

Intermediates with Copper–Carbon Bonds Formed by the Reaction of Aliphatic Free Radicals with a Copper–Peptide Complex in Aqueous Solutions

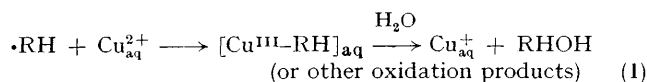
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Summary The reactions of $\cdot\text{CH}_2\text{CO}_2^-$ and $\cdot\text{CH}_2\text{CMe}_2\text{OH}$ solutions yield relatively long lived intermediates with copper–carbon σ bonds. with a copper–peptide complex in neutral aqueous

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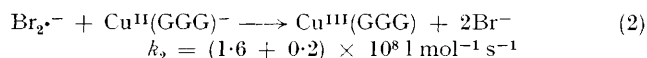
RECENTLY it was shown that Cu^{II} peptide complexes can be easily oxidized to Cu^{III} peptide complexes in aqueous solutions at physiological pH,¹ and it has also been shown that aliphatic free radicals react with Cu^{2+} ions in aqueous solutions according to the mechanism in equation (1).²



The intermediates $\text{Cu}^{\text{III}}\text{-RH}$ are relatively long lived for aliphatic radicals like $\cdot\text{CH}_2\text{CO}_2^-$, $\cdot\text{CH}_2\text{CH}_2\text{OH}$, and $\text{CH}_2\text{CMe}_2\text{OH}$.² These results have led us to investigate the possibility that aliphatic free radicals would react with a copper peptide complex *via* a similar mechanism. Such reactions are of special interest as copper ions sensitize radiation damage under oxidizing conditions, though it is difficult to believe that $\cdot\text{OH}$ radicals will react directly with the copper ions and not with the organic compounds present in biological media.

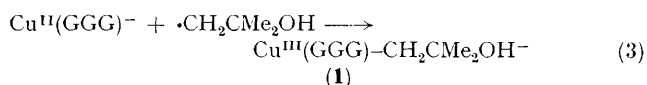
Nitrous oxide-saturated solutions containing $1\text{--}5 \times 10^{-4}$ M copper(II) sulphate triglycine complex, $\text{Cu}^{\text{II}}(\text{GGG})^-$ ($\text{GGGH}_3 = \text{NH}_2\text{CH}_2\text{CONHCH}_2\text{CONHCH}_2\text{CO}_2\text{H}$) in the pH range 6–10.2, maintained using 0.01 M sodium phosphate or sodium borate, and 0.01 M NaBr, 1.0 M Me_3COH , or 1 M MeCO_2Na were irradiated by a 4–40 ns pulse of 15 MeV electrons from the linear accelerator at Argonne. (In all solutions the concentration ratio $[\text{GGGH}_3]/[\text{CuSO}_4] = 1.1$ was maintained.) Under these conditions 6×10^{-6} – 6×10^{-5} M of the free radicals $\text{Br}_2\cdot^-$,³ $\cdot\text{CH}_2\text{CMe}_2\text{OH}$,⁴ and $\cdot\text{CH}_2\text{CO}_2^-$ ⁴ were formed in the solutions containing NaBr, Me_3COH , and MeCO_2Na respectively. (In all these solutions hydrogen atoms were also formed with a yield of *ca.* 10% of the total free radical yield.⁵) The formation of relatively long lived unstable intermediates with absorption bands in the near u.v. was observed

under these conditions. The Figure shows the absorption spectra⁶ observed in the NaBr and Me_3COH containing solutions. In the former the transient has $\lambda_{\text{max}} 385 \pm 5$ nm ($\epsilon_{\text{max}} 4200 \pm 600 \text{ l mol}^{-1} \text{ cm}^{-1}$). This spectrum is attributed to $\text{Cu}^{\text{III}}(\text{GGG})^2$ formed in reaction (2). After



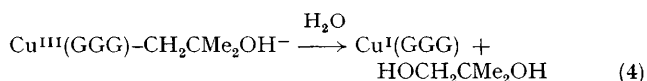
this reaction a further reaction with *k ca.* 10^3 s^{-1} occurred. This reaction caused a slight (*ca.* 10%) decrease in the absorbance, and could therefore not be studied in detail.[†] The $\text{Cu}^{\text{III}}(\text{GGG})$ complex thus formed decomposes slowly in a first-order reaction the rate of which increases with pH: *k ca.* 0.02, *ca.* 0.08, 0.25 ± 0.05 , 3.0 ± 0.5 , and 10 ± 1 at pH 6.2, 7.3, 7.8, 9.0, and 10.0 respectively, in accord with the reported properties of $\text{Cu}^{\text{III}}(\text{peptide})$ complexes.¹

The transient formed in the solutions containing Me_3COH ($\lambda_{\text{max}} 320$ nm; $\epsilon_{\text{max}} 5000 \pm 700 \text{ l mol}^{-1} \text{ cm}^{-1}$) was identified as compound (1) formed in reaction (3). The transient (1) is clearly different from $\text{Cu}^{\text{III}}(\text{GGG})$ as can be deduced



$$k_3 = (2.6 \pm 0.4) \times 10^7 \text{ l mol}^{-1} \text{ s}^{-1}$$

from their respective spectra (Figure) and from the differences in their rates of decomposition (see later). It is also not $\text{Cu}^{\text{I}}(\text{GGG})$, as the yield of (1) in He-saturated solutions, where only $\text{HO}\cdot$ is effective in producing the aliphatic radical, is only *ca.* 40% of that observed in N_2O -saturated solutions where both the primary radiolysis products, e_{aq}^- , as well as $\text{HO}\cdot$, do so (the former *via* N_2O scavenging, $\text{N}_2\text{O} + e_{\text{aq}}^- + \text{H}^+ \rightarrow \text{N}_2 + \text{HO}\cdot$). The rate of decomposition of (1) in the He saturated solutions is slightly higher than in the N_2O saturated solutions. The mechanism of decomposition of (1) is believed to be as shown in reaction (4), by analogy with the mechanism of decomposition of



$(\text{H}_2\text{O})_n\text{Cu}^{\text{III}}\text{-RH}$.² $k_4 = 0.75 \pm 0.10$, 1.5 ± 0.2 , 2.3 ± 0.3 , and 2.5 ± 3 at pH 7.3, 7.8, 9.0, and 10.2 respectively. The product of reaction (4) cannot be $\text{Cu}^{\text{III}}(\text{GGG})$ as the rate of decomposition of the latter at pH 7.3 and 7.8 is considerably lower than k_4 . At 380 nm the absorption coefficient of $\text{Cu}^{\text{III}}(\text{GGG})$ is considerably larger than that of $\text{Cu}^{\text{III}}(\text{GGG})\text{-CH}_2\text{CMe}_2\text{OH}^-$; reaction (4) causes a decrease and not an increase in absorption at 380 nm.

In solutions containing 1 M MeCO_2Na instead of Me_3COH similar results were observed: $k[\text{Cu}^{\text{II}}(\text{GGG}) + \cdot\text{CH}_2\text{CO}_2^-] = (1.6 \pm 0.2) \times 10^7 \text{ l mol}^{-1} \text{ s}^{-1}$. The absorption band of the transient in these solutions is broader, with $\lambda_{\text{max}} < 350$ nm. The decomposition of the transient identified as $\text{Cu}^{\text{III}}(\text{GGG})\text{-CH}_2\text{CO}_2^-$ consists of two consecutive steps; the ratio of the relative contribution of the first to that of the second reaction decreases with pH, from 50% at pH 7.8 to 20% at pH 10.2. The rate constant of the first

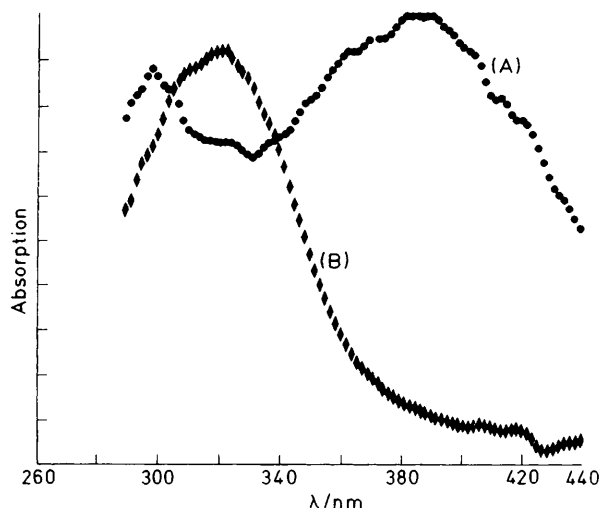


FIGURE. Absorption spectra 180 μs after a 3×10^4 rad pulse into a 5 cm cell as observed using a streak camera. Arbitrary absorbance scale. Solution composition: (A) 2.5×10^{-4} M $\text{Cu}^{\text{II}}(\text{GGG})^-$, pH 7.3, 1×10^{-2} M NaBr, N_2O saturated; (B) 5×10^{-4} M $\text{Cu}^{\text{II}}(\text{GGG})^-$, pH 7.3, 1.0 M Me_3COH , N_2O saturated.

† One possibility is that first $\text{Cu}^{\text{III}}(\text{GGG})\text{Br}$ is formed and that the reaction observed is the loss of Br^- .

step is 7.6 s^{-1} at pH 7.8 and 3.5 s^{-1} at pH 10.2 while that of the second step is 0.89 and 0.06 s^{-1} at these pH values. The exact nature of these two reactions is not clear; however, the decomposition of $(\text{Cu}^{\text{III}}\text{-CH}_2\text{CO}_2^-)_{\text{aq}}$ also consists of two steps.² It is also of interest that the rate of decomposition of $\text{Cu}^{\text{III}}(\text{GGG})\text{-CH}_2\text{CO}_2^-$ decreases with increasing pH while that of $\text{Cu}^{\text{III}}(\text{GGG})$ and $\text{Cu}^{\text{III}}(\text{GGG})\text{-CH}_2\text{CMe}_2\text{OH}^-$ increases.

The results obtained in this study indicate that copper proteins might act as scavengers of 'non-reducing' aliphatic

free radicals *via* the formation of relatively long-lived intermediates containing copper-carbon σ bonds. This mechanism might also contribute to the role of copper ions as radio-sensitizers.

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