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LETTER

EPR evidence of intermediate peroxo complexes formed in a SOD model system

Satoshi Fujii, Hiroaki Ohya-Nishiguchi* and **Noboru Hirota**

Department of Chemistry, Faculty of Science, Kyoto University, Kyoto 606 (Japan)

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Oxygen activation by transition metal complexes has been studied extensively in recent years. As one of such complexes, FeEDTA (EDTA: ethylenediaminetetraacetate) has been previously investigated [1–12]. Fee and coworkers reported that FeEDTA catalyzes superoxide dismutation and suggested the formation of an intermediate peroxo complex, $[Fe^{3+}\text{–EDTA–}O_2]^{3-}$, in the catalytic cycle [1, 2]. This peroxo complex was first described by Cheng and Lott in 1956 [3] and characterized in considerable detail by others [4–8]. However, the mode of dioxygen binding to the ferric ion is still in controversy [2, 7, 8]. Thus, it is desirable to apply EPR (electron paramagnetic resonance) spectroscopy to such systems to obtain more detailed information about the microscopic structural mode of the intermediate peroxo complex.

During the EPR investigations of the reactions of Fe^{3+}EDTA with hydrogen peroxide, t-butyl hydroperoxide (t-BuOOH), and n-butyl hydroperoxide (n-BuOOH), we first succeeded in finding the existence of two types of intermediate peroxo complexes which have different crystal field parameters, and of a ferrous superoxo complex. We report here the detection and structural characterization of these complexes and propose a possible reaction mechanism based on the experimental results obtained.

Experimental

$NaFeEDTA\cdot 3H_2O$ (Dohjin), Na_2EDTA (nacalai tesque), 30% H_2O_2 (Wako Pure Chemicals) and t-BuOOH (nacalai tesque) were used as received. n-BuOOH was offered by Professor Ishizu of Ehime University. All peroxo complexes were prepared by dissolving $NaFeEDTA\cdot 3H_2O$ in H_2O followed by the addition of peroxides to give a solution 2 mM in Fe and 200 mM in peroxides. Na_2EDTA was also added to stabilize peroxo complexes [5]. In this study a ratio of [FeEDTA]:[Na_2EDTA] = 1:5 was employed. In spite of the addition of Na_2EDTA, the peroxo complexes derived from the reaction with H_2O_2 were unstable. Especially the species in neutral pH solution were very unstable, so the samples were frozen rapidly in liquid nitrogen.

The pH of the solution was controlled with a NaOH solution by using a Horiba $L\text{-}7_{LC}$ pH meter. X band EPR measurements were carried out with a JES-FE3X spectrometer equipped with 100-kHz field modulation at 77 and 4.2 K. Cr^{3+} in MgO ($g = 1.9800$) was used as a standard g marker.

Results and discussion

In a weak alkaline solution, Fe^{3+}EDTA behaves as a weak acid [9, 10].

$$[Fe^{3+}\text{EDTA-}OH_2]^- \rightleftharpoons [Fe^{3+}\text{EDTA-OH}]^{2-} + H^+ \quad (1)$$

The EPR spectrum of $[Fe^{3+}\text{EDTA-OH}]^{2-}$ (**1**) in H_2O (pH 10.0) is shown in Fig. 1(a)*. The spectrum exhibits a sharp signal at $g = 4.2$ which is characteristic of a high-spin non-heme ferric system. Addition of H_2O_2 to a nearly neutral solution (pH 7.6) resulted in the appearance of a new signal, as shown in Fig. 1(b). The sharp signal at $g = 4.2$ appearing in Fig. 1(b) corresponds to original FeEDTA uncoordinated by the peroxo anion. This is probably due to an equilibrium reported by Ringbom *et al.* [4]. The g values of the new signal ($g_1 = 4.39$, $g_2 = 4.15$ and $g_3 = 3.90$) indicate that this new species (**2**) formed by adding H_2O_2 has high rhombic distortion similar to but distinguishable from **1**. On the contrary, the addition of H_2O_2 to an alkaline solution (pH 11.2) led to a different species (**3**) with a less rhombic distortion, $g_1 = 5.08$, $g_2 = 3.81$ and $g_3 = 3.37$, as shown in Fig. 1(c). It is suggested from the comparison of

*Author to whom correspondence should be addressed.

*The EPR spectrum of FeEDTA in aqueous solution shows pH dependence. The spectrum is very broad at low pH and becomes sharp as the solution becomes basic. A sharp signal can be observed at neutral region if the solution is frozen rapidly. (See the signal of uncoordinated species in Fig. 1(b).) The spectrum at pH 10.0 is chosen for good correspondence to the uncoordinated species in Fig. 1(b). The broad signal at pH 7 is, of course, different from that of species **2**.

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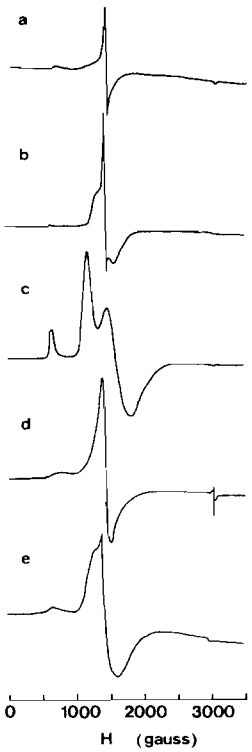


Fig. 1. EPR spectra of FeEDTA complexes in water at 77 K: (a) FeEDTA, pH 10.0; (b) FeEDTA + H_2O_2, pH 7.6; (c) FeEDTA + H_2O_2, pH 11.2; (d) FeEDTA + t-BuOOH, pH 11.9; (e) FeEDTA + n-BuOOH, pH 11.9.

the g values of **3** with those of **2**, that the structure of **3** in alkaline solution is different from that of **2** in nearly neutral solution. Indeed, the nearly neutral solution was colorless whereas the alkaline solution was purple ($\lambda_{max} = 520$ nm), as has been previously reported [1, 3–5, 7].

As the reaction of Fe^{3+}EDTA with H_2O_2 proceeded, the signal intensity of **3** decreased, and then disappeared. At this stage of the reaction only a small signal due to a trace amount of **1** could be seen. After a while the signal of **1** reappeared and its intensity gradually increased.

In order to clarify the reaction and the structure of the intermediate species **2** and **3**, we have tried to react Fe^{3+}EDTA with typical organic peroxides. The solutions were prepared at high pH to make organic peroxides serve as anionic ligands, $^-$OOR (generally $pK_a \geqslant 11.5$ for alkyl peroxides). Figure 1(d) shows an EPR spectrum of FeEDTA with t-BuOOH in H_2O at pH 11.9. The major component of the species formed (**4**) has a little broad linewidth, the g values being $g_1 = 4.33$, $g_2 = 4.18$ and $g_3 = 3.98$. The signal around $g = 2$ in the Figure is due to the t-BuOO$^\cdot$ radical ($g_\parallel = 2.03$, $g_\perp = 2.008$), which is generated from an excess amount of t-BuOOH. The spectrum of the FeEDTA–n-BuOOH system (**5**) in alkaline solution (pH 11.9) is shown in Fig. 1(e). Its g values, $g_1 = 4.60$, $g_2 = 4.18$ and $g_3 = 3.75$, are similar to those of **2** shown in Fig. 1(b).

The EPR data obtained were analyzed by $S = 5/2$ spin Hamiltonian

$$\mathscr{H} = D[S_z^2 - 35/12 + E/D(S_x^2 - S_y^2)] + g_0\beta S \cdot H \qquad (2)$$

where D and E in the first term are zero-field splitting (ZFS) parameters. E/D were determined by a numerical calculation of the relation between the apparent g and E/D by using a NEC PC-9801 personal computer. Binding of peroxides should cause changes in D as well as E/D. However, observed g values change only slightly if $D > 0.4$ cm^{-1}. We adopt here, therefore, $D = 0.83$ cm^{-1} for Fe^{3+}EDTA reported by Aasa [13].

The g values and calculated E/D of these species are summarized in Table 1. The E/D values of species **1**, **2**, **4** and **5** are nearly equal. This fact suggests that the metal environments in these complexes resemble each other. Consequently it is expected that the ferric ions in these species have the same coordination number. Thus, we conclude that the structure of these peroxo complexes is a monodentate or 'end-on' type, [FeEDTA–$^-$OOR]$^{2-}$ (R = H, t-Bu and n-Bu).

On the other hand, species **3** gives a unique value of $E/D = 0.20 \pm 0.01$. Similar EPR spectra were observed above pH 8 and increased in intensity with pH. According to Francis *et al.*, the intensity of the absorption at 520 nm which characterizes the 'FeEDTA peroxo complex' increased gradually around pH 8 and came to a plateau at pH 10 [7]. Thus it is concluded that species **3** corresponds to the 'FeEDTA peroxo complex'. Considering the E/D value of **3**, the structure of **3** is evidently different from others. In an alkaline solution, deprotonation of $^-$OOH is expected and the resultant O_2^{2-} is probably in existence. If O_2^{2-} ligated in an end-on mode, the E/D value of **3** should be nearly equal to those of **1**, **2**, **4** and **5**. Hence it is most likely that the peroxo ligation mode to FeEDTA of **3** is a cyclic or ' side-on' fashion. Our results obtained by EPR measurements support the results of Bull *et al.* [2] and Ahmad *et al.* [8].

The reason for the decrease in E/D from 0.31 in **1** to 0.20 in **3** is considered as follows. The seven-coordinated ferric ion of [FeEDTA–OH_2]$^-$ in the crystalline state has a distorted pentagonal-bipyramidal geometry as reported by Lind *et al.* [14]. Two nitrogens from amine and three oxygens (one from the water molecule and the others from carboxylates) occupy the pentagonal plane, whereas the remaining two carboxylate oxygens coordinate to the axial position. If a nucleophilic ligand exchange of $^-$OH with $^-$OOH results in the 'side-on peroxo complex'

TABLE 1. EPR parameters for FeEDTA complexes with peroxides

Species	Peroxides	pH	g value			E/D
1[a]		10.0	4.20	4.15	4.14	0.31±0.01
2	H_2O_2	7.6	4.39	4.15	3.90	0.29±0.01
3[a]	H_2O_2	11.2	5.08	3.81	3.37	0.20±0.01
4	t-BuOOH	11.9	4.33	4.18	3.98	0.30±0.01
5	n-BuOOH	11.9	4.60	4.18	3.75	0.28±0.01

[a]These species were also measured at 4.2 K.

$[FeEDTA–O_2]^{3-}$ (species **3**), it presumably has a distorted hexagonal-bipyramidal geometry. Being occupied by six atoms, crystal field symmetry on the plane may be improved. Therefore the value of E/D, as well as E, is expected to become smaller.

At higher pH (above *c.* 12), where the superoxide ion becomes relatively stable, a typical EPR spectrum of O_2^- ($g_\parallel = 2.060$, $g_\perp = 2.0069$) could be detected (Fig. 2(a) and (b)). A signal derived from **3** was also seen with a low intensity in Fig. 2(a). These signals suggest two interesting facts. First, through the examination of the reported g values of O_2^- which interacts with the metal ions, we found that the value of $g_\parallel$ reflects the O_2^- environment well [15]. Free O_2^- in solution exhibits the signal at $g_\parallel \geqslant 2.10$. If O_2^- has an interaction with the metal ion, $g_\parallel$ becomes smaller, e.g. $g_\parallel = 2.084$ for Co^{3+}–Mb–O_2^- [16], $g_\parallel = 2.081$ for Fe^{3+}–xanthine oxidase–O_2^- [15], $g_\parallel = 2.079$ for Co^{3+}–$P450_{cam}$–O_2^- [17], $g_\parallel = 2.076$ for Co^{3+}–Hb–O_2^- [18]. Compared with these $g_\parallel$ values, the observed $g_\parallel = 2.060$ is still smaller. This demonstrates clearly that O_2^- contained in our system interacts with the iron more tightly (Fe^{2+}–O_2^-). Second, the decrease of the signal intensity of **3** and concomitant increase of the Fe^{2+}–O_2^- complex (see Fig. 1(c) and Fig. 2(a)) indicates the existence of a charge transfer process

$$Fe^{3+}EDTA–O_2^{2-} \rightleftharpoons Fe^{2+}EDTA–O_2^- \quad (3)$$

The EPR signal intensity change described above supports the charge transfer process. This ferrous superoxo complex is a key intermediate in the catalytic cycle both of FeEDTA and FeSOD systems [2, 19, 20]. Although the mode of superoxo coordination to FeEDTA is unknown, it is presumably in an end-on fashion.

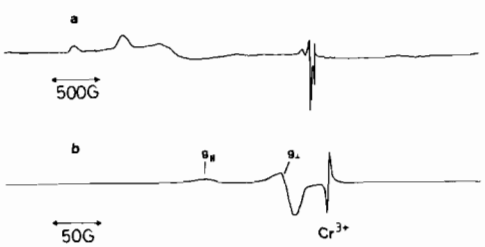
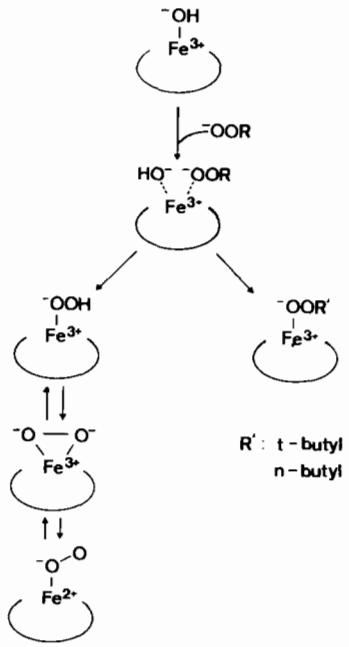


Fig. 2. EPR spectra of FeEDTA with H_2O_2 at pH 11.9: (a) 2500±2500 G; (b) spectrum of O_2^-, 3250±250 G.



Scheme 1. Possible reaction mechanism in a SOD model system.

Thus, we propose a possible reaction mechanism on the basis of our investigation (Scheme 1). In summary, the present EPR study reveals that (i) the bound dioxygen of the 'FeEDTA peroxo complex' is in a side-on peroxo configuration, but a transient end-on hydroperoxo complex also exists, and (ii) Fe^{3+} is reduced to Fe^{2+} by O_2^{2-} and forms a Fe^{2+}EDTA superoxo complex. In order to examine the reaction mechanism, more detailed studies are now under way.

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References

1 G. J. McClune, J. A. Fee, G. A. McClusky and J. T. Groves, *J. Am. Chem. Soc., 99* (1977) 5220.
2 C. Bull, G. J. McClune and J. A. Fee, *J. Am. Chem. Soc., 105* (1983) 5290.
3 K. L. Cheng and P. F. Lott, *Anal. Chem., 28* (1956) 462.
4 A. Ringbom, S. Siitonen and B. Saxen, *Anal. Chim. Acta, 16* (1957) 541.
5 C. Walling, M. Kurz and H. J. Schugar, *Inorg. Chem., 9* (1970) 931.
6 Y. Ilan and G. Czapski, *Biochim. Biophys. Acta, 498* (1977) 386.
7 K. C. Francis, D. Cummins and J. Oakes, *J. Chem. Soc., Dalton Trans.,* (1985) 493.
8 S. Ahmad, J. D. McCallum, A. K. Shiemke, E. H. Appelman, T. M. Loehr and J. J. Sanders-Loehr, *Inorg. Chem., 27* (1988) 2230.
9 G. Schwarzenbach and J. Heller, *Helv. Chem. Acta, 34* (1951) 576.
10 P. N. Balasubramanian and T. C. Bruice, *J. Am. Chem. Soc., 108* (1986) 5495.
11 P. N. Balasubramanian and T. C. Bruice, *Proc. Natl. Acad. Sci. U.S.A., 84* (1987) 1734.
12 J. D. Rush and W. H. Koppenol, *J. Inorg. Biochem., 29* (1987) 199.
13 R. Aasa, *J. Chem. Phys., 52* (1970) 3919.
14 M. D. Lind, M. J. Hamor, T. A. Hamor and J. L. Hoard, *Inorg. Chem., 3* (1964) 34.
15 P. F. Knowles, J. F. Gibson, F. M. Pick and R. C. Bray, *Biochem. J., 111* (1969) 53.
16 M. R. Green, H. A. O. Hill and D. R. Turner, *FEBS Lett., 103* (1979) 176.
17 G. C. Wagner, I. C. Gunslus, M. Y. R. Wang and B. M. Hoffman, *J. Biol. Chem., 256* (1981) 6266.
18 T. Yonetani, H. Yamamoto and T. Iizuka, *J. Biol. Chem., 249* (1974) 2168.
19 C. Bull and J. A. Fee, *J. Am. Chem. Soc., 107* (1985) 3295.
20 D. M. Dooley, J. L. Karas, T. F. Jones, C. E. Cote and S. B. Smith, *Inorg. Chem., 25* (1986) 4761.