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Formation of Phenoxy Radical by Binuclear Iron(III) Complex and Hydrogen Peroxide System

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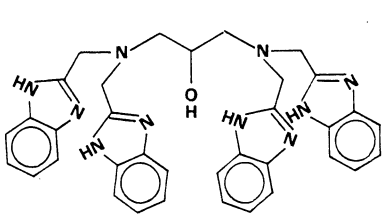
We have found that several peroxide adducts of binuclear iron(III) compounds react with phenols to yield a phenoxy radical, and concluded that the formation of the radical may be attributed to hydrogen atom abstraction by the peroxide adduct with electrophilic nature.

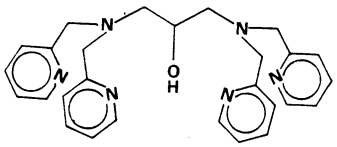
Binuclear non-heme iron centers are utilized in living system organism for reversible oxygen binding(hemerythrin) and for oxygen activation(methane monooxygenase and ribonucleotide reductase).[1] Ribonucleotide reductase from Escherichia coli consists of two subunits, B1 and B2; active enzyme is a 1:1 complex of the two subunits and it carries a stable radical on tyrosine-122 and a binuclear iron center, both of which are required for enzyme activity.[2] The radical is an oxidized form of the tyrosine residue and the iron center is an antiferromagnetically coupled pair of high-spin ferric center connected by a μ-oxo bridge.[3] There are many spectroscopic similarities between the binuclear iron center of hemerythrin and ribonucleotide reductase. In hemerythrin the normal reaction between the reduced iron center of deoxyhemerythrin and oxygen is the formation of oxyhemerythrin with a reversible di-ferric peroxy configuration. In ribonucleotide reductase the reaction between the reduced iron center and molecular oxygen might lead to a similar di-ferric peroxy form, which however is not stable but immediately oxidizes the nearby tyrosine residue, yielding a tyrosine radical. However, there is no report on tyrosine radical formation by the synthetic di-ferric peroxy compounds. In this article we wish to show the first phenoxy radical formation by the binuclear iron(III)-peroxide adducts which was detected by the ESR spectroscopy.

The binuclear iron(III) compounds used in this study are as follows: $Fe_2(HPTB)(OH)(NO_3)_4$,[4] $Fe_2(HPTP)(OH)(NO_3)_2(ClO_4)_2$,[5] $[(n\text{-}Bu)_4N][Fe_2(L\text{-}pac)$

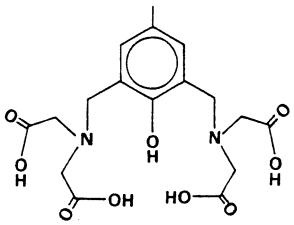
$(CH_3COO)_2]$,[6] $K_3Fe_2O(nta)_2(CH_3COO)$,[7] $Fe_2O(tpa)_2(CH_3COO)(ClO_4)_3$,[8] and $(enH_2)[Fe_2O(Hedta)_2]$,[9] where $H_3(nta)$, (tpa) and $H_3(Hedta)$ represent nitrilotriacetic acid, tris(2-pyridylmethyl)amine, and N-(2-hydroxyethyl)-ethylenediamine-N,N',N'-triacetic acid, respectively, and the chemical structures of some ligands are illustrated below. It has been reported that former three binuclear iron(III) complexes react with hydrogen peroxide to yield a (1:1) peroxide adduct.[4-6] The phenols, 2,6-di-tert-butyl-4-methylphenol(BHT) and 2,6-di-tert-butyl-4-hydroxyanisole (DTBHA) were obtained commercially, and once recrystallized from ethanol prior to study. NADH(reduced form) was obtained commercially (Nacalai Tesque). The intensity of the ESR signal was normalized in terms of that of MnO(standard marker).



H(HPTB)



H(HPTP)



H_5(L-pac)

As shown in Fig. 1, the solution of DTBHA and hydrogen peroxide gives negligible ESR signal(trace A in Fig. 1). It is clear that the formation of the phenoxy radical(g-value(2.0039) and peak-to-peak separation(0.081 mT) observed are quite consistent with those reported previously[10]) is greatly enhanced by the presence of the binuclear iron(III) complexes

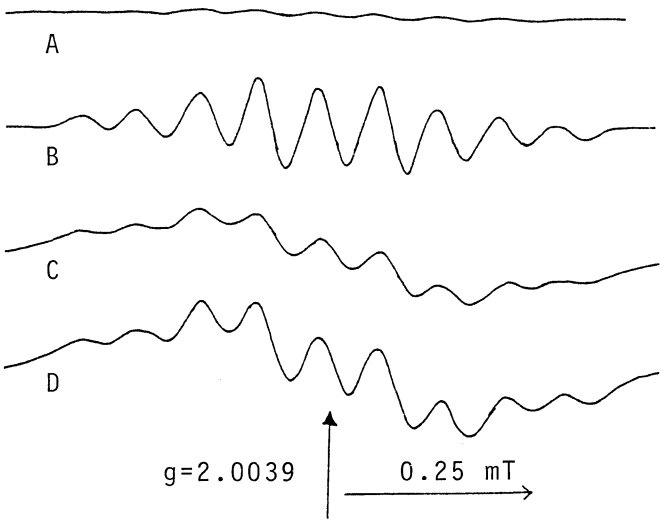


Fig. 1. ESR spectra(X-band, 25 °C) of equi-volume mixtures of DTBHA(in ethanol, 1/25 M), H_2O_2(in ethanol/water=1/1, 1/50 M), and Fe(III) complex(in ethanol/water=1/1, 1/500 M).
A: no Fe(III) complex, x100
B: $Fe_2(HPTP)(OH)(NO_3)_2^{2+}$, x1
C: $Fe_2(HPTB)(OH)(NO_3)_2^{2+}$, x20
D: $Fe_2(L\text{-}pac)(CH_3COO)_2^{-}$, x100

with H(HPTP) and H(HPTB)(cf. traces B and C in Fig. 1). It should be noted here that the formation of phenoxy radical is much lower in the mixed solution of Fe_2(L-pac)$(CH_3COO)_2^-$ and DTBHA(cf. trace D in Fig. 1). This indicates that the presence of both H_2O_2 and binuclear iron(III) complex does not always lead to facile phenoxy radical formation.

A similar fact was also observed for the binuclear iron(III) compounds with μ-oxo bridge; notable formation of phenoxy radical is observed for the solution of $Fe_2O(tpa)_2(CH_3COO)^{3+}$(cf. trace A in Fig. 2), but the formation is much lower in the solutions of $Fe_2O(nta)_2(CH_3COO)^{3-}$ (trace B in Fig. 2) and $Fe_2O(Hedta)^{2-}$. The formation of the phenoxy radical was also observed in the solutions containing phenol, binuclear iron(III) complex and reducing reagents such as ascorbic acid or NADH (cf. trace C in Fig. 2). In the latter case, the order of ESR signal intensity is almost the same as that observed for the solutions with hydrogen peroxide, that is, (tpa) $\gg$ (nta) $\sim$ (Hedta). It seems likely that the phenoxy radical formation in the solution with NADH may proceed as follows; at first two iron(III) atoms are reduced to an iron(II) state and the reduced binuclear iron(II) species reacts with dioxygen molecule, yielding a di-ferric peroxy species, and this reacts with phenol. The latter assumption can be supported by the work obtained in this study.

Several authors have pointed out that the ferryl intermediate(Fe(IV)=O) is important for the formation of tyrosine radical in ribonucleotide reductase.[11,12] The formation of ferryl state should be greatly dependent on the electronic state of the iron(III) ion, and the

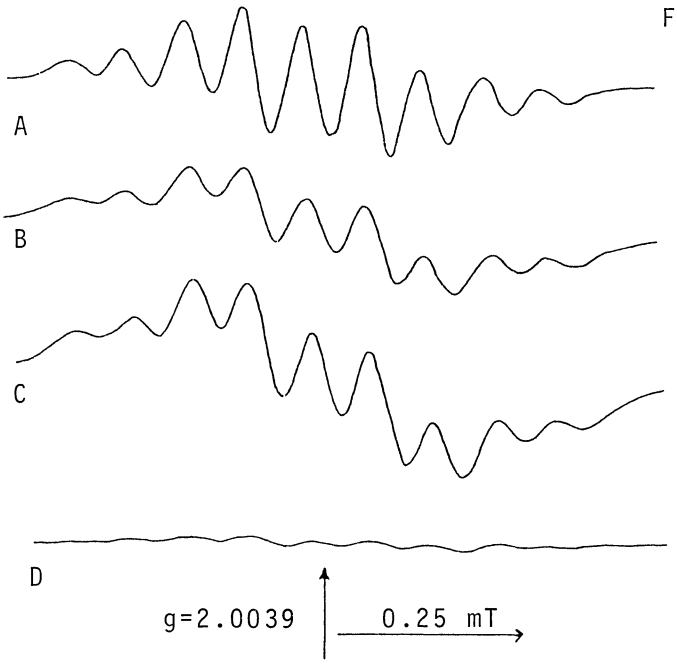


Fig. 2. ESR spectra(X-band, 25 °C) of equi-volume mixtures of DTBHA(in ethanol, 1/25 M), Fe(III) complex(in ethanol/water=1/1, 1/500 M) and H_2O_2 (in ethanol/water=1/1, 1/50 M) or NADH(in water, 3/500 M).
A: $Fe_2O(tpa)_2(CH_3COO)^{3+}$ + H_2O_2 + DTBHA, x1
B: $Fe_2O(nta)_2(CH_3COO)^{3-}$ + H_2O_2 + DTBHA, x40
C: $Fe_2O(tpa)_2(CH_3COO)^{3+}$ + NADH + DTBHA, x25
D: $Fe_2O(nta)_2(CH_3COO)^{3-}$ + NADH + DTBHA, x100

formation seems to be more favorable when the iron(III) is more oxidizable. The electrochemical data have revealed that the iron(III) ion in $Fe_2(L\text{-pac})(CH_3COO)_2^-$ is more oxidizable than others in binuclear compounds such as with H(HPTB) and H(HPTP).[5] In the present study we have found that the more reducible the iron(III) is, the higher the intensity of ESR signal due to phenoxy radical is, in the reaction mixture of DTBHA, H_2O_2 and binuclear iron(III) compound. This may rule out the possible role of a ferryl intermediate. In the previous papers, we have reported that the peroxide ion in the adduct with $Fe_2(HPTP)(OH)(NO_3)_2^{2+}$ is more activated(i.e., with stronger electrophilic nature) than those in $Fe_2(HPTB)(OH)^{4+}$ and $Fe_2(L\text{-pac})(CH_3COO)_2^-$ on the basis of the study for catalase- and bleomycin-like functions of these compounds.[5] Thus, we would like to propose that the activation of peroxide ion by the binuclear iron(III) compound is closely related with the formation of phenoxy radical observed in this study, and also tyrosine radical formation in ribonucleotide reductase.

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