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Functional modeling of manganese-containing O_2 evolution enzymes with manganese porphyrin dimers

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Abstract

As a functional model of manganese catalyses, manganese porphyrin dimers exhibited high catalytic activity. This modeling system of the enzymatic H_2O_2 dismutation was supposed to involve the corresponding Mn_2^{III}/Mn_2^{IV} couple in its catalytic cycle. A Mn^{IV} porphyrin dimer, $[Mn^{IV}(OR)_2P]_2$ (R = OMe or OH), as a candidate of the high-valent Mn intermediate was prepared by the oxidation of the Mn complex with ClO^- in order to establish the detailed reaction mechanism of the modeling reaction with a manganese porphyrin dimer. The resultant high-valent Mn complex was characterized by UV–vis, ESR, and IR spectroscopies. Its reduction rate by H_2O_2 was measured photometrically at low temperatures and compared with that observed by the catalytic dismutation of the corresponding Mn^{III} complex. The reduction rate of $[Mn^{IV}(OR)_2P]_2$ was higher than the overall rate of the catalytic dismutation. Thus, these results suggest that $[Mn^{IV}(OR)_2P]_2$ or the related complex could be the intermediate in the dismutation reaction and its reduction by H_2O_2 is a fast process in the overall catalytic cycle. This is in agreement with the previous mechanistic studies and a similar intermediate is likely to be involved in the catalytic cycle of Mn catalyses.

Keywords: Manganese catalase; Manganeseporphyrin; Dismutation; Hydrogen peroxide

1. Introduction

Manganese-containing enzymes: manganese superoxide dismutase (Mn-SOD), manganese catalase (Mn-CAT), and photosynthetic oxygen-evolving complex (OEC) play important roles in oxygen evolution in biological systems [1–3]. Among them Mn-CATs, they are a family of

$$2O_2^- + 2H^+ \xrightarrow{\text{Mn-SOD}} O_2 + H_2O_2 \quad (1)$$

$$2H_2O_2 \xrightarrow{\text{Mn-CAT}} O_2 + 2H_2O \quad (2)$$

$$2H_2O \xrightarrow{\text{OEC}} O_2 + 4H^+ + 4e^- \quad (3)$$

manganese-containing catalyses isolated from bacteria, *Lactobacillus plantarum* [4–6], *Thermoleophilum album* [7], and *Thermus thermophilus* [8]. They catalyze the dismutation of H_2O_2 to O_2 in high efficiency and their active center is a subunit consisting of two manganese ions. Low-resolution X-ray crystallographic analysis has been done for only one, isolated from *T. thermophilus* [9]. Spectroscopic studies using EXAFS and ESR [1] have indicated that

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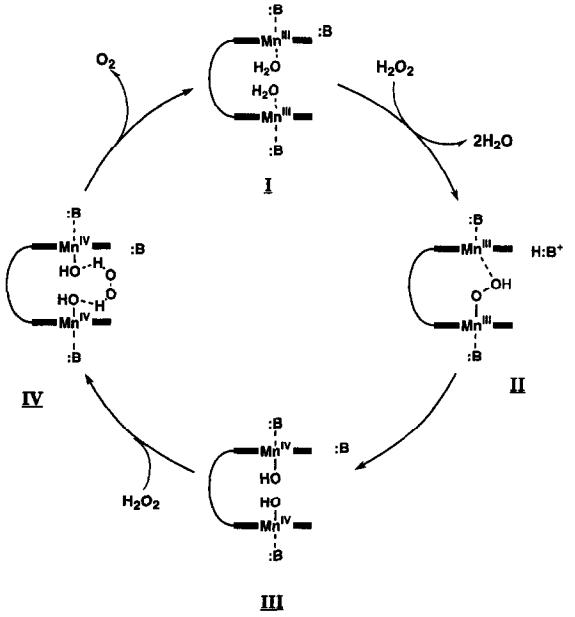
two manganese ions are assumed to form the μ-oxo, bicarbonate-bridged binuclear core. Thus, the structure of the active site is completely different from the well-known heme catalase. The S_2 state of OEC exhibits catalase activity [10,11]. Mn-CATs are also interesting because of their implication in photosynthetic water oxidation in higher plants and cyanobacteria.

Many modeling studies have been performed with the aim of understanding the role of these polynuclear manganese clusters in the reaction [12–20], though no systematic study has been carried out with simple manganese complexes. These manganese clusters do not involve porphyrin ligand. However, manganese porphyrin *monomers* are known to dismutate H_2O_2 [21], while their activity is not high compared with dinuclear manganese complexes. Porphyrin ligands are easy to synthesize and one can prepare dimers exhibiting various metal–metal separation. Furthermore, its supramolecular structure could form a cavity in which an optimum reaction site ensures high activity. We have reported the attainment of high 'catalase' activity with manganese porphyrin dimers having an appropriate metal–metal separation in the presence of a nitrogen base [22–26]. Optimum metal separation has been determined to be about 4 Å [27], which is in agreement with a putative μ-peroxodimanganese complex (Mn–O–O–Mn). This leads either to the simultaneous interaction of two oxygen atoms in one H_2O_2 molecule at a suitable reaction stage or to the formation of a certain μ-peroxide complex. A mechanistic study suggested that the initial H_2O_2 forms a higher-valent manganese complex $[Mn^{IV}(OR)_2P]_2$ as a reactive intermediate, which oxidizes the second H_2O_2 molecule to evolve O_2 (Scheme 1). In this study, we report the preparation of these two possible manganese(IV) complexes as the reaction intermediate and the kinetics of their reaction with H_2O_2 [28]. By comparison of the resulting rates with that of the catalytic reaction, we established the reaction scheme.



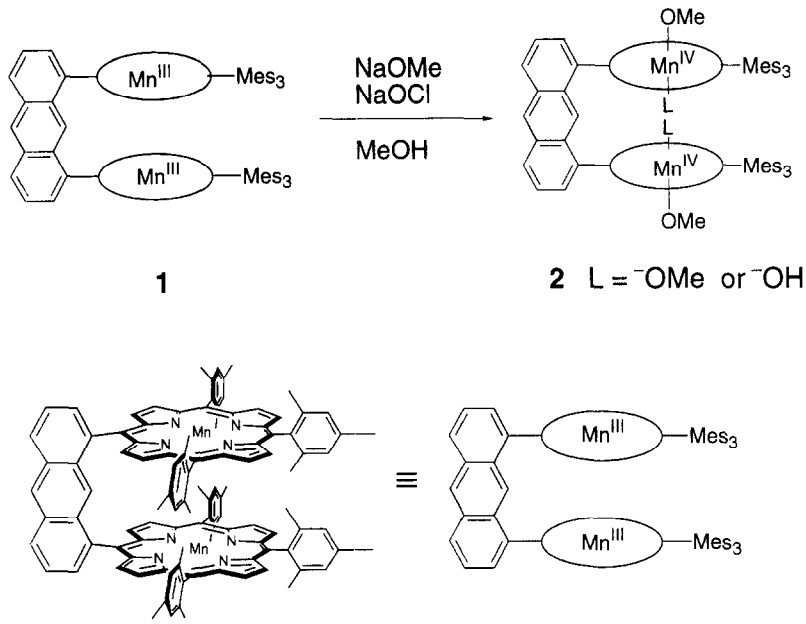
Scheme 1. Proposed catalytic cycle of dismutation of hydrogen peroxide with manganese porphyrin dimers.

2. Results and discussion

In order to determine the reaction pathway of the dismutation in our model system, we chose anthracene-linked manganese porphyrin dimer **1**. By incorporation of mesityl groups at *meso* positions, one could prevent undesirable μ-oxo dimer formation both intra- and intermolecularly.

2.1. Preparation and characterization of manganese(IV) (L)(OMe) complex of the porphyrin dimer

Manganese(III) *meso*-tetramesitylporphyrin was converted to the corresponding $Mn^{IV}(OMe)_2$ complex by treatment with sodium hypochlorite in basic methanolic solution [29]. By a similar method, 1,8-anthracene-linked trimesityl porphyrin dimer **1** was converted to the corresponding Mn_2^{IV} complex **2** (Scheme 2). A methanolic solution of **1** was mixed with NaOMe. After



Scheme 2. Synthesis of the manganese(IV) derivative of anthracene-linked porphyrin dimer by the treatment of NaOCl.

removal of the resultant insoluble NaCl by filtration, aqueous NaOCl was added at −10°C. The brown precipitate was filtered to give the corresponding Mn_2^{IV} complex **2**. its Uv–vis spectrum at −65°C showed a Soret band at $\lambda_{max} = 423$ nm (CH_2Cl_2), which is similar to that of the corresponding TMP monomer (Fig. 1). The ESR spectrum showed a strong and broad band characteristic of a Mn^{IV} porphyrin at $g = 4.4$ (CH_2Cl_2, 4 K). its shape and g-value were similar to those of the corresponding monomer (Fig. 2). This spectrum showed the absence of any magnetic interactions, e.g., any μ-oxo bond, between the two manganese(IV) ions. The IR spectrum supported the presence of a Mn–O bond in complex **2**, $\nu_{Mn-O} = 558\ cm^{-1}$.

The Mn^{IV} dimer **2** was somewhat unstable compared to the corresponding monomer. One can store **2** as a fine powder at −80°C for several hours without any decomposition. However, during its storage for a longer period, it decomposed to the corresponding Mn^{III} derivative. As an CH_3CN solution at −20°C, its decomposition was negligibly small for several hours. Thus, one can use it for the rate measurement experiment with H_2O_2.

2.2. Heat measurement of the reaction between 2 and H_2O_2

The reduction rate of **2** with H_2O_2 was determined by the time-course of its UV–vis-spectral change in CH_3CN at the temperature range between −20 to −40°C. In the kinetic analysis, we assumed that each manganese ion in the dimer independently reacts with H_2O_2. Its rate is expressed as Eq. (4), where $[Mn^{III}P]$ and $[Mn^{IV}P]$ are the concentrations of the Mn^{III} and Mn^{IV} porphyrin monomer residues, respectively.

$$\frac{d[Mn^{III}P]}{dt} = k_3[Mn^{IV}P]^2[H_2O_2] \tag{4}$$

$$k_3 t = R \tag{5}$$

$$R = \frac{1}{A}\frac{2[Mn^{III}P]}{[Mn^{IV}P]_0([Mn^{IV}P]_0 - 2[Mn^{III}P])} + \frac{1}{B^2}\ln\left\{\frac{[H_2O_2]_0([Mn^{IV}P]_0 - 2[Mn^{III}P])}{([H_2O_2]_0 - [Mn^{III}P])[Mn^{IV}P]_0}\right\}$$

$$A = 2[H_2O_2]_0 + [Mn^{IV}P]_0$$

$$B = 2[H_2O_2]_0 - [Mn^{IV}P]_0$$

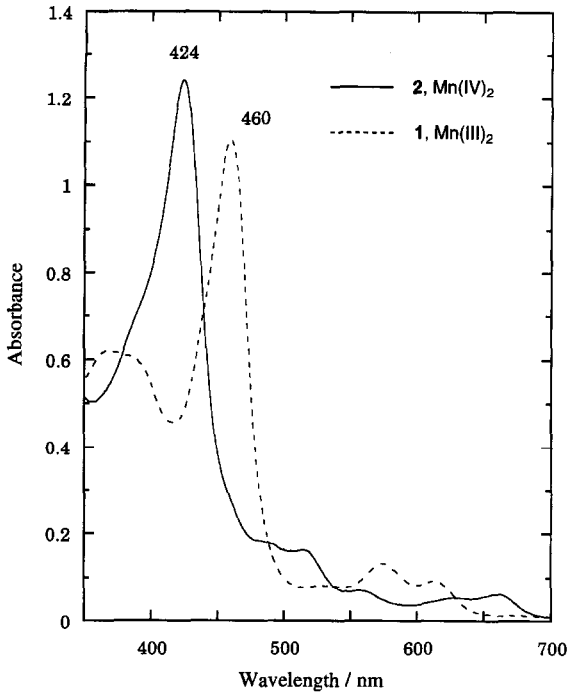


Fig. 1. UV–vis spectrum of Mn^{IV} (————) and Mn^{III} (---) complex.

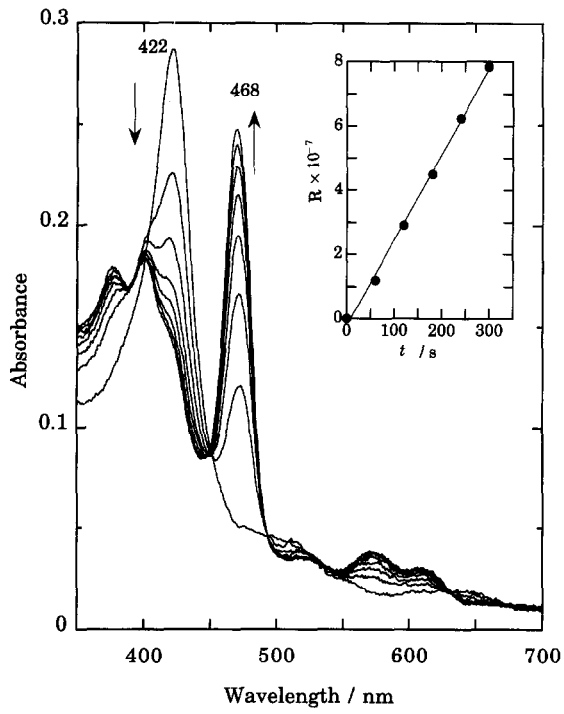


Fig. 3. Time-course of the UV–vis spectrum of the reaction between 2 and H_2O_2 at −40°C. Inset is the plot according to Eq. (5).

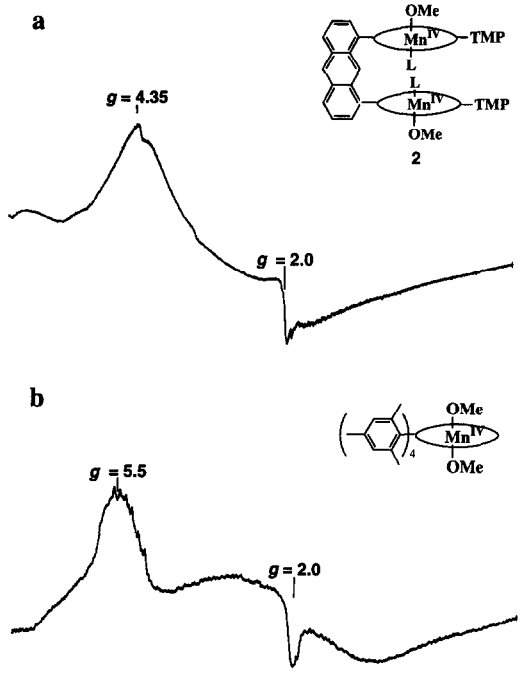


Fig. 2. ESR spectrum of (a) 2 and (b) $Mn^{IV}(OMe)_2$ derivative of MnTMP: in CH_2Cl_2 at 4 K.

The rate constant k_3 was estimated from Eq. (5). The spectral change of this reaction at −40°C is shown in Fig. 3. Absorption at 422 nm corresponding to $Mn^{IV}P$ decreased in accordance with the increase of one at 468 nm ($Mn^{III}P$). Isosbestic points were observed and the plot according to Eq. (5) exhibited a good linear correlation (see inset of Fig. 3). This confirms that the rate analysis was correct. The rate constant was determined to be $k_3 = 2.61 \times 10^5$ mol^{-2} dm^6 s^{-1} at −40°C. Similarly, rate constants at −20 and −30°C were obtained. The activation parameters were determined from their Arrhenius plot (Fig. 4): $\Delta E^{\neq} = 9.1$ kcal mol^{-1} and $\Delta S^{\neq} = 4.8$ eu (283 K).

With use of the rate constant k_3 and the activation parameters, one can determine the rate at 10°C: k_3 (283) $= 6.9 \times 10^6$ mol^{-2} dm^6 s^{-1}. When one uses the substrate concentration applied for the catalytic disproportionation reaction, one can estimate the rate of the latter O_2

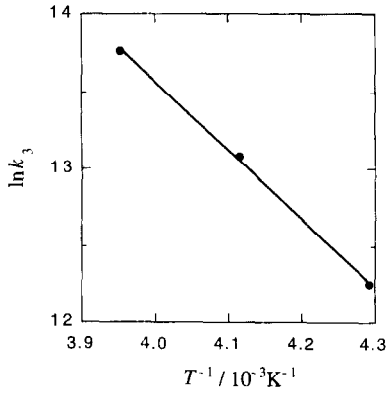


Fig. 4. Arrhenius plot of the reduction of 2 with H_2O_2; $\Delta E^{\neq} = 9.1$ kcal mol^{-1}, $\Delta S^{\neq} = 4.8$ cu (283 K).

evolution reaction and directly compared it with the rate of the overall O_2 evolution reaction. Thus, under the conditions, $[Mn_2P] = 1.25 \times 10^{-4}$ mol dm^{-3}, $[H_2O_2] = 6.4 \times 10^{-2}$ mol dm^{-3} at 10°C in acetonitrile, the reduction rate of the Mn^{IV}_2P **2** by H_2O_2 to the corresponding Mn^{III} and O_2 is estimated to be 1.3×10^{-2} mol dm^{-3} s^{-1}, which is 6.5 times higher than the overall disproportionation rate under catalytic conditions. This result confirms that the reduction process by the 2nd H_2O_2 molecule in the catalytic cycle is a fast one in the overall reaction, as predicted before.

The coordinating axial ligand at the external position of the manganese complex **2** was a MeO^- group, which is more strongly electron donating than nitrogen bases such as imidazole or pyridine derivatives. Consequently, the oxidation potential of the Mn^{IV} complex **2** is considered to be lower than the possible intermediate III (Scheme 1) in the catalytic cycle (Scheme 1). Thus, the reduction rate of III by H_2O_2 could be much faster than the model compound **2**.

Based on the kinetic analysis mentioned above, we conclude that the catalytic disproportionation reaction of H_2O_2 with manganese porphyrin dimers could involve $[Mn^{IV}(OH)X]_2$ as a high-valent intermediate, where two coordinating OH groups to the Mn ions are inside the cavity. This convergent conformation of the OH groups is favorable for the proton and electron transfer from a H_2O_2 molecule and could allow the efficient oxidation of H_2O_2. Thus, the resultant high catalytic turnover is attributed to this supramolecular character of the applied manganese porphyrin dimers (Fig. 5).

Finally, we mention the mechanistic relevance of this result to the enzyme reaction of OEC. By flash illumination of thylakoids isolated from plant chloroplast, the OEC can be converted to a so-called S_2 state [30], which involves $[Mn^{III}]_3[Mn^{IV}](OH)_2$. This high valent Mn cluster as well as Mn-CAT shows O_2 evolu-

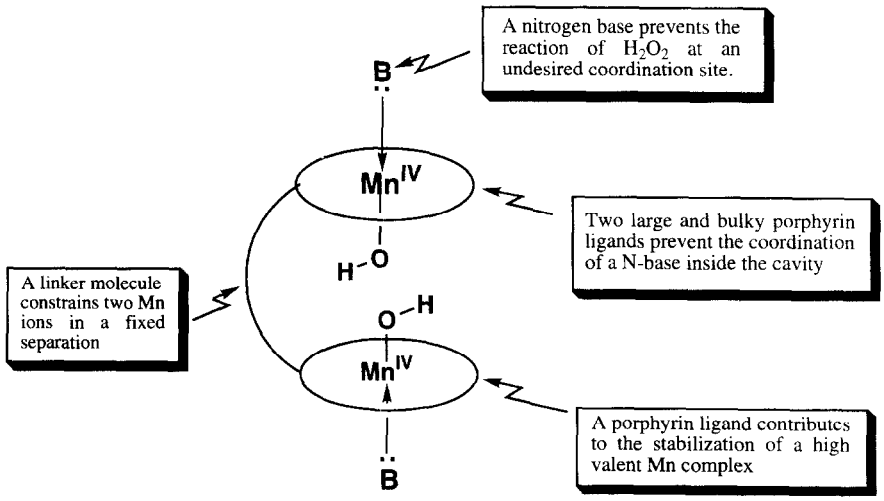


Fig. 5. Schematic drawing of the supramolecular structure of a manganese porphyrin dimer.

tion activity in the presence of H_2O_2 and converts one H_2O_2 molecule to O_2 and two H_2O molecule. The present result is similar to this enzyme reaction in the simultaneous electron transfer to higher-valent Mn ions from one H_2O_2 molecule and successive formation of O_2 and water without any other H^+ sources.

As another possible high valent dinuclear Mn complex involved in Mn-CAT, an $[Mn^{IV}(=O)]_2$ complex has been proposed [15]. However, to the best of our knowledge, no evidence for the formation of the $Mn^{IV}(=O)$ complex has been reported both in OEC or in Mn-CATs. Furthermore, if the $[Mn^{IV}(=O)]_2$ complex is an intermediate, the resultant products in its reaction with H_2O_2 could be O_2 and $[Mn^{III}(OH)]_2$, This Mn^{III} complex is highly basic and requires further proton transfer from some other H^+ sources in order to form H_2O and to complete its catalytic cycle. Thus, the $[Mn^{IV}(=O)]_2$ complex is less likely to be the intermediate in the H_2O_2 dismutation by these enzymes.

3. Experimental

3.1. General

UV–vis absorption spectra were measured on a Shimadzu UV-3100 PC spectrometer equipped with a thermostated cell-holder, and the temperature was controlled by a NESLAB ULT-80DD. Infrared spectra were recorded on a Perkin–Elmer 1640 spectrometer. Electron spin resonance spectra were obtained on a Bruker ESP-300E operating in the X-band with 100 kHz modulation equipped with an Oxford cryostat and a Hewlett–Packard frequency meter, Model 5352B. Acetonitrile was purified by distillation twice from P_2O_5 and once from K_2CO_3 under nitrogen atmosphere. Hydrogen peroxide was fractionally distilled from ca. 70% water solution under reduced pressure (38°C/6 mmHg) at the bath temperature below 40°C. Purity of the distilled H_2O_2 was about 95%, which was determined by iodometric titration. The stock solution of hydrogen peroxide in acetonitrile (1.0 M) was used for the reaction. Dimanganese 1,8-bis[5,10,15-tri(2,4,6-trimethylphenyl)porphyrinyl]anthracene (**1**) was prepared as reported [28].

3.2. Preparation of Mn^{IV} derivative 2

To the methanolic solution (2.0 ml) of **1** (1.0 μmol), 4.4 M NaOMe (166 μl) was added and then filtered under nitrogen atmosphere. At 10°C, 0.7 M NaOCl (0.16 ml) was added to the filtrate and then the resulting brown precipitate was filtered and washed with cold methanol to give Mn^{IV} derivative **2** (yield 70%), which was dried at −60°C in vacuo; IR (KBr) $\nu = 558$ cm^{-1} (Mn–O); UV–vis (CH_2Cl_2, −65°C) $\lambda_{max} = 423$ nm. The purity of freshly prepared **2** was confirmed by its ESR and visible spectrum at low temperature and it did not contain any Mn^{III} impurities. This complex can be stored at −80°C for several hours, however, it rapidly decomposes mainly to the corresponding Mn^{III} complex at room temperature. Thus, satisfactory elemental analysis was not obtained.

3.3. Measurement of the reduction rate of Mn^{IV} dimer 2 with H_2O_2

In a 1.0 × 1.0 cm cuvette for UV–vis spectrometer, the Mn^{IV} complex **2** (ca. 10^{-5} mol dm^{-3}) in dry CH_3CN (3.0 ml) was cooled to −40°C (internal temperature) and a stock solution of H_2O_2 in CH_3CN was added at once and the UV–vis spectral change was monitored in suitable time intervals. The same measurement was also done at different temperatures (< −20°C). Since spontaneous decomposition of **2** was not negligible at higher temperatures than −20°C, kinetic experiments were done at lower temperatures.

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