{-2.5}$ M) gives a slope of unity in accord with eq 18 rather than a slope of

$$2Cu^{II}(H_{-2}G_4)^- \rightleftarrows Cu^I(H_{-1}G_4)^- + Cu^{III}(H_{-3}G_4)^- \quad (18)$$

$^3/_2$ expected from eq 17. The concentrations used in the present study are much lower than those used by Österberg and therefore monomeric species are more likely. The identity of the Cu(I) complex is uncertain and might also be $Cu^IG_4(OH)$ or even $Cu^I(OH)$. An equilibrium constant 10^{-8} for eq 18 fits our data. The fact that Cu(III) persists in trace quantities no matter how carefully $[Cu^{II}G_4]_T$ solutions are prepared or how long they are kept under N_2 strongly supports initiation by a disproportionation mechanism.

Comparison of $Cu^{II}G_4$ and $Ni^{II}G_4$ Autoxidations. The mechanisms for autoxidation for both the $Cu^{II}G_4$ and $Ni^{II}G_4$ reactions are dependent on the decomposition of the trivalent metal complex. The rate of decomposition of $Ni^{III}(H_{-3}G_4)^-$ is approximately 20 times greater than that of $Cu^{III}(H_{-3}G_4)^-$ and this accounts for the faster autoxidation of $Ni^{II}G_4$. The $Ni^{III,II}(H_{-3}G_4)$ potential of 0.79 V is higher than the $Cu^{III,II}(H_{-3}G_4)$ potential of 0.63 V. This may be related to the fact that decarboxylation occurs in the nickel system but not in the copper system. The need for a higher potential to generate the reactive intermediate (a carbon-centered free radical or a Cu(I) species) is consistent with the hypothesis that the reactive copper species is $Cu^{III}(H_{-2}G_4)$ formed by uphill electron transfer.

Conclusions

The overall autoxidation rate expression (except at low concentrations of O_2 and of $Cu^{II}(H_{-2}G_4)^-$) is given in eq 19.

$$-d[O_2]/dt = \frac{k_2k_4k_5[Cu^{III}(H_{-3}G_4)^-][Cu^{II}(H_{-2}G_4)^-][O_2]}{\{(K_3/K_w)k_{-4}[Cu^{II}(H_{-2}G_4)^-] + k_5\}\{k_2[O_2] + k_3[Cu^{III}(H_{-3}G_4)^-]\}} \quad (19)$$

The $Cu^{III}(H_{-3}G_4)^-$ appears to be generated at trace levels by the disproportionation of $Cu^{II}(H_{-2}G_4)^-$ not shown in eq 19. The $Cu^{II}(H_{-2}G_4)^-$ complex also catalyzes the Cu(III) decomposition process which is the rate-determining step for the entire autoxidation. This catalysis requires rapid electron-transfer reactions. The multiple oxidation states of copper, the ability to use copper(III) peptides as a reservoir of oxidizing power, and the rapid electron-exchange reactions of copper make it an effective catalyst in this autoxidation.

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Registry No. $Cu^{II}(H_{-2}G_4)^-$, 67180-35-2; $Cu^{III}(H_{-3}G_4)^-$, 57692-61-2.

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