Efficient Oxygen Consumption by Hydroxo(protoporphyrinato)iron(III) Adsorbed on Magnesium Oxide Powder in the Presence of Cysteine

Hiroyuki Noda,* Hiroaki Ohya,† and Hitoshi Kamada

Institute for Life Support Technology, Yamagata Technopolis Foundation, 2-2-1 Matsuei, Yamagata 990-2473

†Faculty of Engineering, Yamagata University, Yonezawa, Yamagata 992-0038

(Received June 4, 1999)

The oxygen reduction ability of hydroxo(protoporphyrinato)iron(III) (HEM) adsorbed on powdery supports in the presence of cysteine was investigated using a Clark-type oxygen electrode. All of the oxygen consumption rates for HEM adsorbed on powdery supports having different surface basicity values ($CaO > MgO > TiO_2$) were larger than the cases of HEM in aqueous solution and of MgO suspension without HEM in the presence of cysteine. Maximum oxygen consumption rate was obtained in the case of the HEM/MgO system. This result suggests that the optimum surface basicity of powdery support is necessary to achieve efficient oxygen consumption. From the measurements for ESR and Raman spectra of the HEM/MgO system, we confirmed the change for the iron valence of HEM from III to II in the presence of cysteine. The production of cystine through the dimerization of cysteinyl radicals in the reaction mixture was also confirmed by the measurements of ESR spectra and capillary electrophoresis (CE). A possible mechanism for accelerating the oxygen consumption rate of the HEM/MgO system in the presence of cysteine is discussed, concerning the effect of the catalytic ability of MgO on the turnovers of iron valence.

Heterogeneous biological systems generally achieve high reactivity and high selectivity in chemical reactions. Conformational changes of protein in the vicinity of protoporphyrinatoiron(III) (HEM) give a variety of biological functions of metalloenzymes such as cytochrome P-450, catalase, and peroxidase.[1—3] These enzymes are also useful in the field of organic synthesis, but are unstable for use as catalysts to some extent. Accordingly, some artificial modifications of the enzymes have been required for their useful applications. Some models of cytochrome P-450, such as binaphthyl modified,[4] steroid modified,[5] and cyclodextrin coupled[6] HEM, have been proposed; their reactivities were quite similar to the reactivities of cytochrome P-450. In particular, the steroid modified HEM in lipid bilayer performed the high reaction selectivity for the epoxidation of steroid.[5] However, these models have the difficulty that their structures are very complex to apply as synthetic catalysts. Alternatively, we reported recently that the formation of some simple heterogeneous systems such as chlorophyll on MgO and hematoporphyrin on the cell wall of yeast played crucial roles for the increase in the reactivity with oxygen under illumination.[7,8] The proposed heterogeneous systems are easy to constitute. Therefore, our systems may also be applicable as model enzymes.

During our study for catalytic applications of HEM adsorbed on powdery supports, we found that efficient oxygen consumption was caused by HEM adsorbed on magnesium oxide powder (HEM/MgO system) in the presence of cysteine. In this paper we will describe the details of the experimental results and discuss a possible mechanism for accelerating the oxygen consumption rate in the HEM/MgO system in the presence of cysteine.

Experimental

Materials. Hydroxo(protoporphyrinato)iron(III) (HEM) was obtained from Aldrich Chem. Co., Inc. Magnesium oxide (MgO, 99.9%, 0.01 μm), calcium oxide (CaO, 99.9%), titanium(IV) oxide (TiO_2, 99.9%, anatase), L-cysteine, and L-cystine were purchased from Wako Pure Chemical Industries, Ltd. As a spin-trap reagent, 5, 5-dimethyl-1-pyrrole 1-oxide (DMPO) was obtained from Dojindo Laboratory. The other reagents used were of the highest grade commercially available. Hydroxo(protoporphyrinato)iron(III) on powdery supports such as MgO (HEM/MgO system), CaO (HEM/CaO system), and TiO_2 (HEM/TiO_2 system) were prepared as follows: powdery supports (50—250 mg cm^{-3}) were suspended in water. Amounts of powdery supports used were able to adsorb all of the HEM addition. One mmol dm^{-3} HEM aqueous solution (HEM_{aq}) or dimethyl sulfoxide solution was added to the suspensions. The sample suspensions were used for experiments after being centrifugally washed by water several times.

Apparatus. A Clark-type oxygen electrode with 1.1 cm^{-3} volume cell (Central Kagaku Co.) was used for the measurements of oxygen consumption profiles. Oxygen consumption profiles were recorded after the addition of the suspension of HEM on supports in the mixed solution of cysteine and phosphate buffer (pH = 7.4) at room temperature. The resonance Raman spectra were recorded using a Japan Spectroscopic Co., Ltd. (JASCO), NR-1800 laser Raman spectrophotometer (Ar^+, 457.9 nm). ESR measurements were conducted using a JEOL Ltd., RE-2X ESR spectrometer at 77 K and at room temperature. ESR spin-trapping measurements were conducted using a flat quartz cell.[7,8] The measurements of capillary electrophoresis (CE) were conducted using a JASCO, CE-

800 CE system. The uncoated fused-silica capillary used was of the following dimensions: an effective length of 50 cm and an inner diameter of 50 μm. Separations were performed at 15 kV at room temperature. Samples were injected into the capillary at its anodic end by electromigration (15 kV, 5 s). Direct UV absorption detection was performed at 280 nm.

Results

Oxygen Consumption of HEM Adsorbed on Powdery Supports in the Presence of Cysteine. In the presence of cysteine, oxygen consumption phenomenon was observed in all of the cases of HEM adsorbed on the powdery supports. Figure 1 shows examples of oxygen consumption profile for both the HEM/MgO system and HEM in aqueous solution (HEM_{aq}) containing 0.045 mmol dm^{-3} HEM and 45 mmol dm^{-3} cysteine. The oxygen consumption rates were determined by the tangents of kinetic curves at the starting point of oxygen consumption, as shown in Fig. 1.

In order to examine an effect of the catalytic ability of powdery supports on the oxygen consumption rate of HEM, we estimated the rates of HEM adsorbed on powdery supports having different surface basicity values such as CaO, MgO, and TiO_2 (CaO > MgO > TiO_2).[9] Figure 2 shows the HEM concentration dependence for the oxygen consumption rates of the HEM/CaO, HEM/MgO, and HEM/TiO_2 systems in the presence of 45 mmol dm^{-3} cysteine. The HEM/MgO system having a medium surface basicity gave the maximum oxygen consumption rate. This means that the acceleration of oxygen consumption rate for HEM adsorbed on supports

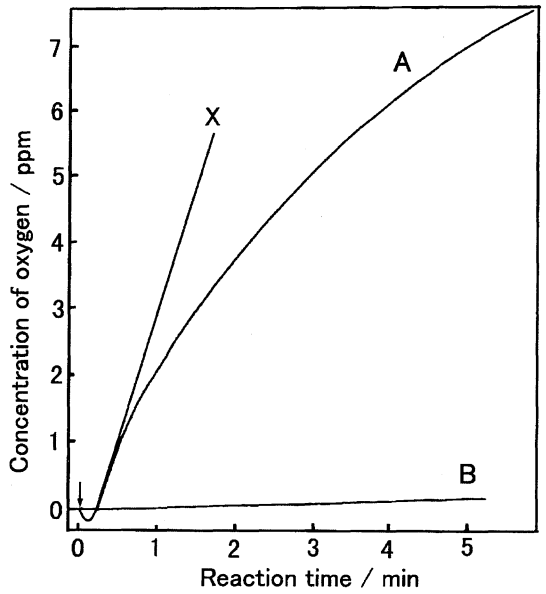

Fig. 1. Time profiles of oxygen consumption obtained from the HEM/MgO system (A) and HEM_{aq} (B) in the presence of 45 mmol dm^{-3} cysteine measured at room temperature. The concentration of HEM in both systems was 0.045 mmol dm^{-3}. The content of HEM/MgO was 4.5 mg cm^{-3}. Oxygen consumption rates were determined by the tangent of the kinetic curves at the starting point of oxygen consumption, and the slope of a linear line X denotes the rate for the HEM/MgO system (A).

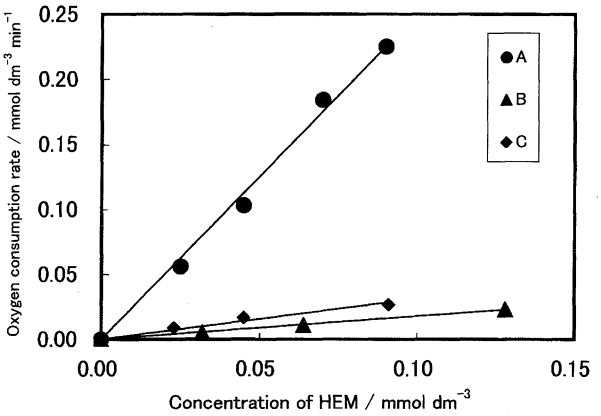

Fig. 2. HEM concentration dependence for the oxygen consumption rates of HEM adsorbed on MgO (A), TiO_2 (B), and CaO (C) in the presence of 45 mmol dm^{-3} cysteine measured at room temperature. The surface basicity values of supports is CaO > MgO > TiO_2.[9]

requires the most suitable surface basicity of powdery support.

The oxygen consumption rates of the powdery supports themselves were 4.8×10^{-3} (5 mg cm^{-3} MgO), 9.7×10^{-3} (5 mg cm^{-3} CaO), and 1.1×10^{-3} (5 mg cm^{-3} TiO_2) mmol dm^{-3} min^{-1} in the presence of 45 mmol dm^{-3} cysteine. This result indicates that a powdery support having higher basicity gives the higher oxygen consumption rate in the presence of cysteine. Even in the case of the HEM/TiO_2 system having the lowest oxygen consumption rate (0.8×10^{-2} mmol dm^{-3} min^{-1}), the rate was about ten times larger than in the case of HEM_{aq}. Thus the adsorption on powdery supports is effective for the acceleration of oxygen consumption rate of HEM.

Figure 3 shows the cysteine concentration dependence for the oxygen consumption rate of the HEM/MgO system containing 0.045 mmol dm^{-3} HEM. The rate of the HEM/MgO system increased with the concentration of cysteine from 20 mmol dm^{-3} to 100 mmol dm^{-3}. The increase in the concentration of cysteine above 100 mmol dm^{-3} led to a decrease

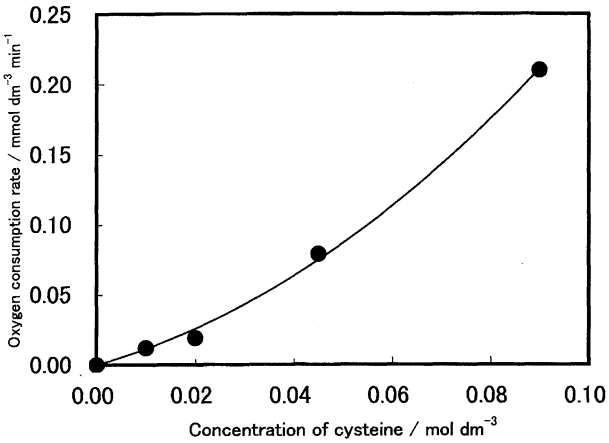

Fig. 3. Cysteine concentration dependence for the oxygen consumption rate of the HEM/MgO system containing 0.045 mmol dm^{-3} HEM measured at room temperature.

of the rate because of the dissolution of MgO (not shown).

Characterization of the HEM/MgO System. In order to clarify the mechanism for the efficient oxygen consumption of the HEM/MgO system in the presence of cysteine in more detail, we examined its electronic and coordination states using ESR and Raman methods.

Figure 4 shows the ESR spectra of 1 mmol dm^{-3} HEM_{aq} (A), the HEM/MgO (B), HEM/CaO (D), and HEM/TiO_2 (E) systems containing 0.5 mmol dm^{-3} HEM, and the HEM/MgO system with 50 mmol dm^{-3} cysteine (C) measured at 77 K. The signal shown in Fig. 4A can be assigned to a high-spin dominant mixed spin state of porphyrinatoiron(III) compound in view of its g-value, 5.4, which is characteristic of five-coordinated iron(III) complexes.[10] The formation of the HEM/MgO system led to an increase of a high-spin state (g = 6.1, Fig. 4B).[11] This means a change of the electronic state of HEM by the formation of a heterogeneous system. A similar phenomenon (g = 6.0) was also observed in the HEM/CaO system (Fig. 4D) having a lower oxygen consumption rate than the case of the HEM/MgO system. On the other hand, an intermediate spin state (g = 4.4) was observed in the HEM/TiO_2 system having a similar rate to that of the HEM/CaO system (Fig. 4E). Therefore, the factor that causes the increase of high-spin states may also be related to the acceleration of oxygen consumption rate of HEM/MgO system. The addition of cysteine in the HEM/MgO system led to the disappearance of ESR signal (Fig. 4C). This clearly indicates that the reduction of the iron valence from III to II occurs, because the iron(II) ion is ESR silent.

The above ESR results show the changes of the axial-ligand field and the valence state of the iron ion, but do not give direct information about their coordination states. We then have conducted the measurements of their resonance Raman spectra in order to examine the coordination states of the iron ion. Figure 5 shows the resonance Raman spectra of the HEM/MgO system containing 0.4 mmol dm^{-3} HEM (Fig. 5A), 50 mmol dm^{-3} cysteine added to A (Fig. 5B), and 1 mmol dm^{-3} HEM_{aq} (Fig. 5C) measured at room temperature. The Raman bands of porphyrinatoiron complexes in the regions of 1360—1380 and 1600—1650 cm^{-1} are sensitive to their coordination states.[12] The Raman bands of HEM_{aq} had the peaks at 1632 and 1377 cm^{-1}. The Raman bands of the HEM/MgO system also had the peaks at 1630 and 1377 cm^{-1}. On the other hand, the Raman bands of the HEM/MgO system with cysteine had the peaks at 1623 and 1362 cm^{-1}. The values of the HEM/MgO system and HEM_{aq} are very close to the values of some five-coordinated porphyrinatoiron(III) high-spin species.[12] The values of the HEM/MgO system with cysteine are close to the values of some six-coordinated porphyrinatoiron(II) low-spin species.[12]

ESR Detection of Radical Intermediates. In general, the direct detection of radical intermediates is difficult due to their short lifetimes. We then carried out the detection of reaction intermediates using an ESR spin-trapping method.[7,8] 5,5-Dimethyl-1-pyrrole 1-oxide (DMPO) was used as a water-soluble spin-trap reagent. Figure 6 shows the ESR spectra of the DMPO spin adducts obtained from the reaction system of the HEM/MgO system containing 0.25 mmol dm^{-3} HEM and 5 mmol dm^{-3} cysteine (Fig. 6A) and from the re-

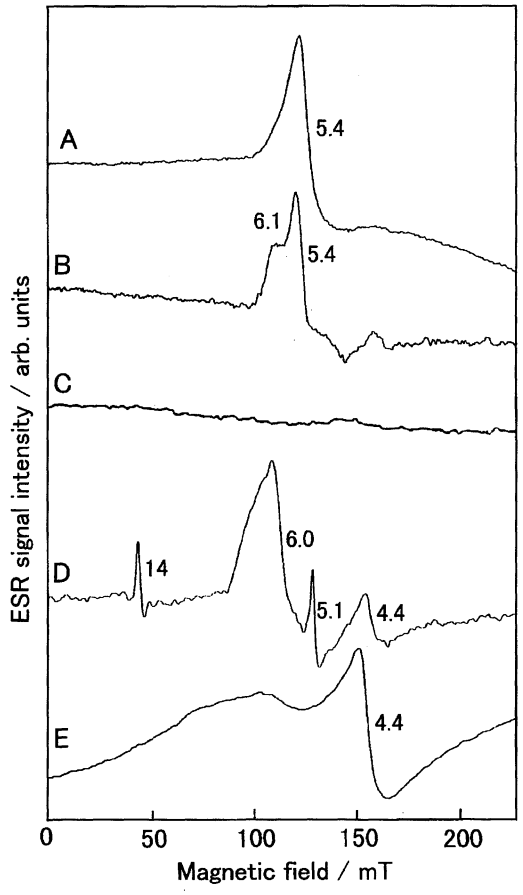

Fig. 4. ESR spectra of 1 mmol dm^{-3} HEM_{aq} (A), the HEM/MgO system (B), the HEM/CaO system (D), and the HEM/TiO_2 system (E) containing 0.5 mmol dm^{-3} HEM, and the HEM/MgO system with 50 mmol dm^{-3} cysteine (C) measured at 77 K.

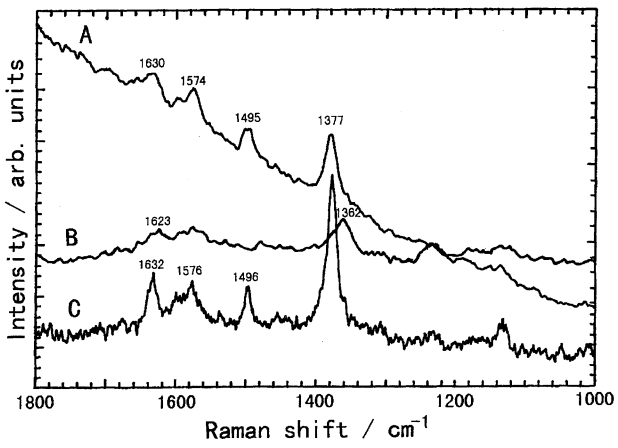

Fig. 5. Resonance Raman spectra of the HEM/MgO system containing 0.4 mmol dm^{-3} HEM (A), A+50 mmol dm^{-3} cysteine (B), and 1 mmol dm^{-3} HEM_{aq} (C) measured at room temperature. Irradiation wavelength was 457.9 nm of Ar^+ laser (80 mW) nearly to the Soret band of HEM.

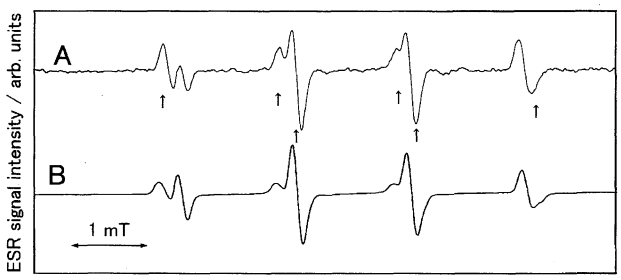

Fig. 6. ESR spectra of the DMPO spin adducts obtained from the reaction mixture of the HEM/MgO system containing 0.25 mmol dm^{-3} HEM and 5 mmol dm^{-3} cysteine (A) and that of 0.68 mmol dm^{-3} iron(II) ion, 3.4 mmol dm^{-3} hydrogen peroxide, and 5 mmol dm^{-3} cysteine (B) measured at room temperature. The concentration of DMPO was 460 mmol dm^{-3}. The cysteiny radical adduct shown as arrows in A was assigned in view of their hyperfine-splitting constant values of a_N = 1.53 mT and a_H = 1.72 mT (a six-line signal).[13]

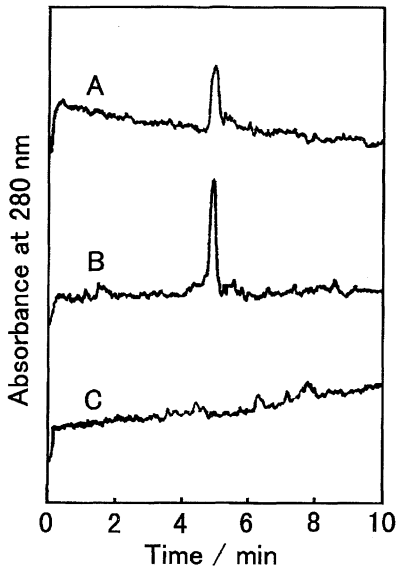

Fig. 7. Electropherogram of the centrifuged reaction mixture of the HEM/MgO system containing 0.25 mmol dm^{-3} HEM and 50 mmol dm^{-3} cysteine (A), 1 mmol dm^{-3} cystine in PBS (pH = 6.4) (B), and 10 mmol dm^{-3} cysteine in PBS (pH = 6.4) (C) measured at room temperature. The measurement of the reaction mixture was conducted using the sample that was ten times diluted by PBS (pH = 6.4). Separation conditions: running buffer, 0.1 mol dm^{-3} PBS (pH = 6.4); effective length of capillary, 50 cm; inner diameter of capillary, 50 μm; detection wavelength, 280 nm; injection, 15 kV, 5 s; running voltage, 15 kV.

action system containing 0.68 mmol dm^{-3} iron(II) ion, 3.4 mmol dm^{-3} H_2O_2, and 5 mmol dm^{-3} cysteine (Fig. 6B) in the presence of oxygen measured at room temperature. The signal shown in Fig. 6A was composed from two sorts of spin-adducts. A six-line signal can be assigned to the cysteinyl radical adduct in view of its hyperfine-splitting constant (hfsc) values (a_N = 1.53 mT and a_H = 1.72 mT).[13] This signal was identical with the signal obtained from the case for the Fenton reaction which produces the hydroxyl radical by the reaction between the iron(II) ion and hydrogen peroxide, in the presence of cysteine (Fig. 6B). A quartet signal of Fig. 6A is quite similar to the hydroxyl radical adduct, but this adduct did not change into a C-center adduct by the addition of ethanol.[8] Therefore, we regarded this adduct as an artifact. The production of the superoxide ion adduct was not confirmed in the reaction mixture.

Products after the Reaction between the HEM/MgO System and Cysteine in the Presence of Oxygen. In order to detect the final product of the reaction between the HEM/MgO system and cysteine in the presence of oxygen, we conducted the measurements of capillary electrophoresis (CE). Figure 7 shows the electropherograms of the centrifuged reaction mixture of the HEM/MgO system containing 0.25 mmol dm^{-3} HEM and 50 mmol dm^{-3} cysteine (Fig. 7A), 1 mmol dm^{-3} cystine (Fig. 7B), which is the oxidized dimer of cysteine, and 10 mmol dm^{-3} cysteine (Fig. 7C) detected at 280 nm. In the reaction mixture of the HEM/MgO system, a peak originated in cystine was observed (Figs. 7A and 7B). At this wavelength, no signal was observed in the solution of 10 mmol dm^{-3} cysteine, which has weak absorption at 280 nm (Fig. 7C). Thus, it can be concluded that cystine was produced by the reaction between the HEM/MgO system and cysteine in the presence of oxygen.

In order to clarify the reduction products of oxygen in the reaction mixture of the HEM/MgO system and cysteine, we examined the accumulation of H_2O_2 in the system. After the addition of catalase, which disproportionates H_2O_2 into H_2O and O_2, the reproduction of oxygen was not confirmed in the reaction mixture (not shown). This result implies that the oxygen consumption goes in such a way that H_2O_2 is not accumulated in the reaction mixture.

Effect of H_2O_2 on the Oxygen Consumption Rate. We examined an effect of H_2O_2 on the oxygen consumption rate of the HEM/MgO system to clarify the reason why H_2O_2 was not accumulated in the reaction mixture. Figure 8 shows the H_2O_2 concentration dependence of the relative oxygen consumption rate. An increase in the concentration of H_2O_2 led to an increase in the relative oxygen consumption rate of the HEM/MgO system. This result suggests that the formation of a complex such as hydroperoxo(protoporphyrinato)iron(III) may be related to the oxygen consumption process for the HEM/MgO system in the presence of cysteine. We attempted the detection of the above complex and oxo(porphyrinato)-iron(IV) complex using the Raman method.[14,15] Unfortunately, we did not succeed in the detection of such complexes.

Discussion

Mechanism for Efficient Oxygen Consumption of the HEM/MgO System in the Presence of Cysteine. Based on the analysis of the results observed in the present study, we present here a possible mechanism for efficient oxygen consumption of the HEM/MgO system in the presence of cysteine. From the results obtained in the present study, the mechanism can be described in the following equations:

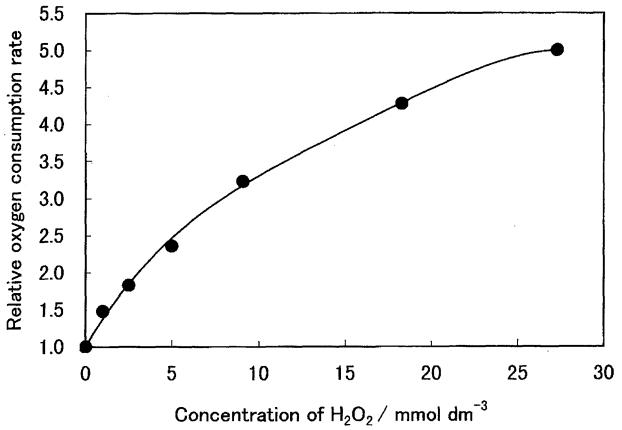

Fig. 8. Hydrogen peroxide concentration dependence for the oxygen consumption rate of the HEM/MgO system containing of 0.045 mmol dm^{-3} HEM and 45 mmol dm^{-3} cysteine measured at room temperature.

$$[Fe^{3+}(OH^-)(por)]/MgO + RSH \rightarrow [Fe^{3+}(RS^-)(OH^-)(por)]/MgO + H^+ \rightarrow [Fe^{2+}(RS\cdot)(OH^-)(por)]/MgO + H^+, \quad (1)$$

$$[Fe^{2+}(RS\cdot)(OH^-)(por)]/MgO + O_2 \rightarrow [Fe^{2+}(O_2)(OH^-)(por)/MgO] + RS\cdot \rightarrow [Fe^{3+}(O_2^-)(OH^-)(por)]/MgO + RS\cdot, \quad (2)$$

$$[Fe^{2+}(RS\cdot)(OH^-)(por)]/MgO + RSH \rightarrow [Fe^{2+}(RS^-)(OH^-)(por)]/MgO + RS\cdot + H^+, \quad (3)$$

$$[Fe^{3+}(O_2^-)(OH^-)(por)]/MgO + RSH + H^+ \rightarrow [Fe^{4+}(O)(OH^-)(por^+)]/MgO + H_2O + RS\cdot, \quad (4)$$

$$2RS\cdot \rightarrow RSSR, \quad (5)$$

$$[Fe^{4+}(O)(OH^-)(por^+)]/MgO + 2RSH \rightarrow [Fe^{3+}(OH^-)(por)]/MgO + H_2O + RSSR, \quad (6)$$

$$4RSH + O_2 \rightarrow 2H_2O + 2RSSR. \quad (7)$$

First, the five-coordinated iron(III) ion in the HEM/MgO system should be reduced to the six-coordinated iron(II) ion by the addition of cysteine [reaction (1)]. The formation of the iron(II) ion was confirmed from the ESR and Raman spectra (see Figs. 4 and 5). In the presence of oxygen, the reaction between $[Fe^{2+}(RS\cdot)(OH^-)(por)]/MgO$ and oxygen leads to the formation of an intermediate of $[Fe^{3+}(O_2^-)(OH^-)(por)]/MgO$ and the release of cysteinyl radical [reaction (2)]. The release of cysteinyl radical was confirmed from the ESR spin-trapping method (see Fig. 6). In the absence of dissolved oxygen, $[Fe^{2+}(RS^-)(OH^-)(por)]/MgO$ may be formed because the existence of a six-coordinated porphyrinatoiron(II) low-spin species was confirmed by the measurement of the Raman spectrum [reaction (3), see Fig. 5]. Moreover, the intermediates of $[Fe^{4+}(O)(OH^-)(por^+)]/MgO$ and cysteinyl radical may be produced by the reaction between $[Fe^{3+}(O_2^-)(OH^-)(por)]/MgO$ and cysteine because the oxygen consumption rate was accelerated by the addition of H_2O_2 [reaction (4), see Fig. 8]. In general, H_2O_2 enhances the formation of $[Fe^{4+}(O)(OH^-)(por^+)]$ and it is thus called a shunt path. Unfortunately, we could not confirm the production of the intermediates of $[Fe^{3+}(O_2^-)(OH^-)(por)]/MgO$ and $[Fe^{4+}(O)(OH^-)(por^+)]/MgO$ in our present study (see results section). Groves et al. reported the existence of the intermediates of $[Fe^{3+}(RO_2)(por)]$ and $[Fe^{4+}(O)(por^+)]$ in the reaction between $[Fe^{3+}(por)]$ and acyl peroxide or perbenzoic acid.[16—18] In our case, therefore, a similar phenomenon is expected to occur. Furthermore, cystine may be produced from the dimerization of the cysteinyl radical produced from reaction (2) to reaction (4) [reaction (5)]. The production of cystine was confirmed by the measurement of CE (see Fig. 7). The recovery of $[Fe^{3+}(por)]/MgO$ is brought about by the reaction between $[Fe^{4+}(O)(por^+)]/MgO$ and two molecules of cysteine [reaction (6)]. That is, a four-electron reduction of oxygen caused by four molecules of cysteine should be performed by the catalytic reactions of the HEM/MgO system [reaction (7)].

We next discuss the mechanism of accelerating the oxygen consumption rate in the HEM/MgO system in the presence of cysteine. To make efficient oxygen consumption possible, the efficient turnovers for iron valence of the HEM/MgO system have to occur. A schematic illustration of a possible mechanism is depicted in Fig. 9. HEM adsorbed on MgO should be constituted by ionic bonds between cationic positions of MgO surface and the propionic acid of HEM, as shown in Fig. 9. In our present study, the maximum oxygen consumption rate was observed in the case of HEM adsorbed on MgO having a medium surface basicity (see Fig. 2). The medium basicity for MgO should lead to the coordination of cysteine to the iron ion of HEM by its ionization. On the other hand, the higher basicity for CaO should lead to the oxidation of cysteine to cystine (see results section) and the lower basicity for TiO_2 should lead to a little ionization of cysteine. Further, the oxygen consumption rates of these two cases were one order of magnitude lower than the rate of the HEM/MgO system (see Fig. 2). Therefore, the ionization of cysteine caused by MgO may be related to the acceleration of oxygen consumption rate of the HEM/MgO system in the

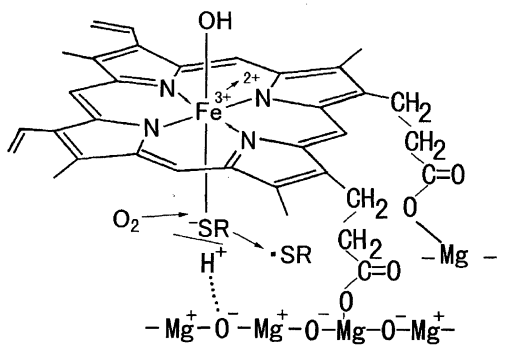

Fig. 9. Schematic illustration of the mechanism accelerating the oxygen consumption rate of the HEM/MgO system induced by the ionization of cysteine catalyzed by MgO.

presence of cysteine. The coordination of cysteinyl ion to HEM leads to reduction of the iron valence of HEM from III to II and the release of the cysteinyl radical (see Figs. 4, 5, and 6). The coordination of oxygen to HEM is brought about by the release of the cysteinyl radical. That is, the ionization of cysteine caused by MgO leads to the efficient reduction for the iron ion of HEM from III to II and the coordination of oxygen to HEM, as shown in Fig. 9. The formation of cysteinyl ion may be a rate-determining step of the oxygen consumption process caused by HEM on powdery supports in the presence of cysteine.

In conclusion, we have demonstrated that efficient oxygen consumption is caused by the HEM/MgO system in the presence of cysteine. The oxygen consumption rate of the HEM/MgO system was two orders of magnitude larger than that of HEM in aqueous solution. A possible mechanism for accelerating the oxygen consumption rate of the HEM/MgO system is proposed concerning the effect for the catalytic activity of MgO on the turnovers of iron valence. The HEM/MgO system is simple but effective for the oxygen consumption in the presence of cysteine. The HEM/MgO system can be applicable to some fields as a reduction catalyst, the reactivity of which is similar to that of cytochrome P-450.

This study was performed through the fund of the Regional Science Promoter of the Japan Science and Technology Corporation.

References

1 P. R. Oritiz de Montellano, "Cytochrome P-450, Structure, Mechanism, and Biochemistry," Plenum, New York (1986).

2 M. R. N. Murthy, T. J. Reid, III, A. Sicignano, N. Tanaka, and M. G. Rossmann, *J. Mol. Biol.*, **152**, 465 (1981).

3 B. C. Finzel, T. L. Poulos, and J. Kraut, *J. Biol. Chem.*, **259**, 13027 (1984).

4 J. T. Groves and R. S. Meyers, *J. Am. Chem. Soc.*, **105**, 5791 (1983).

5 J. T. Groves and R. Neumann, *J. Am. Chem. Soc.*, **111**, 2900 (1989).

6 Y. Kuroda, T. Hiroshige, and H. Ogoshi, *J. Chem. Soc., Chem. Commun.*, **1990**, 1594.

7 H. Noda, K. Oikawa, H. Ohya-Nishiguchi, and H. Kamada, *Chem. Lett.*, **1994**, 1949.

8 H. Noda, H. Ohya-Nishiguchi, and H. Kamada, *Bull. Chem. Soc. Jpn.*, **70**, 405 (1997).

9 K. Tanaka and A. Ozaki, *J. Catal.*, **8**, 1 (1967).

10 M. M. Maltempo and T. H. Moss, *Q. Rev. Biophys.*, **9**, 181 (1976).

11 A. Ehrenberg, *Ark. Kemi*, **19**, 119 (1962).

12 T. G. Spiro and J. M. Burke, *J. Am. Chem. Soc.*, **98**, 5482 (1976).

13 G. R. Buettner, *Free Radicals Biol. Med.*, **3**, 259 (1987).

14 S. Hashimoto, Y. Tatsuno, and T. Kitagawa, *Proc. Natl. Acad. Sci. U.S.A.*, **83**, 2417 (1986).

15 S. Hashimoto, Y. Tatsuno, and T. Kitagawa, *J. Am. Chem. Soc.*, **109**, 8096 (1987).

16 J. T. Groves and Y. Watanabe, *J. Am. Chem. Soc.*, **108**, 7834 (1986).

17 J. T. Groves and Y. Watanabe, *J. Am. Chem. Soc.*, **108**, 7836 (1986).

18 J. T. Groves, R. C. Haushalter, M. Nakamura, T. E. Nemo, and B. J. Evans, *J. Am. Chem. Soc.*, **103**, 2884 (1981).