

Preparation and Catalase-Like Function of a Binuclear Iron(III) Complex with N,N,N',N'-Tetrakis(2-pyridylmethyl)-2-hydroxy-1,3-diaminopropane

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Z. Naturforsch. **47b**, 115–120 (1992); received June 6/July 22, 1991

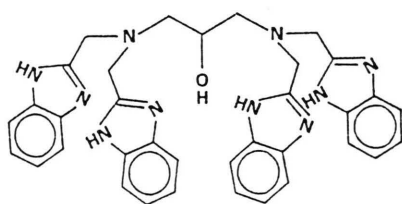
Binuclear Iron(III) Complex, Catalase-Like Function, DNA Cleavage

The complex derived from $\text{Fe}(\text{NO}_3)_3 \cdot 9\text{H}_2\text{O}$ and perchlorate salt of N,N,N',N'-tetrakis(2-pyridylmethyl)-2-hydroxy-1,3-diaminopropane (abbreviated as H(HPTP)) is formulated as a binuclear species with μ -alkoxo and μ -hydroxo bridges, $[\text{Fe}_2(\text{HPTP})(\text{OH})(\text{NO}_3)_2](\text{ClO}_4)_2$, on the basis of analytical data, magnetic properties, and conductivity data. This complex reacts with hydrogen peroxide in water to form a violet adduct with new charge transfer bands in the range 520–600 nm. This adduct is very unstable at room temperature, indicating the high catalase-like function of this complex. The complex exhibits a high ability to degradate DNA in the presence of H_2O_2 . The observed high catalase-like and bleomycin-like functions of the complex are discussed in terms of the activation of the peroxide ion by the binuclear iron(III) compound.

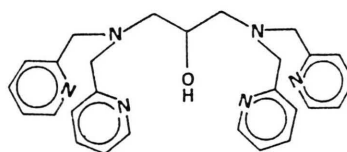
1. Introduction

Diiron centers that are known to interact with dioxygen have been found in hemerythrin [1], methane monooxygenase [2–4], and ribonucleotide reductase [5–7]. Deoxyhemerythrin reversibly binds dioxygen with an active site consisting of a (μ -hydroxo)bis(μ -carboxylato)diiron(II) core and five terminal histidines. Dioxygen binds to the diferrous center at the remaining vacant coordination site with concomitant electron and proton transfer to form a (μ -oxo)diferric center with a coordinated hydroperoxide moiety that is hydrogen bonded to the oxo bridge [8, 9]. Dioxygen may bind in similar or related fashion to the diferrous center of methane monooxygenase and ribonucleotide reductase to effect the necessary oxidative chemistry associated with these enzymes [4, 10].

A number of ligand systems [11–14] have been used to model the spectroscopic and structural properties of the diferric sites in the crystallographically characterized azidomethemerythrin [15] and ribonucleotide reductase [16]. While there are many examples of structurally characterized dinuclear cobalt and copper peroxide complexes [17, 18], the study on the nonheme diferric peroxide complexes is limited at present [19]. In our previous paper [20], we have reported the synthesis of the binuclear iron(III) complex with N,N,N',N'-tetrakis(2-benzimidazolylmethyl)-2-hydroxy-1,3-diaminopropane (abbreviated as H(HPTB), see the Figure below), $\text{Fe}_2(\text{HPTB})(\text{NO}_3)_5 \cdot \text{solvate}$, and have reported that this complex reacts with hydrogen peroxide to form a blue 1:1 adduct with a new charge transfer band at 600 nm ($\epsilon = 1300$ in methanol) [20, 21]. This blue peroxide adduct exhibits



H (HPTB)



H (HPTP)

* Reprint requests to Dr. Y. Nishida.

unique reactivities such as transformation of phenol to *o*-quinone [20], cleavage of DNA [22], and oxygenation of adamantane [23]. In order to get

more information on the reactivity of peroxide adducts of the binuclear iron(III) compounds, we have prepared a new binuclear complex with the ligand, N,N,N',N'-tetrakis(2-pyridylmethyl)-2-hydroxy-1,3-diaminopropane (abbreviated as H(HPTP), see the above figure), and investigated the reaction between the diferric complex and hydrogen peroxide. It was found that the present complex with H(HPTP) also reacts with hydrogen peroxide to give a violet adduct in water, which is very unstable, contrary to that of the complex with H(HPTB). The instability of the peroxide adduct of the present compound was discussed in terms of the possible activation of hydrogen peroxide by the binuclear iron(III) complexes.

2. Materials and Method

Preparation. The ligand, H(HPTP) was obtained as the perchlorate salt according to the slightly modified method reported by Toftlund *et al.* [24].

Analysis for H(HPTP) · 4HClO₄ · H₂O

Calcd C 37.00 H 4.14 N 9.59,

Found C 36.80 H 4.09 N 9.51.

The iron(III) complex was obtained as follows: To 50 ml methanol solution of H(HPTP) · 4HClO₄ (0.002 mol) and triethylamine (0.01 mol), 10 ml of methanol solution of Fe(NO₃)₃ · 9H₂O (0.004 mol) was added with stirring. After one hour, yellow crystals precipitated and were filtered, and recrystallized from ethanol solution.

Analysis for Fe₂(HPTP)(OH)(NO₃)₂(ClO₄)₂ · H₂O

Calcd C 35.15 H 3.49 N 12.14 Fe 12.1,

Found C 34.97 H 3.31 N 12.16 Fe 11.6.

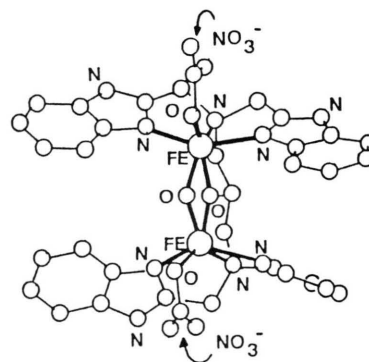
Measurements: Magnetic susceptibility data were recorded over a temperature range 81.1–296.8 K by Dr. T. Tokii at Saga University. Absorption spectra were recorded with a Hitachi spectrophotometer model 330 at 25 °C. ESR spectra were obtained with a JEOL ESR apparatus model JES RE-2X using X-band at room temperature. The cyclic voltammograms (CV) were measured in N,N-dimethylsulphoxide (dmsO), 0.1 M tetra(*n*-butyl)ammonium tetrafluoroborate as supporting electrolyte, 0.001 M metal complex, at 25 °C, and using a glassy carbon electrode. The potential was referenced to a saturated sodium chloride calomel electrode (SSCE).

DNA degradation by binuclear iron(III) compounds in the presence of H₂O₂. The reaction mixture (30 ml) containing 10 ml of DNA (1 mg/1 ml),

10 ml of Fe(III) complex (0.004 M), and 10 ml of H₂O₂ (0.1 M), was kept at room temperature. At appropriate time after mixing, 4 ml of the reaction mixture were combined with 1 ml of a 2 N HCl solution containing 40 mg of 2-thiobarbituric acid (TBA), and heated to 93 °C for 15 min. After cooling, the absorbance at 532 nm of the resulted solution was recorded [22].

3. Results and Discussion

Nishida and co-workers [20] first formulated the complex of Fe(III) and H(HPTB) as Fe₂(HPTB)(NO₃)₅ · solvate based on its elemental analysis. Conductivity measurements indicated a 1:2 complex and led to the suggestion of a (μ-alkoxo)(μ-nitrato)diiron core for the complex with two terminal nitrates. Very recently Que *et al.* [25] have determined the crystal structure of this complex, and found that two iron atoms are bridged by alkoxo and hydroxo oxygen atoms, as depicted below, and pointed out that this complex should be formulated to be Fe₂(HPTB)(OH)(NO₃)₂²⁺.



The analytical data of the present complex with H(HPTP) is coincident with an assumed formula, Fe₂(HPTP)(OH)(NO₃)₂(ClO₄)₂. In Fig. 1, the temperature dependence of the magnetic susceptibility is shown. The magnetic moments are 2.10 μ_B and 3.98 μ_B at 81.1 and 296.8 K, respectively, and the magnetic behaviour can be rationalized using the Heisenberg–Dirac–van Vleck spin Hamiltonian, $\mathcal{H} = -2JS_1 \cdot S_2$ with $-J = 23.9 \text{ cm}^{-1}$, $g = 2.00$, and $N\alpha = 0$. This clearly demonstrates that the complex is of a binuclear structure with alkoxo or hydroxo bridges [26]. All these data including the conductivity (200 ohm⁻¹ cm² mol⁻¹ in 1 mM methanol solution at 25 °C) suggest that the structure of the

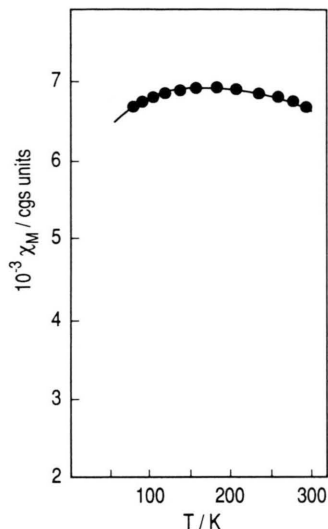


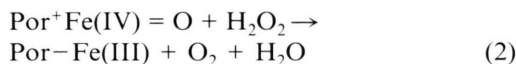
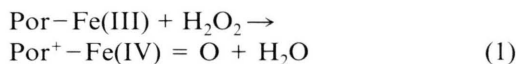
Fig. 1. Temperature dependency of magnetic susceptibility of $\text{Fe}_2(\text{HPTP})(\text{OH})(\text{NO}_3)_2(\text{ClO}_4)_2$. ●●●: experimental value —: theoretical curve based on the theoretical expression derived from spin Hamiltonian $\mathcal{H} = -2JS_1 \cdot S_2$ with $-J = 23.9 \text{ cm}^{-1}$, $g = 2.00$, $N\alpha = 0$ with 1% of monomeric contamination.

present complex, $\text{Fe}_2(\text{HPTP})(\text{OH})(\text{NO}_3)_2(\text{ClO}_4)_2$ is almost the same as that of the corresponding H(HPTB) complex with μ -alkoxo and μ -hydroxo bridges as depicted above.

In Fig. 2, the absorption spectrum of this complex is shown. In all the solvents available, no bands are observed in the range 900–450 nm. The addition of hydrogen peroxide to the yellow solution caused a drastic color change, *e.g.* a deep blue species formed in solvents such as methanol, dimethylsulphoxide, and acetonitrile. These blue species are very unstable, however, and turn to the original yellow color within a few minutes. In water, the violet species, obtained by the addition of hydrogen peroxide, is rather stable and the absorption spectrum of this species is shown in Fig. 2, new absorption bands being observed in the region 520–600 nm. But this violet species is much less stable (it decomposes within 10 min at 25 °C as shown in Fig. 2) than the violet species of the corresponding H(HPTB) complex [22]. The absorption spectrum of the latter violet species remains unchanged for more than 3 h under the same experimental conditions as described for the H(HPTP) complex in Fig. 2. Since the violet spe-

cies derived from the present compound and hydrogen peroxide is very unstable, we cannot apply the continuous variation method to this system, but it seems reasonable to consider that the violet species derived from the complex with H(HPTP) is also a (1:1) peroxide adduct, as assumed for the case of H(HPTB) [21], based on the similarity in the spectral properties between two species. Thus, the instability of the peroxide adduct of the H(HPTP) complex demonstrates that the present binuclear iron(III) complex exhibits high catalase-like function.

Catalase is a heme-containing enzyme which catalyzes the decomposition of hydrogen peroxide in the following manner [27]:



The details of each step, especially step (1), remain unclear at present. The formation of a $\text{Fe(IV)} = \text{O}$ species cited in equation (1) suggests the presence

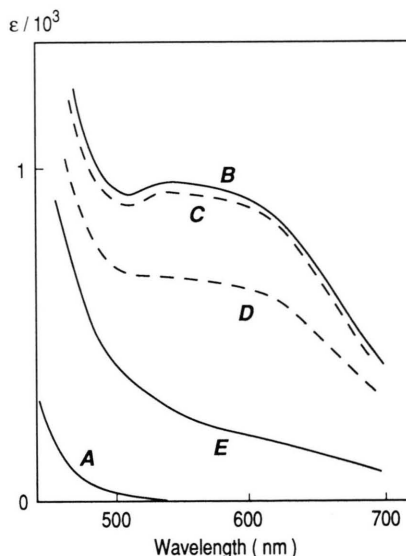


Fig. 2. Absorption spectra of $\text{Fe}_2(\text{HPTP})(\text{OH})(\text{NO}_3)_2(\text{ClO}_4)_2$ in water at 25 °C. A: $\text{Fe}_2(\text{HPTP})(\text{OH})(\text{NO}_3)_2(\text{ClO}_4)_2$ (1 mM); B: mixed solution of the iron(III) complex (1 mM) and hydrogen peroxide (10 mM), measured immediately after mixing; C: above solution, measured 2 min after mixing; D: above solution, measured 5 min after mixing; E: above solution, measured 10 min after mixing.

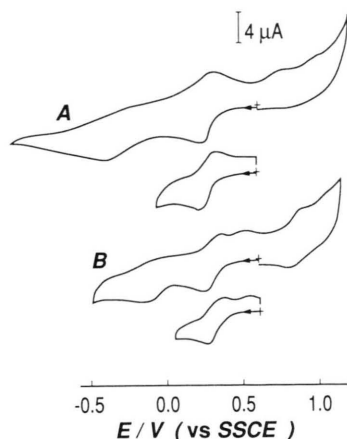
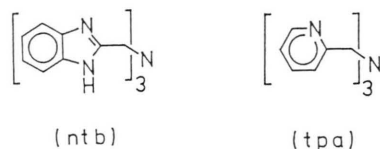


Fig. 3. CV of the compounds (in dmso, 25 °C, scan speed 100 mV/s).

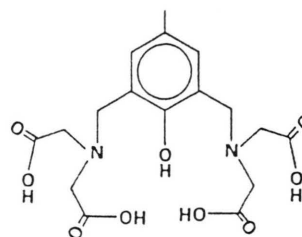
A: $\text{Fe}_2(\text{HPTB})(\text{OH})(\text{NO}_3)_4$;
B: $\text{Fe}_2(\text{HPTP})(\text{OH})(\text{NO}_3)_2(\text{ClO}_4)_2$.

of an electron transfer reaction between heme and hydrogen peroxide. If we assume that such an electron transfer step is also important in the catalase-like function of $\text{Fe}_2(\text{HPTP})(\text{OH})(\text{NO}_3)_2^{2+}$, we can expect that there should be much difference in the electrochemical properties between the two compounds with H(HPTB) and H(HPTP), since only the (HPTP)-complex exhibits high catalase-like function. In order to confirm the above suggestion, we have measured the cyclic voltammograms (CV) of the compounds (Fig. 3). The redox couple, observed around +0.3 V (vs SSCE) for both compounds, should be due to $\text{Fe}(\text{III}) \rightleftharpoons \text{Fe}(\text{II})$, according to studies on the electrochemical properties of mononuclear iron(III) complexes with similar ligand systems [28], *i.e.* corresponding redox couples were observed at +0.42 and +0.20 V (vs SSCE) for $[\text{Fe}(\text{ntb})\text{Cl}_2]^+$ and $[\text{Fe}(\text{tpa})\text{Cl}_2]^+$, respectively, where (ntb) and (tpa) are tripod-like ligands as shown below:



Thus, since the electrochemical properties of both complexes are similar (Fig. 3), there clearly should be another reason for the catalase-like

function being observed only for the H(HPTP) complex, without taking the redox reaction between binuclear iron(III) complex and hydrogen peroxide into consideration. In addition to the above facts, it is known that the binuclear iron(III) complex with $\text{H}_5(\text{L-pac})$ (see below), $\text{Fe}_2(\text{L-pac})(\text{CH}_3\text{COO})_2^-$, forms a stable peroxide adduct [29], and also that the reduction potential, $\text{Fe}(\text{III}) \rightarrow \text{Fe}(\text{II})$ is more negative (*ca.* -0.32 V (vs



$\text{H}_5(\text{L-pac})$

SSCE)) [30] than those of complexes with H(HPTB) and H(HPTP). Since the iron(III) ion in the complex with $\text{H}_5(\text{L-pac})$ is more readily oxidized than those in complexes with H(HPTP) and H(HPTB), the fact that the peroxide adduct of the former complex is very stable clearly implies that the formation of a $\text{Fe}(\text{IV}) = \text{O}$ species is unlikely in the reaction between the binuclear iron(III) compounds with H(HPTB) and H(HPTP) and hydrogen peroxide, although the formation of a $\text{Fe}(\text{IV}) = \text{O}$ species has been proposed for the reaction mechanism of methane monooxygenase [31] and as an explanation for the catalase-like function of ribonucleotide reductase [10].

In order to get more detailed informations on the reactivity of the peroxide adducts of binuclear iron(III) compounds, we have examined the DNA degradation by these peroxide adducts, because we already reported that some binuclear iron(III)-peroxide adducts can cleave DNA [22]. In Fig. 4, the time dependency of the absorbance at 532 nm of the TBA-treated reaction mixtures (*cf.* experimental section) is provided, and the result shows that the peroxide adduct of the H(HPTP)-complex is more effective in degrading DNA among the three compounds. It should be noted here that the order of ability of compounds for DNA degradation is roughly parallel to that of the catalase-like

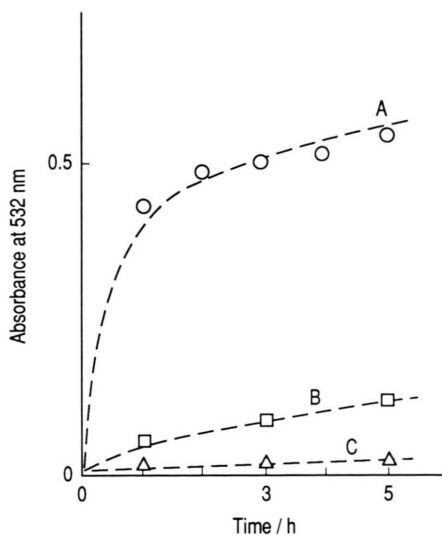


Fig. 4. Time dependency of absorbance at 532 nm in the reaction mixture (DNA, binuclear iron(III) complex, and hydrogen peroxide) treated by TBA (*cf.* experimental section).

A: Fe₂(HPTP)(OH)⁴⁺;
 B: Fe₂(HPTB)(OH)⁴⁺;
 C: Fe₂(L-pac)(CH₃COO)₂⁻.

function; H(HPTP) ≫ H(HPTB) > H₅(L-pac). Since the addition of NaClO to the reaction mixture containing DNA and H₂O₂ (without iron(III) complex) did not cause any degradation of DNA, it is clear that singlet oxygen (¹O₂) is not an “active species” in the present case, because the “NaClO–H₂O₂ system” is known as a famous singlet oxygen generator [32]. Based on these facts, we would like to propose that the catalase- and bleomycin-like function of the complex with H(HPTP) should have the same origin. The activated peroxide coordinating to the iron atoms reacts directly with substrates such as DNA or H₂O₂, as assumed in our previous paper [22]. Our assumption seems to be consistent with the suggestion that OH[•] or O₂^{•-} are not “active species” in the degrading DNA by the Fe(II)-bleomycin system [33, 34]. The reason for the lower reactivity of peroxide in the complex with H₅(L-pac) will be a future problem to solve.

We are thankful for the supports from the Inamori Foundation and a Grant-in-Aid for Scientific Research No. 02403012 from the Ministry of Education, Science and Culture.

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