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Mechanism of reaction of peroxomethyl radicals with copper(II)(glycine)₂ and copper(II)(glycylglycylglycine) in aqueous solutions

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Abstract

The reactions of O_2CH_3 radicals with Cu^{II}(glycine)₂ and Cu^{II}(GGG), GGG = glycylglycylglycine, in aqueous solutions were studied.

The results demonstrate that the peroxyl radicals oxidize the copper complexes forming relatively stable intermediates of the type $L_m Cu^{III}$ -OOCH₃. These intermediates decompose via oxidation of the ligands glycine and GGG, respectively. Substituents on the alkyl of the peroxyl radical affect somewhat the kinetics of reaction but not the mechanism of oxidation. It is suggested that analogous reactions are probably contributing to the radical-induced deleterious biological processes. © 2005 Elsevier B.V. All rights reserved.

Keywords: Copper complexes; Peroxyl radicals; Biological ligands

1. Introduction

Copper ions are critically important in a wide variety of catalytic, synthetic and biochemical processes in which complexes with carbon–copper bonds are formed [1–8]. Copper salts have been shown to affect different catalytic reactions in which radicals are involved in both aqueous and non-aqueous media. As the enzyme superoxide dismutase contains Cu-ions, there is significant interest in the reactions of oxygen containing radicals with Cu-complexes [9]. Furthermore, in all catalytic processes involving alkyl radicals, alkyl-peroxyl radicals are formed in the presence of dioxygen. Intermediates of the type $L_m M^{n+1}$ -OOR can be formed in biological and catalytic systems where alkyl radicals are formed in the presence of molecular dioxygen [8,10–14]. These complexes are strong oxidizing agents.

Because of the importance of copper complexes in biological systems, it was decided to study the chemical activity of alkyl-peroxyl radicals with copper model complexes. The results might be relevant to radicalinduced biological deleterious processes [8,15]. The properties of the transient complexes LCu^{III}-OOR might also be relevant to the understanding of the properties of complexes of the type Cu–OO–Cu [16–23]. The copper complex with glycylgylcylglycine (GGG) was chosen as a model of protein-bonded copper. For comparison purposes, the reactions with the analogous complex $Cu^{II}(Gly)_2$ were studied.

The pulse radiolysis technique was employed to produce the LCu^{III}-OOR complexes from the corresponding LCu^{II} complexes and the radicals R in the presence of dioxygen.

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2. Experimental

2.1. Materials

All materials used were of AR grade and were purchased from Merck, Aldrich, B.D.H., Fluka or Frutarom. The solutions were prepared using distilled water that was further purified using a Millipore Milli-Q system. The final resistance was better than 10 M Ω /cm. All pH measurements were done using a Corning 220 pH meter.

 N_2O gas was purchased from Maxima. All solutions were saturated with the desired gases by bubbling the gas through the solution in a glass syringe for 20 min [24,25]. Solutions of 50% O_2 and 50% N_2O were prepared by mixing (1:1) the solutions saturated with N_2O or dioxygen.

2.2. Irradiations

The pulse radiolysis experiments were carried out using the Varian 7715 linear electron accelerator of the Hebrew University of Jerusalem. The pulse duration was $0.1-1.5 \,\mu$ s with a 200-mA current of 5 MeV electrons. The dose per pulse was dependent on its length. A 4-cm Suprasil cell was used, with the analyzing light passing through 3 times to yield an optical path length of 12.3 cm. A 150-W xenon arc lamp produced the analyzing light. The entire experimental setup has been described previously [26,27].

The exact dose was determined using a 1×10^{-3} M KSCN solution saturated with N₂O. The yield of $(SCN)_2$ ⁻⁻ was measured using a molar extinction coefficient of $\varepsilon_{475} = 7600$ M⁻¹ cm⁻¹, and the dose per pulse was calculated assuming $G(SCN)_2$ ⁻⁻ = 6.0 [28]. The dose per pulse was set so that the initial radical concentration was 2–20 μ M.

The values of the molar extinction coefficients calculated from the dosimetry measurements have an error limit of 15% due to the scatter in the pulse intensity and due to uncertainties in *G* values. The dose delivered to vials, identical to those irradiated for final product analysis, was measured using the Fricke dosimeter for high dose rates using G(Fe(III)) = 0.68 and $\varepsilon_{302} =$ 2205 M⁻¹ cm⁻¹ [29].

A Noratom 60 Co γ -source with a dose rate of 1400 rad/min was used for low-dose-rate experiments and product analysis.

2.3. UV-Vis measurements

All UV–Vis measurements were performed on a Hewlett Packard 8452A diode array spectrophotometer.

Analysis of formaldehyde was performed according to the literature procedure [30]. Formaldehyde reacts with a reagent containing 0.05 M acetylacetone, 1 M ammonium acetate and 0.033 M acetic acid, to form a yellow complex. The concentration of formaldehyde was determined colorimetrically at the wavelength of maximum absorption (412 nm).

2.4. GC analysis

GC analyses of the product CO_2 were done on a Varian 3700 gas chromatograph with FID and TCD detectors.

As the solubility of CO_2 is high at pH 7, slightly acidic solutions containing all the components were degassed and then solid phosphate salt was added so that pH 7.0 was obtained. These solutions were irradiated and then acidified for analysis. It should be noted that the amount of CO_2 detected in non-irradiated solutions was negligible.

2.5. Formation of radicals with ionizing radiation

The radicals were formed by irradiating the solutions with ionizing radiation (γ radiation or fast electrons).

When ionizing radiation is absorbed by a dilute aqueous solution, the following initial products are formed [31]:

$$\begin{aligned} H_2 O &\xrightarrow{\gamma,e} {}^{\bullet} H(0.60), {}^{\bullet} OH(2.65), e_{aq}^{-}(2.65), H_2 O_2(0.75), \\ H_2(0.45), H_3 O^{+}(2.65) \end{aligned} \tag{1}$$

where the *G* values are given in parentheses (*G* values are defined as the number of molecules of each product per 100 eV of radiation absorbed by the solution). The distribution of these products in the solution after 1×10^{-7} s is homogeneous [31]. The measurements were performed in times longer than 1×10^{-6} s.

Reactions of hydroxyl radicals were studied by saturating the solutions with N₂O (0.022 M) to reduce interference from other initial radicals. This is due to the reaction of N₂O with the solvated electron (e_{aq}^{-}) [32]:

$$e_{aq}^{-} + N_2 O \rightarrow N_2 + OH + OH^-$$

 $k = 8.7 \times 10^9 \text{ M}^{-1} \text{ s}^{-1}$ (2)

It is important that $[H_3O^+]$ be held below 1×10^{-3} M as e_{aq}^- reacts diffusion controlled with H_3O^+ (reaction 3) [32]. Under these conditions, the solvated electrons will all react with N₂O yielding 'OH as the major product:

$$e_{aq}^{-} + H_3 O^+ \rightarrow H + H_2 O \quad k = 2.2 \times 10^{10} \text{ M}^{-1} \text{ s}^{-1} \quad (3)$$

2.6. Preparation of CH_3 and organic radicals R

The 'R radicals were produced via the reaction between the hydroxyl radicals and the hydrogen atoms with RH:

$$\mathbf{R}\mathbf{H} + \mathbf{O}\mathbf{H}/\mathbf{H}^{\bullet} \to \mathbf{R} + \mathbf{H}_2\mathbf{O}/\mathbf{H}_2 \tag{4}$$

Thus, in N₂O saturated solutions containing, e.g., 0.1 M $C(CH_3)_3OH$, the only radical formed is $CH_2C(CH_3)_2-OH$.

The methyl radicals were formed via the following reactions [33]:

$$(CH_3)_2 S^{\bullet}(O)OH \rightarrow CH_3 S(O)OH + {}^{\bullet}CH_3$$

$$k = 1.5 \times 10^7 \text{ s}^{-1}$$
(6)

In solutions containing dioxygen, alkyl ('R) radicals are converted into the corresponding alkyl-peroxyl RO_2 • radicals. Thus, over 90% of the initial radicals are converted to the desired peroxyl radicals in less than 10^{-6} s in solutions saturated with N₂O/O₂ (1:1).

3. Results and discussion

3.1. Reaction of $Cu^{II}(gly)_2$ with O_2CH_3 radicals

When 50% N₂O/50% O₂ saturated aqueous solutions containing CuSO₄ (1.0–20.0 × 10⁻⁴ M), (CH₃)₂SO (0.10 M), KH₂PO₄ (1.0 × 10⁻³ M) and glycine (2 × [Cu^{II}] + 1.0 × 10⁻⁴ M; this slight excess of ligand is sufficient to form the complex Cu^{II}(gly)₂ quantitatively, as verified by the fact that no Cu(OH)₂ precipitated at this pH) at pH 7–10 are irradiated by a short pulse, three distinct time-resolved reactions are observed in the wavelength region of 310–480 nm (Figs. 1 and 2). At 350 nm, a formation followed by two decomposition reactions is observed. In the first reaction, an unstable intermediate is formed within several μ s with $\lambda_{max} = 350 \pm 5$ nm and $\varepsilon_{max} = 870 \pm 50 \text{ M}^{-1} \text{ cm}^{-1}$ (Fig.



Fig. 1. Computer trace of the time-dependence of the first two reactions taking place after irradiation of an N₂O/O₂ (50:50) saturated solution by a short electron-pulse. The solution contained 5.0×10^{-4} M Cu^{II}(gly)₂, 0.10 M (CH₃)₂SO and 0.010 M KH₂PO₄ at pH 7.0. Observation wavelength: 350 nm. (Inset shows the kinetic trace and second-order fit of the decomposition reaction.)



Fig. 2. Computer trace of the time-dependence of the two decomposition reactions taking place after irradiation of an N₂O/O₂ (50:50) saturated solution by a short electron-pulse. The solution contained 1.0×10^{-3} M Cu^{II}(gly)₂, 0.10 M (CH₃)₂SO and 0.010 M KH₂PO₄ at pH 7.0. Observation wavelength: 350 nm. (Inset shows the kinetic traces and respective second and first-order fits.)

3(a)). Its kinetics of formation could not be followed directly as the reaction is too fast.

In accord with similar reactions of alkylperoxyl radicals with transition metal complexes [34–39], it is proposed that the methylperoxyl radicals formed by the radiolysis (under the experimental conditions all of the primary radicals are converted into O_2CH_3 radicals during the time of the pulse) react with $Cu^{II}(gly)_2$ to form an unstable intermediate [(gly)₂- $Cu^{III}-O_2CH_3$].



Fig. 3. Absorption spectra of the intermediates obtained in the first (a) and the second (b) reactions after irradiation of N₂O/O₂ (50:50) saturated solutions containing 1.0×10^{-3} M Cu^{II}(gly)₂, 0.10 M (CH₃)₂SO and 0.010 M KH₂PO₄ at pH 7.0 by an electron pulse. Spectrum (a) was corrected using the equilibrium constant of reaction (7). Spectrum (b) is uncorrected, as the contribution of reaction (8) to the process is not known accurately.

$$\operatorname{Cu}^{\mathrm{II}}(\operatorname{gly})_{2} + {}^{\bullet}\operatorname{O}_{2}\operatorname{CH}_{3} \stackrel{k_{1}}{\underset{k_{-1}}{\rightleftharpoons}} (\operatorname{gly})_{2}\operatorname{Cu}^{\mathrm{III}} {}^{\bullet}\operatorname{O}_{2}\operatorname{CH}_{3}$$
(7)

From a plot of 1/O.D._{max} versus 1/[Cu^{II}(gly)₂] (Fig. 4), the equilibrium constant of this process could be derived as $K_1 = 650 \pm 130 \text{ M}^{-1}$. If $k_1 \ge 6.5 \times 10^7 \text{ M}^{-1} \text{ s}^{-1}$ (a reasonable assumption as $k(\text{Cu}^{II}(\text{gly})_2 + \text{`CH}_3) = (2.5 - 7.0) \times 10^7 \text{ M}^{-1} \text{ s}^{-1}$ [40,41]) one concludes that $k_{-1} \ge 10^5 \text{ s}^{-1}$, therefore the kinetics of reaction (7) are too fast to be studied.

In the second reaction, which takes place in the ms timescale under our experimental conditions, the intermediate formed in the first reaction decomposes in a process obeying a second-order rate law. The spectrum derived after this process is similar, though not identical, to the spectrum of the intermediate derived after the initial formation reaction. The decomposition probably involves the biradical reaction $(2CH_3O_2^{\bullet} \rightarrow \text{products}, \text{ as the steady-state concentration of the CH}_3O_2^{\bullet}$ is relatively high), explaining the second-order rate law, in parallel to an isomerization of the first intermediate. The observed rate of this reaction $\Delta \varepsilon \times (2.4 \pm 0.4) \times 10^6 \text{ M}^{-1} \text{ s}^{-1}$ has clearly contributions of the second-order rate rocess and the isomerization reaction: $(\text{gly})_2 \text{Cu}^{\text{III}}$ - $O_2 \text{CH}_3 \text{-}R$.

The resemblance of the spectra of the intermediates formed in the first and second reaction (Fig. 3(a) and (b)) indicates that they are similar, but not identical. It is therefore reasonable to assume that the second intermediate is the reorganization product of the first intermediate. There is no way to determine the exact structure of this intermediate, thus it is represented as $[(gly)_2Cu^{III}-O_2CH_3]_R$. Similar isomerizations have been reported for the transients formed in the reactions of other radicals such as $\cdot CH_2CO_2^{-1}$ and $\cdot CH_2C(CH_3)_2OH$ with $Cu^{II}(GGG)$ [42] and for $\cdot CH_3$ with $Cu^{II}(gly)_2$ [40,41].



Fig. 4. Determination of the equilibrium constant of the formation of the first intermediate, $(gly)_2Cu^{III}$ -O₂CH₃. Irradiated solutions were saturated with N₂O/O₂ (50:50) and contained Cu^{II}(gly)₂ (5.0–20.0 × 10⁻⁴ M), 0.10 M (CH₃)₂SO and 0.010 M KH₂PO₄ at pH 7.0.

In the third reaction (Fig. 2), which is relatively slow and obeys a first-order rate law with a rate constant of $k = 0.70 \pm 0.14 \text{ s}^{-1}$, the latter intermediate [(gly)₂Cu^{III}-O₂CH₃]_R decomposes to yield the final products.

The final products CO₂ and formaldehyde were analyzed for solutions containing [Cu^{II}] = 1.0×10^{-3} M, glycine (2×[Cu^{II}] + 1.0×10^{-4} M), [(CH₃)₂SO] = 0.10 M and [KH₂PO₄] = 1.0×10^{-3} M at pH 7.0. The solutions were irradiated for 10 min in the ⁶⁰Co γ -source, 1400 rad/min, i.e., at a low dose rate, which decreases the contribution of the second-order process. Formaldehyde and CO₂ are produced at equal yields, $G = 2.5 \pm 0.5$ was derived for both products.

In accordance with the above observations, the following mechanism is suggested:

$$Cu^{II}(gly)_{2} + O_{2}CH_{3} \stackrel{k_{1}}{\underset{k_{-1}}{\leftarrow}} (gly)_{2}Cu^{III} O_{2}CH_{3}$$
$$K_{7} = 650 \text{ M}^{-1}$$
(7)

 $^{\bullet}O_{2}CH_{3} + ^{\bullet}O_{2}CH_{3} \rightarrow CH_{3}OOOOCH_{3}$

 \rightarrow non-radical products [43]

$$k_8 = 4 \times 10^8 \text{ M}^{-1} \text{ s}^{-1}$$
 [44] (8)

$$(gly)_2Cu^{III}-O_2CH_3 \rightleftharpoons [(gly)_2Cu^{III}-O_2CH_3]_R$$
 (9)

$$[(gly)_2Cu^{III}-O_2CH_3]_R \to \text{products} \quad k_{10} = 0.7 \text{ s}^{-1}$$
 (10)

The final products CO_2 and formaldehyde are most probably decomposition products of the glycine ligand. It is proposed that the rearranged intermediate $[(gly)_{2-}$ $Cu^{III}-O_2CH_3]_R$ decomposes in the following way to yield the final products:

$$\begin{split} [(gly)_2Cu^{III}-O_2CH_3]_R & \stackrel{H_2O}{\rightarrow} Cu^{III}(gly)_{aq}^{2+} + CO_2 \\ & + CH_2 = NH + CH_3OH + 2OH^- \end{split}$$
(11)

 $CH_2 = NH + H_2O \rightarrow CH_2O + NH_3 \tag{12}$

i.e.,

$$\begin{aligned} (gly)_2 Cu^{III} - O_2 CH_3 &\rightarrow Cu^{III} (gly)_{aq}^{2+} + CO_2 + CH_2 O \\ &+ NH_3 + CH_3 OH + 2OH^- \end{aligned} \tag{13}$$

The Cu^{III}(Gly) decomposes via: [45]

$$Cu^{III}(gly)_{aq}^{2+} \rightarrow Cu_{aq}^{2+} + CO_2 + {}^{\bullet}CH_2NH_2$$
(14)

$$CH_2NH_2 + Cu^{III}(gly)_{aq}^{2+}$$

$$\rightarrow Cu^{II}(gly)_{aq}^{+} + CH_2 = NH + H^+$$
(15)

and probably by some oxidation of CH_2O by the Cu^{III} complexes.

As the intermediate $(gly)_2Cu^{III}-O_2CH_3$ is a strong oxidizing agent, it is proposed that the intermediate

decomposes via a mechanism that involves oxidation of the ligand in accord with the determined decomposition products. The yield of CO₂ and CH₂O is considerably lower than the initial yield of the CH₃O₂• radicals, $G(CH_3O_2•) \sim 6$. This is probably due to some contribution of reaction (8) and other side reactions in the relatively long irradiation in the ⁶⁰Co γ -source.

3.2. Reaction of $Cu^{II}(GGG)$ with O_2CH_3 radicals

When 50% $N_2O/50\%$ O_2 saturated aqueous solutions containing CuSO₄ ((1.0–20.0) × 10^{-4} M), (CH₃)₂SO $(1.0 \times 10^{-2} \text{ M})$ (0.10 M), KH₂PO₄ and GGG $([Cu^{II}] + 1.0 \times 10^{-4} \text{ M}; \text{ this slight excess of ligand is suf-}$ ficient to form the complex Cu^{II}(GGG) quantitatively, as verified by the fact that no Cu(OH)₂ precipitated at this pH) at pH 7-10 are irradiated by a short pulse, four distinct time-resolved reactions are observed (Figs. 5-7). Table 1 summarizes selected observed rate constants for these four processes under different conditions of pH (7-9) and initial concentrations of Cu^{II}(GGG). The spectra of the intermediates formed in the first three processes are shown in Fig. 8.

In the first reaction, an unstable intermediate is formed within several hundred μ s (under the experimental conditions) with $\lambda_{max} = 395 \pm 5$ nm. Its kinetics of formation follows a first-order rate law. Surprisingly, this rate is independent of the concentration of Cu^{II} and other components of the solution, pH or wavelength of observation. However, the reaction rate depends slightly on pulse intensity. The rate constant of this reaction was determined as $(2.0 \pm 1.6) \times 10^4$ s⁻¹. In analogy with the previous system, the first reaction is inferred to be the formation of the unstable intermediate [(GGG)Cu^{III}-O₂CH₃]. The equilibrium constant of the formation of this first intermediate was derived form



Fig. 5. Computer trace of the time-dependence of the first two reactions taking place after irradiation of an N₂O/O₂ (50:50) saturated solution by a short electron-pulse. The solution contained 6.0×10^{-4} M Cu^{II}(GGG), 0.10 M (CH₃)₂SO and 0.010 M KH₂PO₄ at pH 7.0. Observation wavelength: 380 nm. (Inset shows the kinetic traces and first-order fit of both reactions.)



Fig. 6. Computer trace of the time-dependence of the first and the third reactions taking place after irradiation of an N₂O/O₂ (50:50) saturated solution by a short electron-pulse. The solution contained 5.0×10^{-4} M Cu^{II}(GGG), 0.10 M (CH₃)₂SO and 0.010 M KH₂PO₄ at pH 7.0. Observation wavelength: 380 nm. (Inset shows the kinetic trace and first-order fit of the third reaction.)



Fig. 7. Absorption spectra during the long term decomposition of (GGG)Cu^{III}-OOCH₃ after irradiation of an N₂O-saturated solution containing 5.0×10^{-4} M Cu^{II}(GGG), 0.10 M (CH₃)₂SO and 0.010 M KH₂PO₄ at pH 7.0. Inset shows the kinetic trace at 302 nm.

the plot of $1/[(GGG)Cu^{III}]$ versus $1/O.D._{max}$ as $K = (2300 \pm 340) M^{-1}$, Fig. 9. As the kinetics of formation is independent of $[Cu^{II}(GGG)]$, the reaction is probably not taking place between the main form of the complex and the peroxyl radical, but with its isomer $Cu^{II}(GGG)'$.

The second reaction takes place in the 100-ms time range. Below ca. 420 nm a formation is observed. Its kinetics follows a first-order rate law. The rate constant of this reaction was determined as $(5.0 \pm 0.2) \times 10^2 \text{ s}^{-1}$. It is independent of the wavelength of observation or the complex concentration but depends on the pH (increasing the H⁺ ion concentration increases the rate, see Table 1).

The third reaction (decay at all wavelengths of observation) takes place in the 0.4-s time range and follows a first-order rate law with a rate constant of $k = (18 \pm 100\%) \text{ s}^{-1}$.

As the spectra of the first three intermediates formed are very similar, it is suggested that the second and third

Table 1 Kinetic results of the system $Cu^{II}(GGG)/O_2CH_3$ radical

[Cu ²⁺] (M)	[GGG] (M)	[DMSO] (M)	pH	$k_1 (s^{-1})$	$k_2 (s^{-1})$	$k_3 (s^{-1})$	$k_4 ({\rm min}^{-1})$
5×10^{-4}	6.0×10^{-4}	0.5	7.0	1.6×10^{4}	4.9×10^{2}	18	0.88
1×10^{-3}	1.1×10^{-3}	0.5	7.0	2.0×10^{4}	5.4×10^{2}	18	1.03
2×10^{-3}	2.1×10^{-3}	0.5	7.0	2.0×10^{4}	4.9×10^{2}	13	0.63
1×10^{-3}	1.1×10^{-3}	0.5	8.0	2.2×10^{4}	3.3×10^{2}	32	0.62
1×10^{-3}	1.1×10^{-3}	0.5	9.0	2.2×10^{4}	1.4×10^{2}	13	0.57



Fig. 8. Absorption spectra of the intermediates obtained in the first (a), second (b) and the third (c) reaction after irradiation of N₂O/O₂ (50:50) saturated solutions containing 1.0×10^{-3} M Cu^{II}(GGG), 0.10 M (CH₃)₂SO and 0.010 M KH₂PO₄ at pH 7.0 by an electron pulse.

observed reactions are reorganization processes. It should be noted that these spectra differ considerably from that of $Cu^{III}(GGG)$ [42]. Thus, one has to conclude that all the observed intermediates are isomers of $(GGG)Cu^{III}-O_2CH_3$.

The 4th reaction, the decomposition of the methylperoxo intermediate, is a slow reaction that takes place on the timescale of minutes. Its kinetics of decomposition is shown in Fig. 7. The reaction follows a firstorder rate law with a rate constant of $k = (1.03 \pm 0.15) \text{ min}^{-1}$.

As no formaldehyde was found as a product in this system, as could have been expected, if the reaction would proceed via the oxidation of $Cu^{II}(GGG)$, it is suggested that the decomposition proceeds via oxidation of the GGG ligand and not the central Cu-ion. Attempts to identify ligand oxidation products by HPLC failed.

The suggested mechanism is summarized in the following scheme:

$$Cu^{II}(GGG) \rightleftharpoons Cu^{II}(GGG)'$$

$$k_{14} = (2.0 \pm 1.6) \times 10^4 \text{ s}^{-1}$$
(16)



Fig. 9. Determination of the equilibrium constant of the formation of the first intermediate, (GGG)Cu^{III}-O₂CH₃. Irradiated solutions were saturated with N₂O/O₂ (50:50) and contained Cu^{II}(GGG) (4.0– 20.0×10^{-4} M), 0.10 M (CH₃)₂SO and 0.010 M KH₂PO₄ at pH 7.0.

$$Cu^{II}(GGG)' + O_2CH_3 \rightleftharpoons (GGG)Cu^{III} - O_2CH_3$$

$$K_{15} = 2300 \text{ M}^{-1}$$
(17)

$$(GGG)Cu^{III}-O_2CH_3 \rightarrow [(GGG)Cu^{III}-O_2CH_3]_{R1}$$

 $k_{16} = (5.0 \pm 0.15) \times 10^2 \text{ s}^{-1}$ (18)

$$[(GGG)Cu^{III} - O_2CH_3]_{R1} \rightarrow [(GGG)Cu^{III} - O_2CH_3]_{R2} k_{17} = (18 \pm 100\%) \text{ s}^{-1}$$
 (19)

$$\begin{split} & [(GGG)Cu^{III}\text{-}O_2CH_3]_{R2} + H_3O^+ \\ & \rightarrow Cu^{III}(GGG) + HO_2CH_3 \quad \text{side reaction} \end{split} \tag{20}$$

$$HO_2CH_3 \to CH_2O + H_2O \tag{21}$$

$$\begin{split} \left[(GGG)Cu^{III}\text{-}O_2CH_3 \right]_{R2} & \rightarrow \left[(GGG-2H)Cu^{III}\text{-}OH \right] \\ & + CH_3OH \quad \text{main reaction} \end{split}$$

$$[(GGG - 2H)Cu^{III}-OH] \rightarrow final \ products$$
(23)

$$^{\bullet}\text{O}_{2}\text{CH}_{3} + ^{\bullet}\text{O}_{2}\text{CH}_{3} \rightarrow \text{non-radical products}$$

$$k_{8} = 4 \times 10^{8} \text{ M}^{-1} \text{ s}^{-1} \text{ [44]}$$
(8)

Table 2 Equilibrium constants of the formation of LCu^{III}-OOR system

1	•			
Copper complex	Peroxyl radical	K(LCu ^{III} -OOR) (M ⁻¹)		
Cu ^{II} (GGG)	·O ₂ CH ₃	2300 ± 340		
Cu ^{II} (GGG)	[•] O ₂ CH ₂ CN	2500 ± 400		
Cu ^{II} (GGG)	'O ₂ CH ₂ C(CH ₃) ₂ OH	700 ± 105		
Cu ^{II} (gly) ₂	·O ₂ CH ₃	650 ± 130		

In this system, the influence of the following substances was also tested: thiosulfate, sulfite, nitrite, and ascorbate. None of these substances reacted with the formed intermediate.

3.3. Reaction of $Cu^{II}(GGG)$ with O_2CH_2CN and $O_2CH_2C(CH_3)_2OH$ radicals

Similar intermediates were formed in the reactions of O_2CH_2CN and $O_2CH_2C(CH_3)_2OH$ radicals. These systems were not studied in detail.

According to the results, the peroxyl radicals derived from the methyl-, acetonitrile- and *tert*-butanol-radical react with the Cu^{II}(GGG) complex to yield the intermediate complex (GGG)Cu^{III}O₂R in equilibria reactions. The results are summarized in Table 2. The weakest binding is that to the peroxyl-*tert*-butanol radical, as expected for a molecule with electron-donating substituents. The peroxyl radicals derived from methyl and acetonitrile yield similar equi- librium constants, although acetonitrile is the more electronegative group. The radicals 'O₂CH₂OH and 'O₂CH₂COO⁻ did not react with the Cu–triglycine complex.

The equilibrium constant of the reaction between the methylperoxyl radical and $Cu^{II}(gly)_2$ is lower than that with $Cu^{II}(GGG)$, indicating that the GGG ligand supports the Cu(III) state in the LCu^{III}-OOR complex better than the glycine ligand.

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