Intermediates with Copper–Carbon Bonds Formed by the Reaction of Aliphatic Free Radicals with a Copper–Peptide Complex in Aqueous Solutions
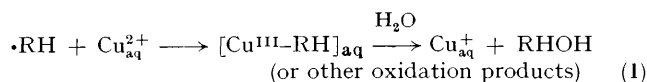
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Summary The reactions of $\cdot CH_2CO_2^-$ and $\cdot CH_2CMe_2OH$ with a copper–peptide complex in neutral aqueous solutions yield relatively long lived intermediates with copper–carbon σ bonds.

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]

$$\cdot RH + Cu^{2+}_{aq} \longrightarrow [Cu^{III}\text{-}RH]_{aq} \xrightarrow{\text{H}_2\text{O}} Cu^+_{aq} + RHOH$$
$$\text{(or other oxidation products)} \quad (1)$$

The intermediates Cu^{III}–RH are relatively long lived for aliphatic radicals like $\cdot CH_2CO_2^-$, $\cdot CH_2CH_2OH$, and CH_2CMe_2OH.[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 $\cdot OH$ radicals will react directly with the copper ions and not with the organic compounds present in biological media.

Nitrous oxide-saturated solutions containing 1—5×10^{-4} M copper(II) sulphate triglycine complex, Cu^{II} $(GGG)^-$ $(GGGH_3 = NH_2CH_2CONHCH_2CONHCH_2CO_2H)$ 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 $[GGGH_3]/[CuSO_4] = 1\cdot1$ was maintained.) Under these conditions 6×10^{-6}—6×10^{-5} M of the free radicals $Br_2^{\cdot-}$,[3] $\cdot CH_2CMe_2OH$,[4] and $\cdot CH_2CO_2^-$ [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
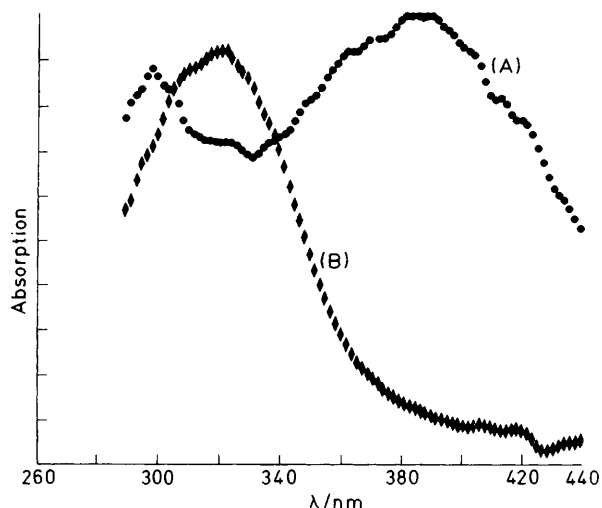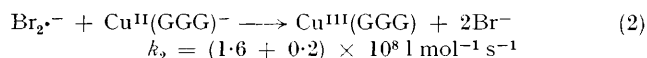

FIGURE. Absorption spectra 180 μs after a 3×10^4 rad pulse into a 5 cm cell as observed using a streak camera. Arbitrary absorbance scale. Solution composition: (A) $2\cdot5 \times 10^{-4}$ M $Cu^{II}(GGG)^-$, pH 7·3, 1×10^{-2} M NaBr, N_2O saturated; (B) 5×10^{-4} M $Cu^{II}(GGG)^-$, pH 7·3, 1·0 M Me_3COH, N_2O saturated.

under these conditions. The Figure shows the absorption spectra[6] observed in the NaBr and Me_3COH containing solutions. In the former the transient has λ_{max} 385 $\pm$ 5 nm (ϵ_{max} 4200 $\pm$ 600 l mol^{-1} cm^{-1}). This spectrum is attributed to $Cu^{III}(GGG)^2$ formed in reaction (2). After

$$Br_2^{\cdot-} + Cu^{II}(GGG)^- \longrightarrow Cu^{III}(GGG) + 2Br^- \quad (2)$$
$$k_2 = (1\cdot6 \pm 0\cdot2) \times 10^8 \text{ l mol}^{-1} \text{ s}^{-1}$$

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 $Cu^{III}(GGG)$ 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 $\pm$ 0·05, 3·0 $\pm$ 0·5, and 10 $\pm$ 1 at pH 6·2, 7·3, 7·8, 9·0, and 10·0 respectively, in accord with the reported properties of Cu^{III}(peptide) complexes.[1]

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

$$Cu^{II}(GGG)^- + \cdot CH_2CMe_2OH \longrightarrow$$
$$Cu^{III}(GGG)\text{-}CH_2CMe_2OH^- \quad (3)$$
$$\text{(1)}$$
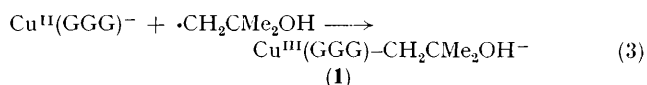
$$k_3 = (2\cdot6 \pm 0\cdot4) \times 10^7 \text{ l mol}^{-1} \text{ s}^{-1}$$

from their respective spectra (Figure) and from the differences in their rates of decomposition (see later). It is also not $Cu^I(GGG)$, as the yield of (1) in He-saturated solutions, where only 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 HO$\cdot$, do so (the former *via* N_2O scavenging, $N_2O + e^-_{aq} + H^+ \rightarrow N_2 + HO\cdot$). 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

$$Cu^{III}(GGG)\text{-}CH_2CMe_2OH^- \xrightarrow{\text{H}_2\text{O}} Cu^I(GGG) +$$
$$HOCH_2CMe_2OH \quad (4)$$
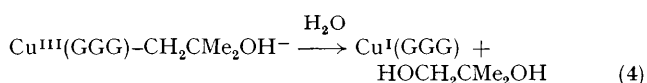
$(H_2O)_n Cu^{III}$–RH.[2] $k_4 = 0\cdot75 \pm 0\cdot10$, $1\cdot5 \pm 0\cdot2$, $2\cdot3 \pm 0\cdot3$, and $2\cdot5 \pm 3$ at pH 7·3, 7·8, 9·0, and 10·2 respectively. The product of reaction (4) cannot be $Cu^{III}(GGG)$ as the rate of decomposition of the latter at pH 7·3 and 7·8 is considerably lower than k_4. At 380 nm the absorption coefficient of $Cu^{III}(GGG)$ is considerably larger than that of $Cu^{III}(GGG)$–$CH_2CMe_2OH^-$; 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: $k[Cu^{II}(GGG) + \cdot CH_2CO_2^-] = (1\cdot6 \pm 0\cdot2) \times 10^7$ l mol^{-1} s^{-1}. The absorption band of the transient in these solutions is broader, with $\lambda_{max} < 350$ nm. The decomposition of the transient identified as $Cu^{III}(GGG)$–$CH_2CO_2^-$ 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

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

step is $7 \cdot 6$ s^{-1} at pH $7 \cdot 8$ and $3 \cdot 5$ s^{-1} at pH $10 \cdot 2$ while that of the second step is $0 \cdot 89$ and $0 \cdot 06$ s^{-1} at these pH values. The exact nature of these two reactions is not clear; however, the decomposition of $(Cu^{III}-CH_2CO_2^-)_{aq}$ also consists of two steps.[2] It is also of interest that the rate of decomposition of $Cu^{III}(GGG)-CH_2CO_2^-$ decreases with increasing pH while that of $Cu^{III}(GGG)$ and $Cu^{III}(GGG)-CH_2CMe_2OH^-$ 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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