Original Article

Conformational effect of thiol-compounds on the formation of a nitrosyl(protoporphyrinato)iron(II) complex adsorbed on a basic oxide of magnesium oxide powder with the nitrite ion

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Abstract

It has investigated using a Clark-type oxygen electrode and an ESR method that the conformation of thiol-compounds such as cysteine (Cys) and glutathione (GSH) effects on the formation of a nitrosyl(protoporphyrinato)iron(II) complex by the reaction between hydroxo(protoporphyrinato)iron(III) complex adsorbed on magnesium oxide (Heme/MgO) powder and the nitrite ion (NO_2^-). The oxygen consumption rate of the Heme/MgO with Cys was slightly higher than that of the Heme/MgO with GSH. However, a nitrosyl(protoporphyrinato)iron(II) complex produced by the Heme/MgO with GSH was lower than that produced by the Heme/MgO with Cys under the concentration of NO_2^- below 0.1 mmol dm^{-3}. This result suggests that the conformational effect of thiol-compounds plays a key role on the formation of the nitrosyl(protoporphyrinato)iron(II) complex during the reduction of NO_2^- to nitrogen monoxide (NO). A possible mechanism for the conformational effect of GSH on the formation of the nitrosyl(protoporphyrinato)iron(II) complex is discussed, as a characteristic feature of the molecular size of GSH and an electronic interaction (repulsion) between GSH and NO_2^-.

Key words

nitrosyl(protoporphyrinato)iron(II) complex, MgO, cysteine, glutathione, conformational effect

1. Introduction

Hydroxo(protoporphyrinato)iron(III) complex (Heme) adsorbed on magnesium oxide (MgO) had higher reactivity than homogeneous systems (Noda et al., 1999; 2004). Efficient oxygen consumption was observed by the system of Heme adsorbed on MgO powder in the presence of cysteine (Cys) (Heme/MgO/Cys) (Noda et al., 1999). Moreover, efficient formation of a nitrosyl(protoporphyrinato)iron(II) complex was observed by the Heme/MgO/Cys system in the presence of the nitrite ion (NO_2^-) (Noda et al., 2004). It is thought that above system is a bifunctional catalyst similar to cytochrome cd_1 (Fueloep et al., 1995; Sjögren et al., 2001), capable of catalyzing the one-electron reduction of NO_2^- to NO, and the four-electron reduction of oxygen to water.

The formation of a nitrosyl(porphyrinato)iron(II) complex as a reaction intermediate plays a key role during the catalytic cycle of nitrite reductase or its model compounds (Yoshimura et al., 1986, Ye et al., 1991, Frangione et al., 1997, Munro et al., 1998). Some reaction models of nitrite reductase were studied using porphrynatoiron complexes (Ozawa et al., 1995, Liu et al., 1997). However, little study about the conformational effect for the fifth or sixth ligand of protoporphyrinatoiron(II) on the formation of a nitrosyl(porphyrinato)iron(II).

During our study using the Heme/MgO with thiol compounds as a reaction model of nitrite reductase to clarify the conformational effects of Cys and GSH on the formation of nitrosyl(protoporphyrinato)iron(II) complex, we found that its formation depend on the structure of thiol-compounds. In this paper, we will describe the details of the experimental results and discuss a possible mechanism for the conformational effect of thiol-compounds such as Cys and GSH on the formation of nitrosyl(protoporphyrinato)iron(II) complex adsorbed on MgO with NO_2^-.

2. Materials and methods

2.1 Materials

Hydroxo(protoporphyrinato)iron(III) (Heme) was obtained from Sigma-Aldrich Co. LCC. Magnesium oxide, as a basic oxide powder (MgO, 99.9 %, 0.01 μm), a standard NO_2^- solution (993 mg dm^{-3}), L-cysteine (special grade, Cys), and glutathione (reduced form, GSH) were purchased from Wako Pure Chemical Industries, Ltd. The Heme adsorbed on MgO (Heme/MgO) system was prepared as described previously (Noda et al., 1999; 2004).

2.2 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 the Heme/MgO in the mixed solution of Cys or GSH, NO_2^-, and 50 mmol dm^{-3} phosphate buffer (pH7.4) at room temperature. The oxygen consumption rates were determined by the tangents of kinetic curves at the starting point of oxygen consumption, as described previously (Noda et al., 1999; 2004).

An ESR measurements was conducted using a JEOL RE-2X ESR spectrometer at room temperature. ESR spectra were recorded after adding the suspension of the Heme/MgO in

the mixed solution of Cys or GSH, NO_2^-, and 50 mmol dm^{-3} phosphate buffer (pH7.4).

3. Results

Figure 1 shows the relationship between the oxygen consumption rate [Heme/MgO/Cys (Closed square) and Heme/MgO/GSH (closed circle)] and the concentration of Cys or GSH (Heme, 45μmol dm^{-3}). The oxygen consumption rate of the Heme/MgO/Cys was slightly higher than that of the Heme/MgO/GSH. This indicates that the size effects of thiol compounds are small on the oxygen consumptions caused by the Heme/MgO/Cys and Heme/MgO/GSH.

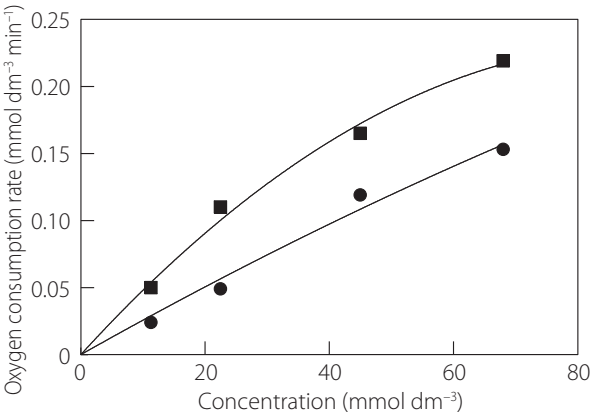

Figure 1: Relationship between oxygen consumption rate and the concentration of thiol-compounds measured at room temperature

Note: Concentration of Heme, 45 μmol dm^{-3}. Closed square, Heme/MgO/Cys; closed circle, Heme/MgO/GSH.

Figure 2 shows the relationship between the oxygen consumption rate [Heme/MgO/Cys (Closed square) and the Heme/MgO/GSH (closed circle)] and the concentration of NO_2^- (Heme, 45 μmol dm^{-3}; Cys and GSH, 50 mmol dm^{-3}). An increase in the concentration of NO_2^- led to an inhibition of the oxygen consumption caused by the Heme/MgO/Cys system. The oxygen consumption caused by the Heme/MgO/Cys was completely inhibited under the concentration of NO_2^- at 50 μmol dm^{-3}. On the other hand, the oxygen consumption caused by the Heme/MgO/GSH was not completely inhibited by the addition of 200 μmol dm^{-3} NO_2^-. These results suggest that the molecular structure of thiol-compounds hardly effect on the formation of nitorosyl(porphyrinato)iron(II).

In order to clarify the mechanism for the reaction between the Heme/MgO/Cys or Heme/MgO/GSH and NO_2^- in more detail, we conducted the measurements for the ESR spectrum of paramagnetic species formed in the system. Figure 3 shows the relationship between the concentration of NO_2^- and the ESR signal intensity of the nitrosyl(protoporphyrinato) iron(II) complex produced by the Heme/MgO/Cys (Closed

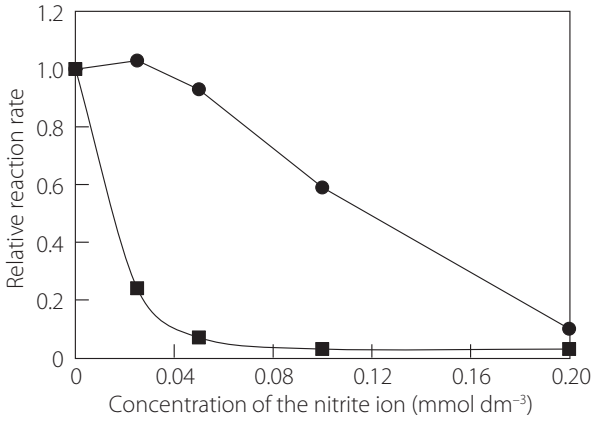

Figure 2: Relationship between the concentration of NO_2^- and relative oxygen consumption rates measured at room temperature

Note: Concentration of Heme, 0.2 mmol dm^{-3}; concentration of Cys and GSH, 50 mmol dm^{-3}. The other conditions are the same as Figure 1.

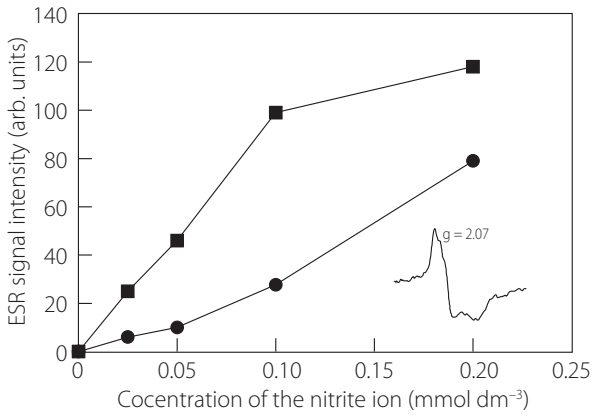

Figure 3: Relationship between the concentration of NO_2^- and the intensities for the ESR signal of nitrosyl(protoporphyrinato) iron(II) complex

Note: Concentration of Heme, 0.2 mmol dm^{-3}; concentration of Cys and GSH, 50 mmol dm^{-3}. The other conditions are the same as Figure 1.

square) and Heme/MgO/GSH (closed circle) (Heme, 0.2 mmol dm^{-3}; Cys or GSH, 50 mmol dm^{-3}) measured after 1-min mixing. The ESR signal observed at room temperature can be assigned to a six coordinated nitrosyl(protpporphyrinato)iron(II) complex by its g-value 2.07 (Yoshimura et al., 1986). The ESR signal intensity of the nitrosyl(protpporphyrinato)iron(II) complex produced by the Heme/MgO/Cys (Heme, 0.2 mmol dm^{-3}; Cys, 50 mmol dm^{-3}) increased with the concentration of NO_2^- up to 0.1 mmol dm^{-3}, and saturated with the concentration of NO_2^- over 0.1 mmol dm^{-3}. On the other hand, the ESR signal intensity of the nitrosyl(protoporphyrinato)iron(II) complex produced by the Heme/MgO/GSH (Heme, 0.2 mmol dm^{-3}; GSH, 50 mmol dm^{-3}) increased with the concentration

of NO_2^- up to 0.2 mmol dm^{-3}. The ESR signal intensity of the nitrosyl(protoporphyrinato)iron(II) complex produced by the Heme/MgO/GSH was lower than that produced by the Heme/MgO/Cys at the same NO_2^- concentration of 0.2 mmol dm^{-3}.

4. Discussion

On the basis of the results obtained in the present study, we present here a possible mechanism for the conformational effect of the thiol-compounds such as Cys and GSH on the formation of nitrosyl(protoporphyrinato)iron(II) complex by the reaction between the Heme/MgO and NO_2^-.

Figure 4 shows a schematic illustration of the conformational effect of the thiol-compounds such as Cys and GSH on the formation of nitrosyl(protoporphyrinato)iron(II) complex adsorbed on a basic oxide of MgO. The five-coordinated iron(III) in the Heme/MgO is reduced to the six-coordinated iron(II) ion by the addition of the thiol-compounds. The six-coordinated nitrosyl(protoporphyrinato)iron(II) complex may be produced by the reaction between the protoporphyrinatoiron(II)-NO_2^- complex and thiol-compound (Noda et al., 2004). In the case of Cys, the six-coordinated nitrosyl(protoporphyrinato)iron(II) complex may easily be produced by the reaction between the porphyrinatoiron(II)-NO_2^- complex and Cys due to its small molecular size and small ionic interference against NO_2^- (see Figures 2 and 3). In the case of GSH, on the other hand, the six-coordinated nitrosyl(protoporphyrinato)iron(II) complex is hard to be able to produce (see Figures 2 and 3) due to its large molecular size and its strong ionic interference against NO_2^-.

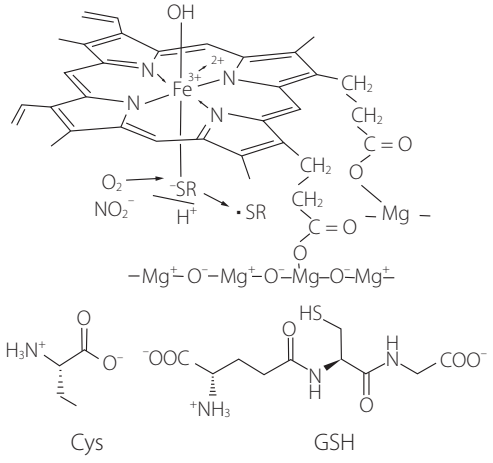

Figure 4: Schematic illustration of the conformational effect of the thiol-compounds such as Cys and GSH on the formation of nitrosyl(protoporphyrinato)iron(II) complex

5. Conclusions

In conclusion, we have demonstrated the conformational effect of thiol-compounds such as Cys and GSH on the formation of nitrosyl(protoporphyrinato)iron(II) complex produced by the reaction between the Heme/MgO and NO_2^-. The conformational effect of GSH on the formation of the nitrosyl(protoporphyrinato)iron(II) complex can be regarded as a characteristic feature of the molecular size of GSH and an electronic interaction (repulsion) between GSH and NO_2^-.

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