$(H_{-3}G_2Aa)$, 62801-38-1; $Cu^{III}(H_{-3}PG_2a)$, 24212-63-3; $Cu^{III}(H_{-3}G_5)^-$, 68550-43-6; $Cu^{III}(H_{-3}G_4A)^-$, 69042-74-6; $Cu^{III}(H_{-3}G_4)^-$, 57692-61-2; $Cu^{III}(H_{-3}AG_3)^-$, 69088-03-5; $Cu^{III}(H_{-3}A_4)^-$, 68628-66-0; $Cu^{III}(H_{-3}V_4)^-$, 62959-93-7; $IrCl_6^{3-}$, 14648-50-1; $IrCl_6^{2-}$, 16918-91-5.

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Oxidative Decarboxylation of Glyoxylate Ion by a Deprotonated-Amine Copper(III)–Peptide Complex

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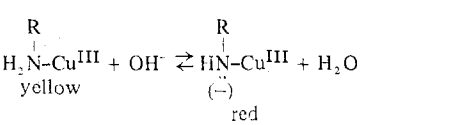
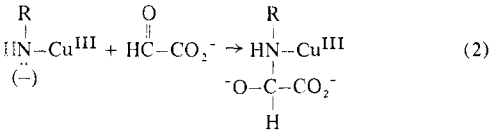
In basic solution copper(III) pentaglycine forms a species, $Cu(H_{-4}G_5)^{2-}$, which has three deprotonated-peptide groups and one deprotonated-amine group coordinated. Acting as a nucleophile, the deprotonated amine attacks glyoxylate ion followed by rapid electron-transfer and decarboxylation steps. The reaction stoichiometry is $2Cu^{III}(H_{-4}G_5)^{2-} + CHOCO_2^- + 2OH^- \rightarrow Cu^{II}(H_{-3}G_5)^{2-} + Cu^{II}(H_{-4}\text{-}N\text{-}fG_5)^{3-} + CO_3^{2-} + H_2O$, where half of the pentaglycine is converted to the *N*-formyl derivative (*N*-fG$_5$). The reaction is first order in the deprotonated-amine species and first order in glyoxylate ion with a rate constant of 1.1×10^6 M^{-1} s^{-1} for the dehydrated form of glyoxylate ion. The rate is pH dependent, with a maximum about pH 13. The rate decreases at higher pH due to the formation of the unreactive glyoxylate dianion. Pyruvate and phenylglyoxylate also react with $Cu^{III}(H_{-4}G_5)^{2-}$ and the relative reactivity is glyoxylate >> pyruvate >> phenylglyoxylate.

Introduction

Copper(III)–peptide complexes[1] can be generated in good yield by chemical or electrochemical oxidation of copper(II)–peptide solutions.[2,3] Above pH 11 the normally yellow copper(III)–peptide solutions turn deep red. Rapid acidification restores the yellow color.[3] Spectral and electrochemical data indicate that reversible deprotonation of the coordinated amine terminus of the peptide[4] occurs (eq 1). Copper(III)–peptide complexes undergo self-redox reactions within a few minutes in strong base. However, the addition of reducing agents can destroy copper(III) more rapidly. When copper(III) pentaglycine is formed in the presence of millimolar concentrations of glyoxylate ion ($CHOCO_2^-$), the red species is immediately lost. Pyruvate ion ($CH_3COCO_2^-$) and phenylglyoxylate ion ($C_6H_5COCO_2^-$) are less reactive and require higher concentrations for rapid quenching to be observed. For the glyoxylate reaction, carbonate ion and *N*-formylpentaglycine are identified as products. It is proposed that the deprotonated-amine copper(III) species, acting as a nucleophile, attacks the carbonyl carbon of glyoxylate to form a carbinolamine species (eq 2). This species undergoes oxidative decarboxylation (eq 3) and reacts rapidly via electron transfer with a second copper(III)–pentaglycine complex to give the observed products.



(1)



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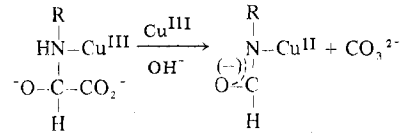

(3)

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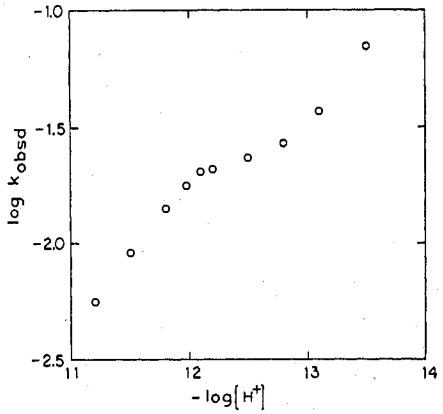


Figure 1. Effect of base on the first-order rate constant for the self-decomposition of copper(III) pentaglycine: 5.0×10^{-5} M $Cu^{III}G_5$, 25.0 °C, μ = 1.0 M (NaOH + $NaClO_4$), reaction followed at 525 nm.

Experimental Section

Pentaglycine (G_5) and L-prolyldiglycinamide hydrobromide (PGGa·HBr) were obtained from Biosynthetika and Vega-Fox Chemicals, respectively. Prior to use, the glyoxylic acid (Eastman) was dried in vacuo. A formula weight of 92.1, determined by titration with NaOH, indicated that the solid was a monohydrate. Freshly prepared solutions were used for glyoxylic acid and the other organic compounds tested in this study.

The copper(II) solutions were prepared from the peptide (in 5–10% mole excess) and copper(II) perchlorate. The copper(III)–peptide solutions were prepared by electrochemical oxidation of the copper(II) complex at pH 10.5, using a flow electrolysis apparatus.[4-6] After electrolysis the eluent was approximately neutral and was adjusted to approximately pH 6 with a few drops of 1 M acetic acid to minimize decomposition of the copper(III) peptide prior to use. A Cary 14 or Cary 16 spectrophotometer was used to measure the absorbance of the solutions at 365 nm, from which the copper(III) concentration could be determined.[2]

Tests with glyoxylate and other organic substrates with copper(III) pentaglycine were carried out in 0.05 M NaOH under nitrogen.

For peptide analysis the samples were acidified to pH 3 with $HClO_4$ and run on a Beckman 121 amino acid analyzer utilizing a glass column (1 cm diameter, 10 cm length) packed with Beckman PA-35 cation-exchange resin. The eluent was a 0.1 M citrate buffer with a pH of 2.8 and ionic strength adjusted to 0.30 M with $NaClO_4$. Separations were carried out at a flow rate of 70 mL h^{-1} and at 55 °C. Peptides and products with amino groups were selectively monitored by a ninhydrin detection system.[7] By use of peptide standards of known composition and peak areas (height times width at half-height[8]), it was possible to identify and quantitate all of the ninhydrin-active species. High-pressure liquid chromatographic separations, using a Varian 4100 HPLC and Waters R401 refractive index detector, were carried out at room temperature on a 50 cm (3 mm i.d.) stainless steel column packed with Aminex Q150S cation-exchange resin (Bio-Rad). A 0.05 M citrate buffer (pH 3, μ = 0.15 M) was used at a flow rate of 80 mL h^{-1}. Chromatograms were traced on a Heath EU20B Servo-Recorder.

A Radiometer E5037-0 pCO_2 electrode connected to a Radiometer PHM26 pH meter was used to measure dissolved carbon dioxide in acidified (0.05 M $HClO_4$) samples at 25.0 °C. The electrode was calibrated immediately prior to use with standard carbonate solutions, also acidified.

Kinetics were studied at 25.0 °C and an ionic strength of 1.0 M ($NaClO_4$ + NaOH) by using a Durrum stopped-flow spectrometer on line with a Hewlett-Packard 2115 computer.[9] An initial copper(III) peptide concentration of 5.0×10^{-5} M was used for these reactions.

N-Formylpentaglycine was isolated by adding electrochemically generated copper(III) pentaglycine directly to a basic glyoxylate solution under nitrogen. The reaction mixture was freeze-dried. The solid was taken up in 0.3 M ammonia and eluted through a 5 × 2 cm diameter column of Chelex 100 (200–400 mesh, Bio-Rad). The eluate was freeze-dried and the solid treated with 10 mL of cold 1 M HCl overnight. The insoluble residue was washed with small volumes of 1 M HCl, 100% ethanol, and ether before drying in vacuo overnight. Samples for NMR spectroscopy (Varian A60A) were dissolved in 1 mL of D_2O with 1 drop of 40% NaOD.

Table I. Decomposition of Copper(III) Pentaglycine in Basic Solution[a]

$[OH^-]$, M	$10^2 k_{obsd}$, s^{-1}	$[OH^-]$, M	$10^2 k_{obsd}$, s^{-1}
0.0025	0.56 ± 0.03	0.025	2.07 ± 0.03[b]
0.0050	0.91 ± 0.06	0.050	2.27 ± 0.04[b]
0.010	1.4 ± 0.1	0.10	2.69 ± 0.05[b]
0.015	1.75 ± 0.07	0.20	3.7 ± 0.1[b]
0.020	2.05 ± 0.07	0.50	7.1 ± 0.3[b]

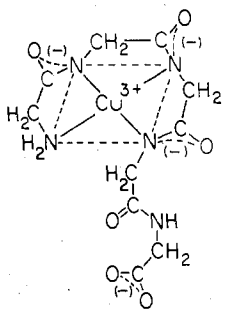
[a] 25.0 °C, μ = 1.0 M ($NaClO_4$), $[Cu^{III}]_0 = 5.0 \times 10^{-5}$ M, 525 nm. [b] Initial rate extrapolation.

Table II. Distribution of Ninhydrin-Active Products Found after the Decay of Copper(III) Pentaglycine in Base

base	% G_5	% Ga	% G_2a	% G_3a	% (G_3 + G_2)	total
0.05 M OH^-	77	10	4	3	5	99
0.05 M OH^-	76	9	7	3	5	100
pH 10.5	76	10	9	3	2	100

Results and Discussion

Reactions of Copper(III) Pentaglycine in Base. In neutral solution the triply deprotonated pentaglycine complex of copper(III), $Cu^{III}(H_{-3}G_5)^-$ as shown in structure I, undergoes



I

a self-decomposition reaction with a half-life of approximately 2 h. The rate of decomposition is base catalyzed as the data in Table I indicate. The amine deprotonation reaction (pK_a = 11.6)[4] to form the red $Cu^{III}(H_{-4}G_5)^{2-}$ species is very rapid and the decomposition of this species is base catalyzed but with a smaller rate constant than $Cu^{III}(H_{-3}G_5)^-$ as shown by the plateau in Figure 1. The rate constants given for hydroxide ion concentrations greater than 0.01 M are taken from initial rate data. The kinetics become more complex as the reaction proceeds because the formation of copper(II) oxidized ligand products catalyze further copper(III) decomposition. The ligand products of the decomposition reaction were analyzed by ion-exchange chromatography, using ninhydrin detection. The results in Table II show a 76% recovery of unoxidized peptide (G_5) and the formation of glycinamide (Ga), diglycinamide (G_2a), triglycinamide (G_3a), and triglycine (G_3) or diglycine (G_2). The latter two species elute together and the 2–5% found refers to their sum. The stoichiometry of the self-decomposition is represented by eq 4, where X refers to

$$4Cu^{III}(H_{-4}G_5)^{2-} \xrightarrow{OH^-} 3Cu^{II}(H_{-3}G_5)^{2-} + Cu^{II}X \quad (4)$$

peptide oxidation products. The initial products in the case of similar decomposition reactions of tetraglycine[10] are dehydropeptides which hydrolyze to form the amides and corresponding carbonyl species. The latter species must be further oxidized by copper(III) pentaglycine in order to ac-

Table III. Reactivity of Copper(III) Pentaglycine under Basic Conditions (0.05 M NaOH) with Organic Substrates

substrate	substrate concn, M	reactivity	peptide products: G_5 recovered	peptide products: others ninhydrin active
none (control)		3 min dec	75 ± 2%	G_3a, G_2a, Ga, and G_2 or G_3
formaldehyde	1×10^{-3}	none[a]	72%	same[a]
formate	1×10^{-3}	none[a]	74 ± 1%	same[a]
benzoate	1×10^{-3}	none[a]	72%	same[a]
oxalate	1×10^{-3}	none[a]	N.D.[b]	N.D.
	3×10^{-2}	none[a]	N.D.	N.D.
glyoxylate	1×10^{-3}	complete upon mixing	49 ± 1%	none
pyruvate	1×10^{-3}	none[a]	N.D.	N.D.
	6×10^{-2}	complete upon mixing	N.D.	N.D.
phenylglyoxylate	1×10^{-3}	none[a]	74 ± 1%	same[a]
	5.4×10^{-2}	~1.5 min dec	N.D.	N.D.

[a] Same as the control sample. [b] Not determined.

count for the stoichiometry in which four Cu(III) are lost for every pentaglycine which is lost. The distribution of amide products in Table II shows that oxidation occurs at each of the carbon atoms adjacent to a coordinated peptide nitrogen. Altogether 99–100% of the initial G_5 concentration is accounted for in terms of the ninhydrin-active products, provided that the 2–5% G_3 (and/or G_2) present is from the hydrolysis of the corresponding amide.

Reactions of Copper(III) Pentaglycine in Base with Glyoxylate Ion and Other Organic Substrates. Table III summarizes the reactivity of the red deprotonated-amine complex, $Cu^{III}(H_{-4}G_5)^{2-}$, with various carbonyl and carboxylate species. In the absence of reducing agents, copper(III) pentaglycine decomposes in about 3 min in 0.05 M NaOH to give pale pink copper(II) species and 75% of the initial G_5 is recovered. The substrates in Table III were mixed with base and then added to solutions of copper(III) pentaglycine, initially at pH 6. The red Cu(III) complex forms within the mixing time and its decay time is unaltered by millimolar concentrations of the substrates, with the exception of glyoxylate ion which immediately destroys the red species. Furthermore, the recovery of G_5 is reduced from 75 to 50% in the presence of glyoxylate and none of the other ninhydrin-active oxidation products (G_3a, G_{2a}, Ga, or G_3/G_2) are found. Although, millimolar concentrations of pyruvate ion ($CH_3COCO_2^-$) and phenylglyoxylate ion ($C_6H_5COCO_2^-$) have no effect, 50–60-fold higher concentrations also lead to a more rapid loss of $Cu^{III}(H_{-4}G_5)^{2-}$. The relative reactivity is glyoxylate ≫ pyruvate ≫ phenylglyoxylate.

Products of the Glyoxylate Ion Reaction. The formation of a $BaCO_3$ precipitate upon the addition of $BaCl_2$ showed that carbonate was a product of the glyoxylate reaction with copper(III) pentaglycine. A pCO_2 electrode was used to determine the stoichiometry (Table IV). The formation of CO_2 corresponds roughly to the amount of ninhydrin-active G_5 which is lost.

Glyoxylate and other α-keto acids are readily oxidized in aqueous solution. Under weakly alkaline conditions periodate,[11,12] ferricyanide,[13] and hydrogen peroxide[14] will oxidize glyoxylate to formate ion and carbon dioxide. In acid, cerium(IV)[15] and dichromate[16] yield the same products. The importance of decarboxylation was indicated by the greatly diminished reactivity of glyoxylic acid ethyl ester with alkaline periodate.[17] The lost peptide was presumed to be the *N*-formyl derivative of pentaglycine, based on work by Nakada and Sund,[18] who isolated a rat liver oxidase which produced *N*-formylglutamic acid and CO_2 from glyoxylate and glutamic acid. Their experiment with labeled glyoxylate confirmed the formyl group transfer. In the present study *N*-formylpentaglycine was isolated from the reaction of copper(III) pentaglycine with glyoxylate ion. This reaction accounts for the loss of 50% of the G_5 and for the carbonate stoichiometry as given in eq 5. *N*-Formylpentaglycine was identified by a limited hydrolysis experiment, NMR spectroscopy, and elemental analysis. Hydrolysis of the isolated product in methanolic HCl by a procedure similar to one developed by Sheehan and Yang[19] results in the recovery of pentaglycine, based on the results of chromatographic analysis using the high pressure LC. The NMR spectrum of the product shows peaks at 8.30 and 8.55 ppm (Me_4Si = 0), corresponding to values observed for aldehydic protons in formamides.[20] Neither pentaglycine nor glyoxylate has peaks in this region. Analysis of the product gave the following. Anal. Calcd for *N*-formylpentaglycine, $C_{11}H_{17}N_5O_7$: C, 39.88; H, 5.17; N, 21.14. Found (adjusted for a 2.5% NaCl impurity): C, 39.91; H, 5.21; N, 20.61.

Table IV. Carbon Dioxide Formed in the Reaction of Copper(III) Pentaglycine with Glyoxylate Ion in 0.01 M NaOH

$[CHOCO_2^-]$, mM	[Cu(III)] used, mM	$[CO_2]$ formed, mM	ratio $[CO_2]/[Cu(III)]$
1.76	0.80	0.35	0.44
1.76	0.80	0.37	0.46
1.76	0.80	0.35	0.44

$$2Cu^{III}(H_{-4}G_5)^{2-} + CHOCO_2^- + 2OH^- \rightarrow Cu^{II}(H_{-3}G_5)^{2-} + Cu^{II}(H_{-4}\text{-}N\text{-}fG_5)^{3-} + CO_3^{2-} + H_2O \quad (5)$$

Formyl group transfer is best explained by nucleophilic attack at the carbonyl group of glyoxylate by the copper(III) deprotonated-amine complex (eq 1 and 2). Only the red complex is reactive with glyoxylate ion. A similar mechanism has been reported for $Ru(NH_3)_6^{3+}$, which reacts with thiosulfate,[21] thiophosphate,[21] or nitric oxide[22] in alkaline solution, presumably as the $Ru(NH_2)(NH_3)_5^{2+}$ complex (pK_a = 12.4 ± 0.5[23]).

The proposed attack also is supported by the order of reactivity of the keto acids. Both steric and electronic factors are involved.[24] For glyoxylate, there is less crowding in the tetrahedral adduct than for pyruvate or phenylglyoxylate. The nucleophilic attack on pyruvate is decreased by the electron-releasing property of the methyl substituent. Although phenyl groups are electron withdrawing, resonance stabilization of the carbonyl group overrides this tendency, making nucleophilic attack even more difficult.

Nakada and Sund proposed a Schiff base intermediate between glyoxylate and glutamate as the reactive species in their oxidase system.[18] This was checked for the copper(III) reaction by using the L-prolyldiglycinamide (PGGa) complex, which cannot form a coordinated Schiff base. The observation of rapid quenching of this species in 1×10^{-3} M glyoxylate rules out Schiff base formation as a necessary step and im-

Table V. Glyoxylate Dependence of $Cu^{III}G_5$–Glyoxylate Reaction[a]

$[CHOCO_2]_T$, mM	k_{obsd}, s^{-1}
0.530	27.5 ± 0.2
0.795	39.2 ± 0.3
1.06	51.1 ± 0.4

[a] 25.0 °C, μ = 1.0 ($NaClO_4$), $[OH^-]$ = 0.05 M, 5.0×10^{-5} M $Cu^{III}G_5$

Table VI. Hydroxide Ion Dependence of $Cu^{III}G_5$–Glyoxylate Reaction[a]

$[OH^-]$, M	k_{obsd}, s^{-1}	$[OH^-]$, M	k_{obsd}, s^{-1}
0.0025	4.35 ± 0.09	0.10	56.6 ± 0.3
0.0040	7.68 ± 0.07	0.125	56.9 ± 0.2
0.0050	10.2 ± 0.2	0.160	54.8 ± 0.2
0.0080	15.5 ± 0.2	0.20	52.3 ± 0.1
0.010	19.3 ± 0.1	0.25	47.5 ± 0.3
0.016	27.1 ± 0.2	0.30	44.8 ± 0.2
0.020	31.0 ± 0.2	0.35	41.1 ± 0.4
0.040	45.2 ± 0.3	0.40	39.6 ± 0.1
0.050	50.6 ± 0.5	0.50	35.7 ± 0.2

[a] 25.0 °C, μ = 1.0 ($NaClO_4$), 5.0×10^{-5} M $Cu^{III}G_5$, 1.0×10^{-3} M glyoxylate.

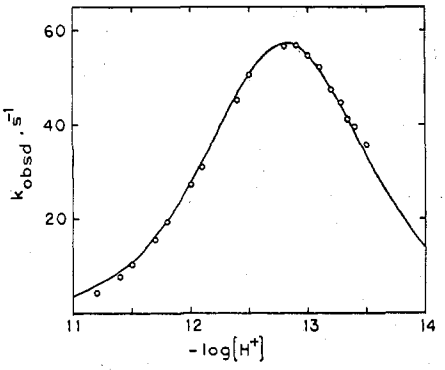


Figure 2. Base dependences of the pseudo-first-order rate constant for the loss of copper(III) pentaglycine in the presence of excess glyoxylate: 5.0×10^{-5} M $Cu^{III}G_5$, 1.0×10^{-3} M glyoxylate, 25.0 °C, μ = 1.0 M (NaOH + $NaClO_4$), 525 nm.

plicates the carbinolamine as the reactive species. The copper(III)–carbinolamine species could decompose to the observed products by several possible intramolecular pathways involving either copper(II) or copper(I) as discussed later.

Kinetics

In the presence of greater than tenfold excesses of glyoxylate, the loss of copper(III) pentaglycine is first order. The glyoxylate dependence was examined in 0.050 M NaOH, where the deprotonated-amine species predominates. The dependence is first order with a slope of 4.5×10^4 M^{-1} s^{-1} (Table V).

The hydroxide dependence of the observed first-order rate constant was examined from 0.0025 to 0.50 M NaOH by using 1.0×10^{-3} M glyoxylate. The results (Table VI) show that k_{obsd} rises rapidly to a maximum before decreasing above 0.10 M OH^-. A symmetrical curve (Figure 2) is obtained when k_{obsd} is plotted against -log $[H^+]$. Thus, two acid–base preequilibria are involved.[25] One is assumed to be the amine deprotonation of copper(III) pentaglycine and the other to be due to the formation of glyoxylate dianion, on the basis of spectral studies of basic glyoxylate solutions. In aqueous solution, glyoxylate ion is extensively hydrated, existing primarily as the *gem*-diol (eq 6). Values of 16.5 ± 2.5[26] and

$$CHOCO_2^- + H_2O \underset{K_{hydr}}{\rightleftharpoons} CH(OH)_2CO_2^- \quad (6)$$

15.1[27] have been determined for K_{hydr}. The dianion can form by either loss of a proton from the *gem*-diol (eq 7) or addition of hydroxide to the aldehyde.[28] The UV spectra imply a pK_a of ~13 (compared to a pK_a of 13.57 for acetaldehyde[28]).

This information, and the assumption that the deprotonated-amine species ($Cu(H_{-4}G_5)^{2-}$) and the dehydrated form of glyoxylate ($CHOCOO^-$) are the reactive species, gives the mechanism (eq 6–10) whose rate expression is shown (eq 11,

$$CH(OH)_2CO_2^- \overset{K_a^{CHO}}{\rightleftharpoons} CH(O)(OH)CO_2^{2-} + H^+ \quad (7)$$

$$Cu^{III}(H_{-3}G_5)^- \overset{K_a^{Cu(III)}}{\rightleftharpoons} Cu^{III}(H_{-4}G_5)^{2-} + H^+ \quad (8)$$

$$Cu^{III}(H_{-4}G_5)^{2-} + CHOCO_2^- \xrightarrow{k} \text{intermediate} \quad (9)$$

$$\text{intermediate} + Cu^{III}(H_{-4}G_5)^{2-} \xrightarrow{\text{fast}} \text{products} \quad (10)$$

12). Equation 12 can be rearranged to give k_{obsd} as a function

$$-d[Cu^{III}]_T/dt = 2k[CHOCO_2^-][Cu(H_{-4}G_5)^{2-}] = k_{obsd}[Cu^{III}]_T \quad (11)$$

$$k_{obsd} = 2k[CHOCO_2^-]([Cu(H_{-4}G_5)^{2-}]/[Cu^{III}]_T) \quad (12)$$

of the total reactant concentrations, $[glyox]_T$ and $[Cu^{III}]_T$ (eq 13 and 14). By use of eq 6, 7, and 14, the concentration of

$$[Cu^{III}]_T = [Cu^{III}(H_{-3}G_5)^-] + [Cu^{III}(H_{-4}G_5)^{2-}] \quad (13)$$

$$[Glyox]_T = [CHOCO_2^-] + [CH(OH)_2CO_2^-] + [CH(O)(OH)CO_2^{2-}] \quad (14)$$

glyoxylate in the dehydrated form can be expressed in terms of $[Glyox]_T$ (eq 15). In a similar manner, the concentration

$$[CHOCO_2^-] = [Glyox]_T/[(K_{hydr} + 1) + (K_{hydr}K_a^{CHO}/[H^+])] \quad (15)$$

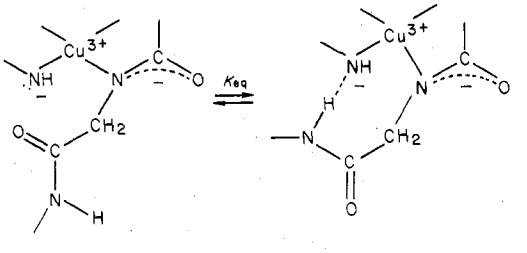
of amino-deprotonated species as a function of $[Cu^{III}]_T$ (eq 16) can be derived using eq 8 and 13. By substitution of eq

$$[Cu(H_{-4}G_5)^{2-}] = [Cu^{III}]_T/[1 + ([H^+]/K_a^{Cu(III)})] \quad (16)$$

15 and 16 into eq 12, the final expression (eq 17) is obtained.

$$k_{obsd} = \frac{2k[Glyox]_T}{((K_{hydr} + 1) + (K_{hydr}K_a^{CHO}/[H^+]))(1 + ([H^+]/K_a^{Cu(III)}))} \quad (17)$$

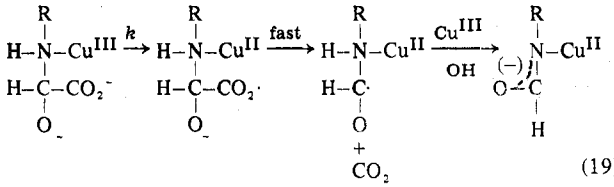
This expression gave a good fit to the experimental data with the following values: K_{hydr} = 15.1,[27] pK_a^{CHO} = 13.05, $pK_a^{Cu(III)}$ = 12.55, and $k = 1.1 \times 10^6$ M^{-1} s^{-1}. These were used to generate the solid curve in Figure 2. While pK_a^{CHO} for glyoxylate is in good agreement with the spectroscopic estimate, a large discrepancy exists between the kinetic (12.6) and the spectroscopic value (11.6 ± 0.1) for $pK_a^{Cu(III)}$. The difference between the spectroscopic and kinetic pK_a for amine deprotonation can be attributed to structural features of $Cu(H_{-4}G_5)^{2-}$. Lower pK_a values are obtained for copper(III) complexes of G_5 and G_6 than for G_4 or G_3a (11.4 and 11.6 vs. 12.1 and 12.3, respectively)[3,4] due to the formation of a hydrogen-bonded species for the longer peptides, which stabilizes the amine-deprotonated form (eq 18). The hydro-



(18)

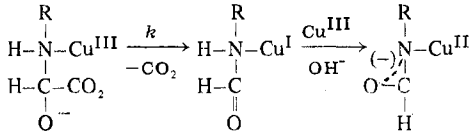
gen-bonded species would not be expected to be reactive with glyoxylate, since the hydrogen-bonded form cannot act as a nucleophile. The value of $pK_a^{Cu(III)}$ obtained kinetically is for the non-hydrogen-bonded species. It is comparable to the spectroscopically determined value for the Cu(III)–G_3a complex, whose deprotonated-amine complex has no hydrogen-bond stabilization. A value of ~9 can be calculated for K_{eq} in eq 18, indicating that only 10% of the amine-deprotonated copper(III) pentaglycine is in the reactive form.

Once the adduct is formed (eq 2), electron transfer takes place, from the carboxylate group of the carbinolamine to the metal center. A one-electron transfer in the rate-determining step results in the formation of a copper(II) carboxyloxy radical, which rapidly decarboxylates[29] to form an alkyl radical (eq 19). The radical can then react with Cu^{III} to yield the



(19)

observed products (eq 3). Similar mechanisms have been proposed for the oxidation of glyoxylate[13,15] and other keto acids[30] by one-electron oxidizing agents. Copper(II) is a sufficiently powerful oxidizing agent to effect the decarboxylation of aminomalonic acid, forming a copper(I) alkyl radical.[31] Indeed, the accessibility of Cu(I) may permit a two-electron process, through the coordinated nitrogen (eq 20).



(20)

A Cu(III) → Cu(I) step is proposed for the enzymic oxidation of galactose.[32] The possibility of copper(I) formation by successive one-electron steps, with copper(II) radical intermediates (eq 20), may also be considered, and would make it difficult to determine the mechanism exactly.

Conclusions

Glyoxylate ion rapidly reduces copper(III) pentaglycine in basic solution. Carbon dioxide and *N*-formylpentaglycine are produced and a second copper(III) pentaglycine is reduced for an overall two-electron redox process. In the proposed mechanism the deprotonated-amine copper(III) complex, $Cu(H_{-4}G_5)^{2-}$, reacts with the dehydrated form of glyoxylate ion to form a carbinolamine copper(III) species which undergoes oxidative decarboxylation. In the reaction the CHO group is transferred to the amine nitrogen of the peptide to form *N*-formylpentaglycine. Pyruvate and phenylglyoxylate are less reactive than glyoxylate.

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Registry No. $Cu^{III}(H_4G_5)^{2-}$, 69204-49-5; glyoxylate, 430-75-1; pyruvate, 57-60-3; phenylglyoxylate, 50572-54-8; OH^-, 14280-30-9.

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