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Detection of Hydroxyl Radical from Photoexcited Chlorophylls on Magnesium Oxide Powder in Aqueous Media Using the ESR Spin Trapping Technique

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A potent active oxygen of hydroxyl radical has been detected in aqueous media of the photoexcited chlorophylls on MgO powder by using the ESR spin trapping technique. The mechanism for the production of the radical is discussed refering to three-electrons reduction of oxygen by the photoexcited chlorophylls on MgO.

Photoexcited porphyrin pigments (Sens), such as chlorophyll (Chl), pheophorbide, hematoporphyrin, etc., have high reactivity for oxygen. There are two processes in the reactions between the photoexcited pigment (Sens*) and oxygen ($^3O_2$), which are described as follows.[1)]

$$\text{Sens}^* + {}^3O_2 \rightarrow \text{Sens} + {}^1O_2 \qquad (1)$$

$$\text{Sens}^* + O_2 \rightarrow \text{Sens}^+ + O_2^- \qquad (2)$$

One is the energy transfer from triplet state of pigment to oxygen and the singlet state of oxygen ($^1O_2$) is produced [reaction (1)]. The other is the electron transfer from triplet state of pigment to oxygen and the superoxide ion (O_2^-) is produced [reaction (2)]. These active oxygen species are important to understand the mechanism for the reaction by photoexcited pigments.

The reactivity of the photoexcited pigments is influenced by their surroundings. For example, the difference of the reactivity of reaction centers, P700 and P680, for photosynthesis is brought by the conformation change of chlorophylls in its visinity.[2)] Therefore, it is significant to examine the reactivity of the photoexcited pigments under various conditions.

A few papers [3-5)] have been published concerning the detection of active oxygen radicals produced from the photoexcited chlorophyll or chloroplast by using the ESR spin trapping technique. Harbour and Bolton[3)] reported the production of the superoxide ion from photoexcited spinach chloroplast. You and Fong[5)] also reported the production of the superoxide ion from the photoexcited chlorophyll a in 50% aqueous aceton solution. The origin of the superoxide ion can be explained by the electron transfer from the photoexcited chlorophyll to oxygen. The production of hydroxyl radical from the photoexcited chlorophyll a in aqueous micellar solution was confirmed by Harbour and Bolton.[4)] However, the mechanism for the production of the hydroxyl radical is not so clear.

We have carried out the ESR spin trapping experiments to examine the reactivity of photoexcited chlorophylls adsorbed on magnesium oxide in the presence of oxygen, and found out effective formation of the radicals in this media.

Magnesium oxide (MgO, 99.9%), catalase (6500 units/mg), hydrogen peroxide (H_2O_2) and ethanol (special grade) were obtained from Wako Pure Chemical Industries, Ltd. As a spin trap reagent, 5,5-dimethyl-3,4-dihydro-2H-pyrole 1-oxide (DMPO), was purchased from Dojindo Laboratory. Superoxide dismutase (SOD, Cu/Zn) was obtained from Sigma. Chlorophyll (Chl) a and b were purchased from Aldrich Chem. Co., Inc. Chlorophylls on MgO (Chl/MgO) was prepared by dropping the chlorophyll ethanol solutions (1.74 mmol dm^{-3}) into the suspension of MgO. As a support, MgO was chosen by the reason that magnesium (II) was the center metal of chlorophylls and the band-gap excitation of the support itself can be avoided because of its high band-gap energy (7 eV). Optical absorption spectra were recorded by using a Hitachi Co., Ltd., U-2000 spectrophotometer, in the wavelength range from 380 to 750 nm. Chl/MgO suspension was mixed with DMPO and various solutions (ethanol, SOD, catalase, and H_2O_2). The suspension was fed into a flat, quartz ESR cell, which was inserted into the cavity of an ESR spectrometer (JEOL RE-2X). The sample was irradiated with a 500 W xenon lamp with 600 nm low pass filter (Toshiba, R-60) or 700 nm band pass filter (Toshiba KL-70). ESR measurements were conducted after or during irradiation.

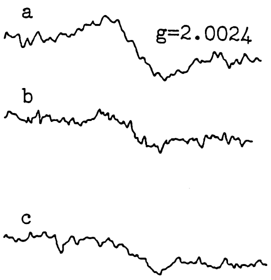

Fig. 2. ESR spectra obtained by irradiation of Chl/MgO suspension.
a: Chl a with 600 nm low pass filter, b: Chl a with 700 nm band pass filter, c: Chl b with 600 nm low pass filter.

The optical absorption spectra of Chl/MgO in water are shown in Fig. 1. A red shift was observed on the Q-band signals of Chl/MgO, compared with those of the chlorophylls in ethanol solutions (Chl a, 660 → 668 nm; Chl b, 638 → 648 nm). This bathochromic effect may have arisen from a conformation change of

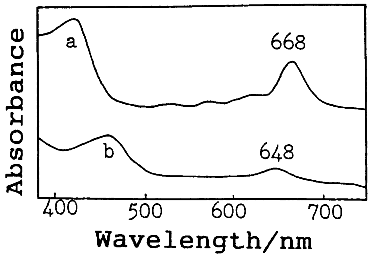

Fig. 1. Optical absorption spectra of Chl/MgO suspension.
a: Chl a, b: Chl b.

chlorophylls by the adsorption on MgO.

The ESR spectra obtained by the photoexcited Chl/MgO suspension during irradiation in the absence of DMPO are shown in Fig. 2. These signals can be assigned to the chlorophyll cation radicals in view of their g value of 2.0024.6) This result supports the occurrence of the reaction (2).

The ESR spectra of the DMPO spin adducts obtained from the photoexcited chlorophylls in 20% ethanol solution are shown in Fig. 3. The superoxide ion adduct was easily assigned by its hyperfine splitting constant (hfsc) values.7) Small amounts of unknown spin adducts were regarded as artifact because no production of these adducts was confirmed by experiments using another spin trap reagent. Moreover, the addition of H_2O_2 did not lead to the production of the hydroxyl radical adduct. Thus, in the homogeneous media only one-electron reduction of oxygen was caused by the photoexcited chlorophyll molecule. The intensity of the superoxide ion adduct from the photoexcited Chl b was larger than that from Chl a.

The ESR spectra of the DMPO spin adducts obtained from photoexcited Chl a/MgO under various conditions are shown in Fig. 4. Since no ESR signal was observed from illuminated MgO suspension in the presence of oxygen, the production of radicals was confirmed to be originated from the photoexcited Chl. In Fig. 4a, these signals can be assigned to the hydroxyl radical and a small amount of the superoxide ion adducts in view of their hfsc values.7,8) An addition of ethanol converted the hydroxyl radical into a carbon(C)-centered radical, as shown in Fig. 4b. This means the production of free hydroxyl radical. The hydroxyl radical adduct appreciably decreased on addition of both SOD and catalase (Fig. 4c). Moreover, the high intensity of the hydroxyl radical adduct was observed on addition of 0.1 mmol dm^{-3} H_2O_2 (Fig. 4d). Therefore, the

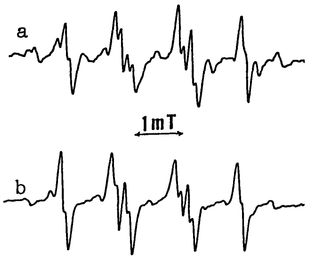

Fig. 3. ESR spectra of the DMPO spin adducts obtained by 60-s irradiation of Chl in 20% ethanol solution.
a: Chl a, b: Chl b, receiver gain: x400 (a), x200 (b), concentration Chl: 0.35 mmol dm^{-3}.

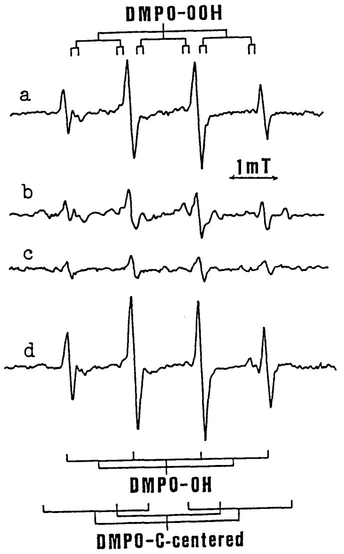

Fig. 4. ESR spectra of the DMPO spin adducts obtained by irradiation of Chl a/MgO suspension.
a: control, b: 10% ethanol, c: 200 units ml^{-1} SOD + 500 units ml^{-1} catalase, d: 0.1 mmol dm^{-3} H_2O_2, irradiation time: 120-s (a-c), 60-s (d), receiver gain: x400, concentration of Chl: 0.017 mmol dm^{-3}.

hydrogen peroxide plays a significant role for the production of the hydroxyl radical. From these results, three-electrons reduction of oxygen is rapidly caused by the photoexcited Chl a/MgO. Similar behavior was observed in photoexcited Chl b/MgO suspension, but the signal intensities in this case were lower than those of the Chl a/MgO. This indicates that the reactivity of the Chl a/MgO is higher than that of the Chl b/MgO. This result is the opposite to the results obtained from chlorophylls in 20% ethanol solution. This may also have arisen from the conformation change of Chlorophylls by the adsorption on MgO. Moreover, no production of the singlet state of oxygen from the photoexcited Chl/MgO in aqueous media was confirmed by the ESR technique using 2,2,6,6-tetramethyl-4-piperidone.⁹⁾ Therefore, only the electron transfer to oxygen is caused by the photoexcited Chl/MgO in aqueous media.

Based on these results, a possible mechanism for the production of the hydroxyl radical from the photoexcited Chl/MgO is proposed as follows.

$$O_2^{-} + e^{-} + 2H^{+} \rightarrow H_2O_2 \qquad (3)$$

$$H_2O_2 + e^{-} + H^{+} \rightarrow \cdot OH + H_2O \qquad (4)$$

$$O_2^{-} + H^{+} + H_2O_2 + Chl^{*} \rightarrow \cdot OH + H_2O + O_2 + Chl \qquad (5)$$

The superoxide ion produced by reaction (2) is further reduced to the hydrogen peroxide by the photoexcited Chl [reaction (3)]. Furthermore, the hydrogen peroxide is reduced to the hydroxyl radical (·OH) by the photoexcited Chl [reaction (4)]. That is, three-electrons reduction of oxygen is performed by the photoexcited Chl/MgO. Such a cascade reduction can be regarded as a characteristic feature of the inhomogeneous media such as in our case. Here the Harber-Weiss reaction¹⁰⁾ [reaction (5)] catalized by photoexcited Chl cannot be excluded because the hydroxyl radical adduct appreciably decreased only by the addition of SOD (not shown). At any rate it can be concluded that the three electrons reduction of oxygen is mainly caused by the photoexcited Chl/MgO systems.

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