

Oxidation of Polyvalent Porphyrin, Tetrakis(3,5-di-tert-butyl-4-hydroxyphenyl)porphyrin by Superoxide Ion

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Superoxide ion, O_2^- , forms the superoxide adduct with non-redox metalloporphyrins such as Zn(II)–, Mg(II)– and Cd(II)–*meso*-tetraphenylporphyrin (TPP)* [1–3], whereas it reduces redox-active metalloporphyrins such as Co(III)–*meso*-tetra-*p*-tolylporphyrin (TPP) [4]. However, there have been no reports concerning the reactions of O_2^- with metal-free porphyrins. Recently, Traylor *et al.* reported [5] that polyvalent porphyrin, tetrakis(3,5-di-tert-butyl-4-hydroxyphenyl)porphyrin (**1**, T^tBHPP) which is sterically protected by the tert-butyl group is a good model for peroxidase to give the radical species. The reason is that at least one of the electrons, *i.e.*, the $2e^-$ equivalent is more oxidized than the resting porphyrin of peroxidase when removed from the porphyrin π system to give a cation radical. This is responsible for much of the electron transfer involved in substrate oxidations [6, 7] when the oxidation of T^tBHPP by *m*-chloroperbenzoic acid in the presence of hemin as a catalyst to obtain the stable intermediate was studied. Whereas, the stable intermediate was not obtained, the $2e^-$ oxidation product of T^tBHPP, quinone (**2**), was. Subsequently, we have examined the reactions of O_2^- with T^tBHPP to detect the stable intermediate by absorption and electron spin resonance (ESR) spectroscopies. In this work, the results of the reactions of O_2^- with T^tBHPP are described.

Experimental

Materials

T^tBHPP was prepared by the condensation reaction between pyrrole and 3,5-di-tert-butyl-4-hydroxybenzaldehyde in propionic acid [8] and was puri-

*Abbreviations used in this paper are as follows: TPP, *meso*-tetraphenylporphyrin; TTP, *meso*-tetra-*p*-tolylporphyrin; T^tBHPP, tetrakis(3,5-di-tert-butyl-4-hydroxyphenyl)porphyrin; O_2^- , superoxide ion; DMSO, dimethyl sulfoxide; CHCl₃, chloroform; ESR, electron spin resonance; IR, infrared.

fied by column chromatography on silica gel with 1:1 benzene–cyclohexane as eluent. TPP [1] and TTP [4] were prepared and purified as described previously. Potassium superoxide (KO₂) was purchased from Alfa Products, Inc. Dicyclohexyl-18-crown-6 (Nippon Soda Ltd.) was used without further purification. 2,4,6-tri-tert-butylphenol was obtained from Aldrich and used after recrystallization from ethanol. Other reagents were commercially available. Dimethyl sulfoxide (DMSO) was distilled at reduced pressure from CaH₂ and stored over freshly activated 4A molecular sieves under argon gas. Other solvents used were distilled immediately prior to use.

Preparation of Solutions of Superoxide Ion

DMSO solutions of O_2^- were prepared as described previously [1, 9]. The concentration of O_2^- was determined by the method as described by Bielski *et al.* [10].

Reaction of Free Base Porphyrin with Superoxide Ion

Two milliliters of *ca.* 10^{-6} M free base porphyrins in DMSO were placed in a 1-cm capped quartz cuvette. Aliquots of 10^{-2} – 10^{-3} M KO₂/dicyclohexyl-18-crown-6 were added rapidly by microsyringe. After the reaction was started by mixing the reactants, visible spectra were recorded immediately.

Spectral Measurements

Absorption spectra were recorded at room temperature with a Union Giken SM-401 spectrophotometer.

ESR spectra were measured at room temperature or at 77 K with a JEOL-PE-1X (X-band) spectrometer with 100 kHz field modulation. ESR parameters were calibrated by comparison with the standard sample of Mn²⁺ doped on MgO and 2,2-diphenyl-1-picrylhydrazyl (DPPH, *g* = 2.0036).

Infrared (IR) spectra were observed in chloroform (CHCl₃) solution using a JASCO IRA-1 diffraction grating infrared spectrophotometer.

Preparation of the Reaction Products between O_2^- and T^tBHPP

8 ml of DMSO solutions of O_2^- (2.6×10^{-4} M) were gently added to 2 ml of CHCl₃ solution of T^tBHPP (4.2×10^{-5} M). After dilution of CHCl₃ into DMSO is carried out, the final concentrations are $[O_2^-] = 2.08 \times 10^{-4}$ M and $[T^tBHPP] = 8.4 \times 10^{-6}$ M in 20% v/v CHCl₃ in DMSO. The reaction mixture was stirred for 30 min at room temperature. The reaction solution resulted in the color change from reddish violet to bluish violet. Afterwards, the reaction mixture was loaded onto a silica gel column and

eluted by CHCl_3 . The first fraction was unreacted $\text{T}^{\text{t}}\text{BHPP}$. The second eluate was collected and concentrated by a rotary evaporator. Excess of methanol (MeOH) was added to the concentrated solution to make crystallines. The resulting greenish crystallines were collected and washed with MeOH several times and dried in the air. The yield was 23.7 mg (50.1%, based on $\text{T}^{\text{t}}\text{BHPP}$).

Results and Discussion

Visible spectra of TPP and TTP did not change in the presence of excess O_2^- , even if the mixtures were kept in the dark for 2 days at room temperature. These results suggest that O_2^- does not react with TPP and TTP.

When DMSO solution of $\text{T}^{\text{t}}\text{BHPP}$ was mixed with O_2^- , a wine-red solution of $\text{T}^{\text{t}}\text{BHPP}$ ($\lambda_{\text{max}} = 429, 526, 566, 605, 660 \text{ nm}$) changed to purple ($\lambda_{\text{max}} = 574, 700 \text{ nm}$) with the isosbestic points, as shown in Fig. 1. As described under 'Experimental', the reac-

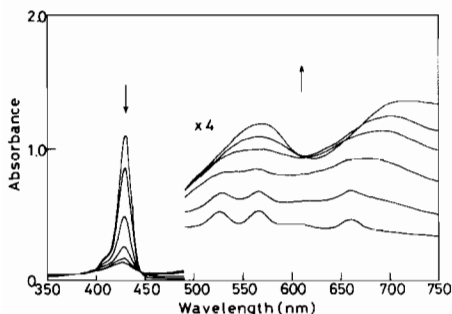


Fig. 1. Visible spectral changes resulting from successive additions of O_2^- to $\text{T}^{\text{t}}\text{BHPP}$ in DMSO. $\text{T}^{\text{t}}\text{BHPP}$ was firstly dissolved in $\text{DMSO}-\text{CHCl}_3$ (3:2) and then the resulting solution was diluted to 1/100-fold concentration with DMSO. Starting concentration of $\text{T}^{\text{t}}\text{BHPP}$: $3.37 \times 10^{-6} \text{ M}$; final concentration of O_2^- : $7.77 \times 10^{-4} \text{ M}$.

tion product gave the green crystallines after being purified by chromatography on silica gel with CHCl_3 as eluent. Both the visible and the IR spectra of the purified product [$\text{O}-\text{H}$ stretching bands ($\nu_{\text{O}-\text{H}}$) at 3700 and 3520 cm^{-1} and a carbonyl absorption ($\nu_{\text{C}=\text{O}}$) at $1600-1610 \text{ cm}^{-1}$ in CHCl_3] were identical with those of the quinone (2) which was prepared from $\text{T}^{\text{t}}\text{BHPP}$ by the two-equivalent oxidation using *m*-chloroperbenzoic acid in the presence of hemin as a catalyst [5]. These results indicate that the $\text{T}^{\text{t}}\text{BHPP}$ is oxidized by O_2^- to the stable species, quinone (2).

Since we have shown that O_2^- can oxidize phenol-type compounds such as tocopherol and its model compounds to give phenoxy-type radicals [11-13], the reactions of O_2^- with $\text{T}^{\text{t}}\text{BHPP}$ were investigated by ESR spectroscopy. At room temperature, no ESR spectrum was observed from the reaction mixture,

even if a high concentration of $\text{T}^{\text{t}}\text{BHPP}$ was used. When the reaction mixture was immediately frozen at 77 K and measured by ESR spectrometer at the same temperature, ESR spectrum due to O_2^- ($g_{\parallel} = 2.104, g_{\perp} = 2.007$) [1] completely disappeared but no new ESR signal could be detected. On the other hand, 2,4,6-tri-*tert*-butylphenol, a model compound of the side chain of $\text{T}^{\text{t}}\text{BHPP}$, gave a stable phenoxy radical [$g = 2.0056, a^{\text{H}}(2) = 2.0 \text{ G}$] during the reaction of O_2^- , as shown in Fig. 2. Furthermore, semi-

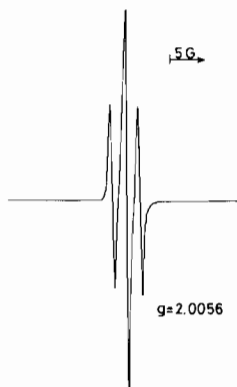
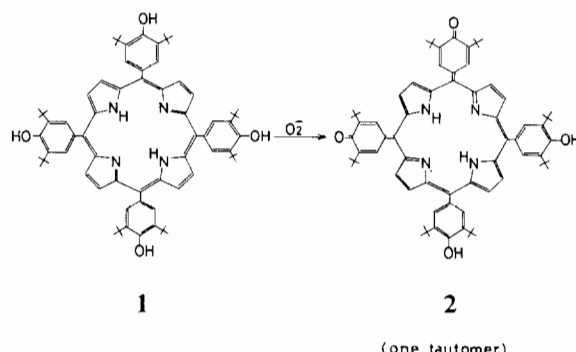


Fig. 2. ESR spectrum observed by the reaction of O_2^- with 2,4,6-tri-*tert*-butylphenol in DMSO. Reaction conditions: $8.1 \times 10^{-2} \text{ M O}_2^-$, $1.6 \times 10^{-2} \text{ M}$ phenol. Instrument settings: microwave power, 10 mW; modulation amplitude, 0.5 G; time constant, 0.1 s; scan time, 4 min.

quinone radicals from the reaction of O_2^- with catechol and its derivatives, which are also considered to be another model compound of $\text{T}^{\text{t}}\text{BHPP}$, have been observed by ESR spectroscopy [14]. These results suggest that the reaction of O_2^- with $\text{T}^{\text{t}}\text{BHPP}$ gives short-lived intermediates which could not be detected by ESR spectroscopy. Also, the stoichiometry of the reaction between O_2^- and $\text{T}^{\text{t}}\text{BHPP}$ could not be determined because the reaction process was dependent both on the concentration of O_2^- and on the reaction time.

In conclusion, the present study has shown that polyvalent porphyrin, $\text{T}^{\text{t}}\text{BHPP}$, undergoes two-equivalent oxidation by superoxide ion in the aprotic



solvent to give the quinone (2) [eqn. (1)], the same oxidation product of T^tBHPP by *m*-chloroperbenzoic acid in the presence of hemin as a catalyst, as reported by Traylor *et al.* [5].

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