Mechanism of Decomposition of Cu^{III}(GlyGlyHis): A Pulse Radiolysis Study

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The trivalent copper complexes of GlyGlyHis, Cu^{III}(H$_{-2}$GlyGlyHis) and Cu^{III}(H$_{-3}$GlyGlyHis), prepared via the oxidation of the corresponding Cu(II) complexes by azide radicals, decompose rapidly to yield CO$_2$, GlyGlyNHCH(OH)CH$_2$Im (Im = imidazole) and Cu(I) in aqueous solutions. The decomposition of Cu^{III}(H$_{-2}$GlyGlyHis) consists of two consecutive first-order reactions. The first reaction is attributed to a decarboxylation process, where CO$_2$ and a complex with a Cu^{III}–carbon σ-bond are formed. This reaction is followed by a heterolytic decomposition of the latter complex to Cu(I) and GlyGlyNHCH(OH)CH$_2$Im. Cu^{III}(H$_{-2}$GlyGlyHis), which is less stable than Cu^{III}(H$_{-3}$GlyGlyHis), decarboxylates with a rate constant of 38 ± 5 s^{-1}, whereas the rate of decarboxylation of Cu^{III}(H$_{-3}$GlyGlyHis) is about 2 orders of magnitude slower. The pK_a of the trivalent complex was determined to be 8.6 ± 0.2. A detailed pressure-dependence study of all the mentioned reaction steps was undertaken. The reported volumes of activation support the above outlined assignments for the nature of the rate-determining steps.

Introduction

The electrode potentials of copper(III) oligopeptide complexes are sensitive to the nature of the ligand and vary from 0.37 to 1.02 V vs NHE.[1] These complexes can undergo self-redox decompositions, where the reaction products are Cu(II) and oxidized peptides.[2–5] The rates of decomposition increase considerably with the increase of the redox potential of the Cu(III)/Cu(II) couple.[2–5]

Histidine is an important amino acid residue with a peptide nitrogen and an imidazole nitrogen available for binding. When histidine is present as the third residue in tripeptide or tetrapeptide complexes, the redox potential for the Cu(III)/Cu(II) couple is high (0.98 V for GlyGlyHis, 1.02 V for GlyGlyHisGly)[1,5] and the Cu(III) complexes decompose so rapidly that they are difficult to prepare.

It has been demonstrated that the Cu(III) tripeptide complexes with terminal histidine residues undergo remarkably fast decar-

boxylation reactions.[5] However, the mechanism of these reactions was not studied due to the short half-life of these Cu(III) complexes.

We have decided to use the pulse radiolysis technique in order to study the mechanism of this interesting process in the case of GlyGlyHis. The effect of pressure on the rates of formation and decomposition of the trivalent copper complex of GlyGlyHis was also investigated in an effort to gain further insight into the intimate nature of the mechanism of these reactions. Such measurements have in recent years greatly assisted the elucidation of the mechanisms of chemical reactions in solution.[6,7] The development of a suitable window for an optical high-pressure cell that allows the penetration of the electron beam into the sample[8] has opened up the possibility to study the pressure dependence of chemical reactions that are induced by free radicals.[9–11]

Experimental Section

Materials. All chemicals employed were of analytical grade and were used as received. Glycylglycylhistidine was purchased from Bachem Feinchemikalien AG. Solutions were prepared with distilled water which

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was further purified using a Milli-Q water purification system. Unless otherwise stated, the ligand concentration exceeded that of copper by 10%, and solutions contained excess concentration of azide over the metal ions and were saturated with N_2O. The pH in the range 6.2–9 was maintained using 2–10 mM phosphate or borate buffers, whereas higher pH's were obtained by using NaOH. Experiments could not be carried out at pH < 6.1, because under these conditions the azide ions compete with the peptide for Cu(II) to form $Cu(N_3)_n^{2-n}$.[12] Spectroscopic measurements indicate that at pH 6.3 in the presence of 0.05 M NaN_3 and 0.2–0.9 mM $Cu^{II}(H_{-2}GlyGlyHis)$ only about 4–6% of the Cu(II) ions are bound to azide ions. We have no spectroscopic evidence for the formation of a ternary complex of $Cu^{II}(H_{-2}GlyGlyHis)(N_3^-)$ under these conditions.

Irradiation. Pulse radiolysis experiments were carried out with the Varian 7715 linear accelerator at the Hebrew University of Jerusalem with a 200-mA current of 5-Mev electrons. The experimental setup was identical to that described earlier.[10]

High-Pressure Experiments. The high-pressure setup, consisting of a 1.5-cm pillbox optical cell, made of suprasil, was placed as near as possible to a thin stainless steel window[8] of the modified high-pressure cell so that the high-energy electrons would have a minimal path through the surrounding water pressurizing medium. The experiments were performed up to 150 MPa pressure at room temperature.

Gas Analysis. Carbon dioxide was analysed by gas chromatography using a Hewlett Packard Model 5890 instrument. The gas samples were separated on a 10 ft × $^1/_8$ in. stainless steel column packed with poropak Q 80/100 mesh at 30 °C with a flow of 35 mL of He/min. The gas was transferred through a thermal conductivity detector. A calibration curve of CO_2 was obtained by irradiation of N_2O-saturated solutions containing 0.5 mM $Cu^{II}(1,10$-phenanthroline) and 0.1 M formate at pH 6.8 (2 mM phosphate buffer). Under these conditions all the primary free radicals produced by the radiation are converted into CO_2^- which subsequently reduce the Cu(II) complex to form CO_2 and the corresponding Cu(I) complex.[14] Before the gas samples were taken, 0.1 mL of concentrated $HClO_4$ was injected into the bulb in order to shift the equilibrium $2H^+ + CO_3^{2-} \rightleftharpoons CO_2 + H_2O$ toward CO_2.
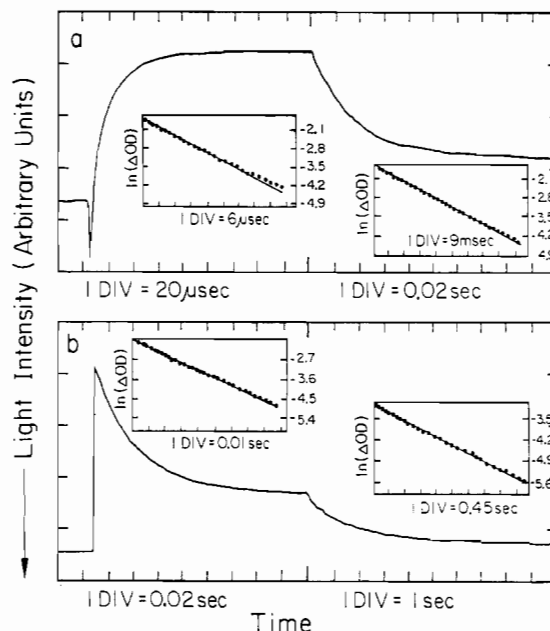
Results

The azide radical is a strong one electron oxidizing agent with a redox potential of 1.3 V vs NHE.[15] This radical exhibits a moderate optical absorption only in the UV region, with a sharp maximum at 274 nm, $\epsilon = 2025$ M^{-1} cm^{-1}, the spectrum obeing only 20–nm wide at half-maximum height.[16] In contrast to $N_3^•$, $Br_2^{•-}$, a stronger oxidizing agent (1.6 V), has a higher absorbance in the near-UV region ($\epsilon_{360} = 9900$ M^{-1} cm^{-1}),[17] and its oxidation reactions are slower than those of $N_3^•$.[18] Since most Cu(III) peptides absorb around 360–390 nm,[1–5,19] we have chosen to use the azide radical as the oxidant of the Cu(II) peptide complex, although some experiments were also carried out with $Br_2^{•-}$ as an oxidizing reagent. The $OH^•$ radical was not used because it oxidizes the metal ion as well as the ligand (see below).[20]

When N_2O-saturated solutions containing azide or bromide ions are irradiated, the following reactions take place:
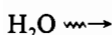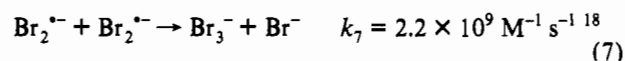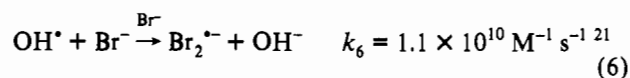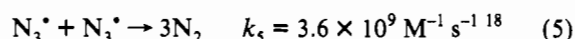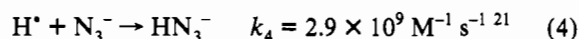
$$H_2O \rightsquigarrow$$
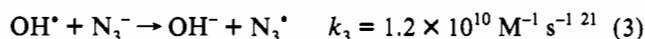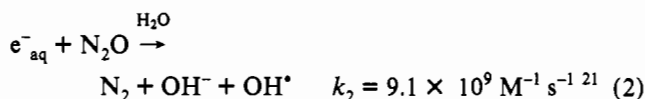$$e^-_{aq} (2.75), OH^• (2.75), H^• (0.60), H_2 (0.45), H_2O_2 (0.75) \quad (1)$$

The numbers in parentheses are G values which represent the

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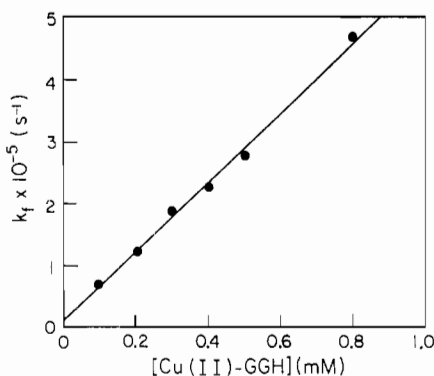(15) Alfassi, Z. B.; Harriman, H.; Huie, R. E.; Mosseri, S.; Neta, P. *J. Phys. Chem.* **1987**, *91*, 2120.
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Figure 1. Computer output of the time dependence of the light transmittance of the sample at 400 nm following the pulse: (a) Formation and the first decay; (b) two decays. The solution contained 0.1 mM $Cu^{II}(GlyGlyHis)$ and 0.05 M NaN_3 at pH 6.8 (2 mM phosphate buffer) and was N_2O-saturated.

number of molecules formed per 100 eV of energy absorbed in the solution.[21]

$$e^-_{aq} + N_2O \xrightarrow{H_2O}$$
$$N_2 + OH^- + OH^• \quad k_2 = 9.1 \times 10^9 \text{ M}^{-1} \text{ s}^{-1} \text{ }^{21} \quad (2)$$

$$OH^• + N_3^- \rightarrow OH^- + N_3^• \quad k_3 = 1.2 \times 10^{10} \text{ M}^{-1} \text{ s}^{-1} \text{ }^{21} \quad (3)$$

$$H^• + N_3^- \rightarrow HN_3^- \quad k_4 = 2.9 \times 10^9 \text{ M}^{-1} \text{ s}^{-1} \text{ }^{21} \quad (4)$$

$$N_3^• + N_3^• \rightarrow 3N_2 \quad k_5 = 3.6 \times 10^9 \text{ M}^{-1} \text{ s}^{-1} \text{ }^{18} \quad (5)$$

$$OH^• + Br^- \xrightarrow{Br^-} Br_2^{•-} + OH^- \quad k_6 = 1.1 \times 10^{10} \text{ M}^{-1} \text{ s}^{-1} \text{ }^{21} \quad (6)$$

$$Br_2^{•-} + Br_2^{•-} \rightarrow Br_3^- + Br^- \quad k_7 = 2.2 \times 10^9 \text{ M}^{-1} \text{ s}^{-1} \text{ }^{18} \quad (7)$$

When N_2O-saturated solutions containing [Cu(II)] = 0.1–0.8 mM, [GlyGlyHis] = 0.11–0.88 mM, and [NaN_3] = 0.01–0.1 M at pH 6.2–11.5 were irradiated, the formation and decomposition of a short-lived transient were observed. A typical kinetic plot at 400 nm is given in Figure 1.

The rate of the formation of the transient obeyed a pseudo-first-order rate law. The observed rate constant was linearly dependent on [$Cu^{II}(H_{-2}GlyGlyHis)$] (Figure 2) and independent of pH and [NaN_3]. A rate constant of $(5.0 \pm 0.2) \times 10^8$ M^{-1} s^{-1} was determined for the formation process from the slope of the line in Figure 2. The small intercept in Figure 2 either is within the experimental error or results from some contribution of reaction 5. The spectrum of the species formed at the end of the formation process depends only on the pH (Figure 3). The two isosbestic points indicate the acid–base equilibrium of the two forms of the Cu(III)–peptide.

The mechanism of decomposition of the transient formed at pH < 8 consists of two consecutive first-order reactions (Figure 1). The rate of both decomposition processes was found to be

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Figure 2. Observed rate constant for the formation of the absorbance (k_f) as a function of [Cu^{II}(GlyGlyHis)] in N$_2$O-saturated solutions containing 0.05 M NaN$_3$ at pH 6.8.
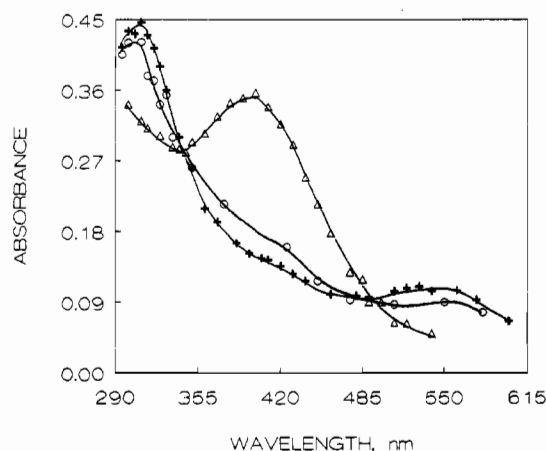
Figure 3. Absorption spectra obtained at the end of the formation process in N$_2$O-saturated solutions containing 0.8 mM Cu^{II}(GlyGlyHis) and 0.05 M NaN$_3$ at pH 6.8 (△), 9.5 (○), and 10.6 (+). The optical pathlength was 12.1 cm, and the dose, 800 rad.
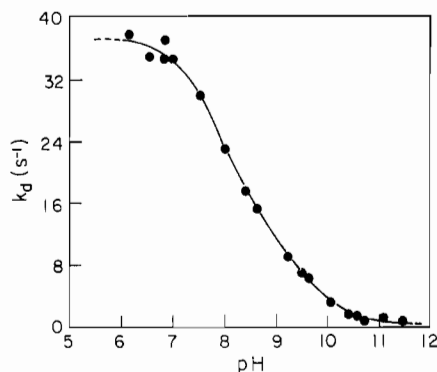
Figure 4. Observed rate constant for the first decay of the absorbance (k_d) as a function of pH in N$_2$O-saturated solutions containing 0.05 M NaN$_3$ and 0.2–0.8 mM Cu^{II}(GlyGlyHis).

independent of [Cu^{II}(H$_{-2}$GlyGlyHis)] = 0.1–0.8 mM and [NaN$_3$] = 0.01–0.1 M and depended only on pH. The rate of the first decomposition reaction decreases with the increase in pH (Figure 4), whereas that of the second reaction increased with the pH and was found to be 2.2, 0.9, 0.65, and 0.3 s^{-1} at pH 7.5, 7.1, 6.8, and 6.2, respectively. At pH > 9 only one decomposition process is observed with a rate decreasing with the increase in the pH (Figure 4).

When the same experiments were carried out in the presence of 0.05 M NaBr instead of NaN$_3$, the disappearance of Br$_2^{\bullet-}$ was followed. The observed rate obeyed a pseudo-first-order rate law and depends linearly on [Cu^{II}H$_{-2}$GlyGlyHis)] only at very low pulse intensity and at [Cu^{II}(H$_{-2}$GlyGlyHis)] > 0.4 mM. A rate constant of (4.0 ± 0.6) × 10^7 M^{-1} s^{-1} was determined for this

process. The spectrum of the species formed at the end of the decomposition of Br$_2^-$ was *identical* to that obtained in the azide system after the end of the formation process at pH 6.8 as well as at pH 10.6. The rate of the decomposition of the latter species in the bromide system followed a first-order rate law with a rate constant of 40 ± 3 s^{-1} at pH 6.3 and 6.8 and 1.3 ± 0.2 s^{-1} at pH 10.6. These rate constants are within experimental error the same as those obtained in the azide system for the first decomposition process (Figure 4).

In the absence of either azide or bromide ions, the spectrum measured immediately after the pulse at pH 6.8 differs from the one obtained in their presence. It seems that two species are formed, one of which absorbs at 395 ± 5 nm and the other at much lower wavelengths. If the absorbance at 395 nm is due to the Cu(III)–peptide complex (Figure 2), then about 40% of the hydroxyl radicals oxidize Cu(II) to Cu(III) and the other 60% oxidize the peptide. The decay of the absorbance obeyed a second-order rate law with a rate almost independent of wavelengths.
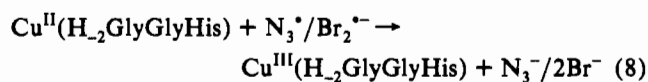
The effect of pressure on the rates of the processes observed was studied under the conditions where [Cu^{II}(H$_{-2}$GlyGlyHis)] = 0.5 mM and [N$_3^-$] = 0.05 M at pH 6.6 (2 mM phosphate buffer) and 11.5 at 0.1 and 150 MPa. At each pressure at least five experiments were performed, and the rate constant is the average. The effect of pressure on the formation of Cu^{III}(H$_{-2}$GlyGlyHis) could be studied accurately at 400 nm at pH 6.6. The observed rate constant for the formation of Cu^{III}(H$_{-2}$GlyGlyHis) increases from (2.1 ± 0.2) × 10^5 to (2.9 ± 0.2) × 10^5 s^{-1} on increasing the pressure from 0.1 to 150 MPa.
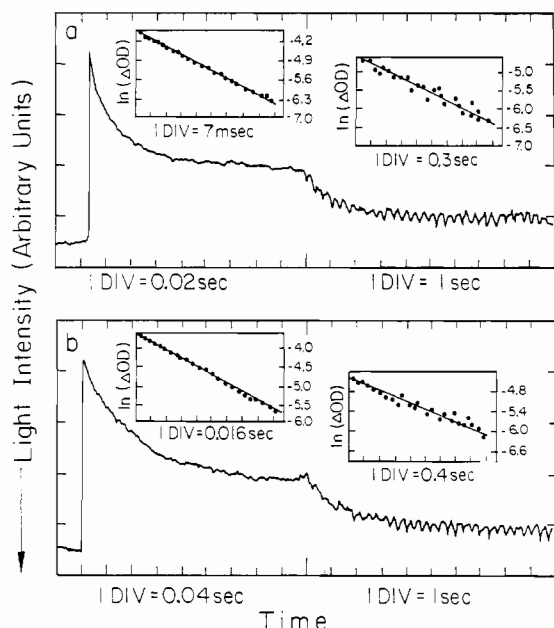
The decomposition of the Cu(III)–peptide complex was studied under similar conditions in the low and high pH independent regions seen in Figure 4. A typical example of the two consecutive first-order decays observed at pH 6.6 and recorded at 0.1 and 150 MPa pressure is given in Figure 5. It can be clearly seen that both processes are significantly decelerated by pressure. The rate constant for the first process decreases from 37 ± 3 to 15 ± 2 s^{-1} over this pressure range. The rate constant for the second decomposition step decreases from 0.63 ± 0.03 to 0.39 ± 0.04 s^{-1} in going from 0.1 to 150 MPa pressure. A series of measurements at pH 11.5 indicates that there is a significant increase in the rate constant of the decomposition of Cu^{III}(H$_{-3}$GlyGlyHis) from 0.40 ± 0.04 to 0.94 ± 0.08 s^{-1} on increasing the pressure from 0.1 to 150 MPa.

Carbon dioxide was found to be one of the final products. N$_2$O-saturated solutions containing [Cu^{II}(H$_{-2}$GlyGlyHis)] = 0.8 mM and [NaN$_3$] = 0.05 M at pH 6.6 (10 mM phosphate buffer) were pulse-irradiated with a total dose of 10–20 krad, and it was found that the yield of CO$_2$ was 60–90% of the yield of all the primary free radicals formed by the radiation. The yield of CO$_2$ was linear with the dose only at low doses. Margerum et al.[5] generated the trivalent copper complex of GlyGlyHis in a different system and found that the main decomposition products of this complex are CO$_2$ and Gly$_2$NHCH(OH)CH$_2$Im.

Discussion

When N$_2$O-saturated solutions containing Cu^{II}(H$_{-2}$GlyGlyHis) and NaN$_3$ or NaBr are irradiated, a short-lived transient is formed, and its spectrum at pH 6.8 (ϵ_{max} = 6500 ± 700 M^{-1} cm^{-1} at 395 ± 5 nm) and at pH 10.6 (ϵ_{max} = 7500 ± 800 and 1850 ± 200 M^{-1} cm^{-1} at 310 ± 5 and 530 ± 5 nm, respectively) is similar to those of various Cu(III)–peptide complexes.[1-5,19] Therefore, the formation process is attributed to reaction 8, where k_8 = (5.0 ± 0.2) × 10^8 and (4.0 ± 0.4) × 10^7 M^{-1} s^{-1} for N$_3^{\bullet}$ and Br$_2^{\bullet-}$, respectively.

$$Cu^{II}(H_{-2}GlyGlyHis) + N_3^{\bullet}/Br_2^{\bullet-} \longrightarrow$$
$$Cu^{III}(H_{-2}GlyGlyHis) + N_3^-/2Br^- \quad (8)$$

Figure 5. Computer output of the time dependence of the light transmittance of the sample at 400 nm following the pulse: (a) Recorded at 0.1 mPa; (b) recorded at 150 mPa. The solution contained 0.5 mM $Cu^{II}(GlyGlyHis)$ and 0.05 M NaN_3 at pH 6.6 (10 mM phosphate buffer) and was N_2O-saturated.
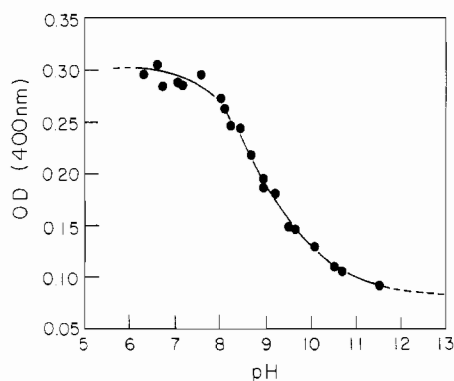
Figure 6. Absorbance at the end of the formation process as a function of pH, measured 20 μs after the end of the pulse at 400 nm in N_2O-saturated solutions containing 0.8 mM $Cu^{II}(GlyGlyHis)$ and 0.05 M NaN_3.

The spectrum of the Cu(III)–peptide complex depends on pH (Figure 3). A plot of OD at 400 nm as a function of pH yields a pK_a plot, where $pK_a = 8.6 \pm 0.2$ (Figure 6). Margerum et al.[5] have shown that the lose of proton from the nitrogen of the histidine is not responsible for the low pK_a value. This indicates that the deprotonation of the terminal amine of $Cu^{III}(H_{-2}GlyGlyHis)$ occurs at considerably lower pH's than those of peptides that do not have a histidyl residue.[5] The latter have pK_a values between 11.5 and 12.5.[5,19,22,23] This low pK_a is most probably due to the strong π-back-bonding properties of the imidazole group.

The mechanism of decomposition of the Cu(III)–peptide complex in the azide system consists of two consecutive processes obeying first-order rate laws at pH < pK_a. The rate of the first process decreases with pH (Figure 4), yielding a pK_a plot. In the bromide system only the first decomposition process was observed at all pH's with rate constants identical to those measured in the azide system. Under our experimental conditions the rate of reaction 7 was never slow enough as compared to that of reaction 8. Therefore, in the bromide system the solutions always contained

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(23) Hinton, J. P.; Margerum, D. W. *Inorg. Chem.* **1986**, *25*, 3248.

Br_3^- and/or BrO^-. The fact that the second intermediate was not observed in this case at pH < pK_a suggests that it reacts, in a fast process, with Br_3^- and/or BrO^-.

Thus, $Cu^{III}(H_{-2}GlyGlyHis)$ is less stable than $Cu^{III}(H_{-3}GlyGlyHis)$, and the following reaction mechanism might explain the experimental results.

(i) As we have shown from Figure 6, the trivalent copper complex of GlyGlyHis has a $pK_a = 8.6 \pm 0.2$ for the terminal amine, in good agreement with the value of 8.2, determined by Margerum et al.:[5]

$$I \overset{K_a}{\rightleftharpoons} II + H^+ \qquad (9)$$

I
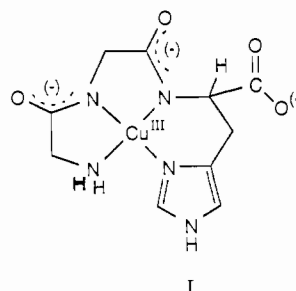
(ii) Both species **I** and **II** are in fast equilibria with the pentacoordinated forms **III** and **IV**, respectively. The possibility of the formation of the transients **III/IV** was checked by carrying out MM2 calculations using the Cache system. The results indicate the feasibility of the entrance of the oxygen of the carboxylate to the plane of the coordination, and the histidyl nitrogen becomes axial. All the angles in the model are reasonable ones. This does not require a breaking of the nitrogen–copper bond and might involve only rearrangement of the coordination sphere. The results are shown in Figure 7.

$$I \overset{K_I}{\rightleftharpoons} III \qquad (10)$$

$$II \overset{K_{II}}{\rightleftharpoons} IV \qquad (11)$$

III
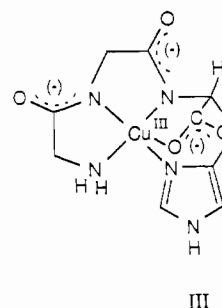
(iii) The transient **III** has a pK_a' for the terminal amine:

$$III \overset{K_a'}{\rightleftharpoons} IV + H^+ \qquad (12)$$

(iv) The protonated form (**III**) decomposes via a decarboxylation reaction to form a short-lived transient with $\epsilon = 1350 \pm 150$ M^{-1} s^{-1} at 375 ± 5 nm at pH 6.8 (Figures 1 and 8). It is proposed that this transient has a Cu^{III}–carbon σ-bond, which is formed via the reaction of Cu(II) with the aliphatic free radical (Scheme 1). This suggestion is in accord with previous observations where aliphatic free radicals reduce Cu(II) to Cu(I) while being oxidized to the corresponding hydroxyl compound, via the formation of a transient with a Cu^{III}–carbon σ-bond as an
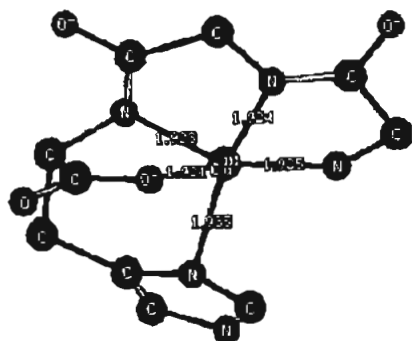
Figure 7. Two-dimensional presentation of species III as calculated by carrying out MM2 calculations using the Cache system. With numbering of the terminal nitrogen as 1, the next is 2, next to 2 is 3, oxygen is 4, and the imidazole nitrogen as 5, the following angles were calculated: $\angle 1Cu2 = 84.7°$; $\angle 2Cu3 = 78.5°$; $\angle 3Cu4 = 82.8°$; $\angle 4Cu1 = 101.4°$; $\angle 5Cu1 = 112.3°$; $\angle 2Cu5 = 119.5°$; $\angle 3Cu5 = 99.2°$; $\angle 4Cu5 = 91.4°$.
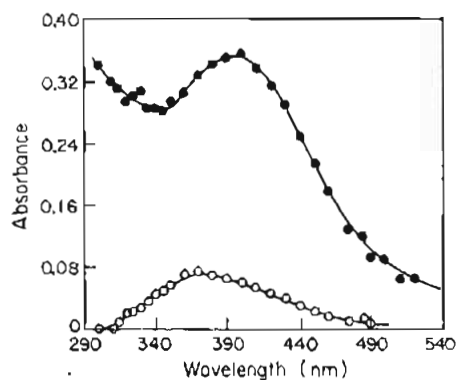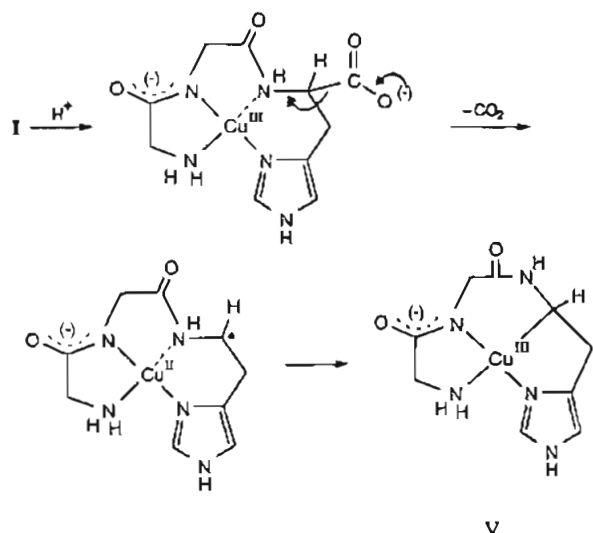

Figure 8. Absorption spectra obtained 20 μs after the end of the pulse, at the end of the formation process (●), and 0.2 s after the end of the pulse, at the end of the first decay (○). The solution contained 0.8 mM Cu^{II}(GlyGlyHis) and 0.05 M NaN$_3$ at pH 6.8 (2 mM phosphate buffer) and was N$_2$O-saturated. The optical path length was 12.1 cm, and the dose, 800 rad.

Scheme 1

intermediate.[24-26] In addition, the half-life of the transient observed is considerably longer than that expected for aliphatic free radicals, and the spectrum of this transient (Figure 8) is similar to those of several complexes with a Cu^{III}–carbon σ-bond.[24-25]

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(25) Freiberg, M.; Meyerstein, D. *J. Chem. Soc., Faraday Trans. 1* **1980**, *76*, 1825.

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Scheme 2
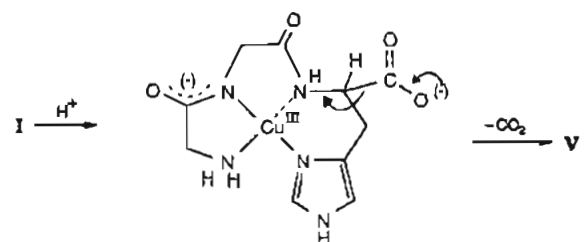
The rate constant of the decarboxylation process is (38 ± 5) s^{-1}, whereas the rate of the decomposition of the deprotonated form (IV) is 2 orders of magnitude slower in both the azide and bromide systems. The rate of the decarboxylation reaction decreases with the increase in pH, and it becomes the rate-determining step.

$$\text{III} \longrightarrow \begin{array}{l} \text{CO}_2 + \text{V} \qquad (13a) \\ \text{CO}_2 + \text{VI} \qquad (13b) \end{array}$$

$$\text{IV} \longrightarrow \begin{array}{l} \text{CO}_2 + \text{VII} \qquad (14a) \\ \text{CO}_2 + \text{VIII} \qquad (14a) \end{array}$$

(VII and VIII are the deprotonated forms of V and VI, respectively.)
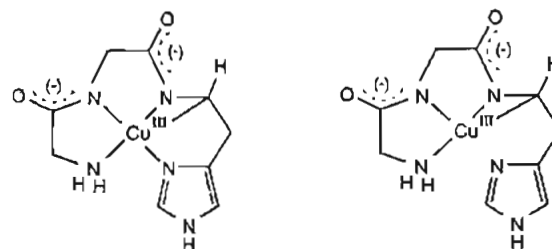
VI

It seems reasonable that reaction 13a (14a) takes place rather than reaction 13b (14b), because a ring containing three atoms has a very large strain and is very unstable.

Alternatively, a direct decarboxylation of I or II to V or VII, respectively, as already suggested by Margerum et al.,[5] could be envisage (Scheme 2). We believe that decarboxylation via an electron transfer to the central metal cation is considerably more facile when the carboxylate ligates to the metal cation. Since our model suggests that the carboxylate can be bound to the copper-(III), we prefer this mechanism, though we cannot rule out Scheme II.

(v) The transient with the Cu^{III}–carbon σ-bond (V) decomposes heterolytically to yield Cu(I) and Gly$_2$NHCH(OH)CH$_2$Im, with a rate which slightly increases with the increase in pH.

$$\text{V} + \text{H}_2\text{O} \rightarrow \text{Cu(I)} + \text{Gly}_2\text{NHCH(OH)CH}_2\text{Im} + \text{H}^+ \quad (15)$$

Cu(I) is not stable in aqueous solutions at the pH's studied. It most probably disproportionates and/or precipitates as CuN$_3$.[12] An attempt to determine [Cu(I)] in the presence of excess Cu^{II}(GlyGlyHis) by adding 2,9-dimethyl-4,7-diphenyl-1,10-phenanthroline (bathocuproine) before or immediately after the irradiation failed because bathocuproine binds Cu(II) and N$_3^-$ reduces Cu(II)–bathocuproine to the corresponding cuprous complex.

According to the above suggested mechanism the rate equation for the decarboxylation process is given by eq 16,

$$-d[\text{I} + \text{II} + \text{III} + \text{IV}]/dt = k_{obs}[\text{I} + \text{II} + \text{III} + \text{IV}] \quad (16)$$

where k_{obs} is given by eq 17.

$$k_{obs} = \frac{k_{13}K_IK_{II} + k_{14}K_IK_{II}K_a'/[H^+]}{K_{II}(1 + K_I) + (1 + K_{II})K_IK_a'/[H^+]} \quad (17)$$

At low pH, $k_{obs} = k_{13}K_I/(1 + K_I)$, whereas, at high pH, $k_{obs} = k_{14}K_{II}/(1 + K_{II})$. If we assume that $K_I, K_{II} \gg 1$, then from Figure 4 one obtains that $pK_a' = 8.3 \pm 0.2$, which is slightly different from the pK_a value of 8.6 ± 0.2, which was determined from Figure 6. However, if $K_I, K_{II} \ll 1$, then $pK_a' + pK_{II} = pK_I = 8.3 \pm 0.2$.

On an increase of the pressure from 0.1 to 150 MPa, the rate of the formation of Cu^{III}(GlyGlyHis) increases from $(2.1 \pm 0.2) \times 10^5$ to $(2.9 \pm 0.2) \times 10^5$ s⁻¹, which corresponds to a ΔV^* value of -5 ± 1 cm³ mol⁻¹. This is in line with the suggested electron-transfer reaction 8. The latter involves the oxidation of Cu(II) to Cu(III) and the reduction of azide radical to N_3^-. These processes should both involve a significant volume collapse and result in a large negative overall reaction volume.[6,7,27,28] In fact, the observed ΔV^* is rather small and indicates that we are most probably dealing with an early transition state along the reaction coordinate. This is quite reasonable for such a fast reaction (5×10^8 M⁻¹ s⁻¹) for which the activation barrier is expected to be low.

The rate constant for the first decomposition process decreases from 37 ± 3 to 15 ± 2 s⁻¹ in going from 0.1 to 150 MPa, which corresponds to $\Delta V^* = +14 \pm 2$ cm³ mol⁻¹. It follows that the first decomposition reaction involves a significant increase in volume and therefore cannot be due to the rate-determining ring closure of the free carboxylate arm as shown in reaction 10. However, this volume is in good agreement with a rate-determining decarboxylation as suggested in reaction 13a, since electron transfer and release of CO_2 will be accompanied by a significant volume increase.[6,7,29,30] The observed volume of activation is also in accord with the decarboxylation occurring via the mechanism proposed by Margerum et al.[5] (Scheme 1). The pressure dependence of the rate constant for the second decomposition step corresponds to $\Delta V^* = +8 \pm 1$ cm³ mol⁻¹. This result also supports the suggested decomposition reaction outlined in (15), where both the reduction of Cu(II) to Cu(I) and the release of the chelate will be accompanied by significant increases in volume.[6,7]

The decomposition reaction at pH 11.5 indicates that there is a significant increase in the rate constant from 0.40 ± 0.04 to 0.94 ± 0.08 s⁻¹ on increasing the pressure from 0.1 to 150 MPa. This corresponds to a ΔV^* value of -14 ± 2 cm³ mol⁻¹. The interpretation of this result is complicated by the fact that increasing the pressure will affect the ionization of water, as reflected by an increase in K_w, for which $\Delta \bar{V} = -22$ cm³ mol⁻¹.[31] At a fixed [OH⁻], this will cause a decrease in pH and, according to the results in Figure 4, an increase in the rate constant of this reaction. Alternatively, this complication may be canceled when K_a for reaction 9 exhibits a pressure dependence similar to that for K_w.

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