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Reactions of Cobalt Complexes of 5,10,15,20-Tetraphenylporphyrin with Peroxide Radicals Generated from Organic Peroxides and Unsaturated Fatty Acids

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The title reactions in aerated benzene solutions were investigated by using ESR and optical-absorption spectrometers. It was found that (1) a benzyl peroxide radical, bpox·, generated photochemically from dibenzoyl peroxide (DBPO) oxidizes [$\mathrm{Co^{II}}$(tpp)] to produce, successively, a five-coordinate cobalt(III) complex, [$\mathrm{Co^{III}}$(bpox)(tpp)]; a π cation radical, [$\mathrm{Co^{III}(tpp)^{\dagger}}$](bpox)$_2$, and isoporphyrin; (2) a *t*-butyl peroxide radical, bupox·, generated from di-*t*-butyl peroxide (DBuPO) oxidizes [$\mathrm{Co^{II}}$(tpp)] to give only a six-coordinate cobalt(III)complex, [$\mathrm{Co^{III}}$(bupox)(tpp)(dbupo)]; (3) lipid peroxide radicals (lpox·: oapox·, lapox·, lnapox·, and aapox·) produced from unsaturated fatty acids, oleic acid (OA), linoleic acid (LA), linolenic acid (LNA), or arachidonic acid (AA), do not react with [$\mathrm{Co^{II}}$(tpp)]; (4) all the peroxide radicals (pox·: lpox·, bpox·, bupox·) react with [$\mathrm{Co^{III}}$(Cl)(tpp)] to produce π cation radicals, [$\mathrm{Co^{III}(tpp)^{\dagger}}$](Cl)(pox), and (5) bpox· and lapox· react further with the π cation radicals to give isoporphyrins. Finally, the present reactions were compared with the reactions of horseradish peroxidase.

It has been well-known that horseradish peroxidase, chloroperoxidase, and cytochrome c peroxidase react with alkyl hydroperoxide, and peroxycarboxylic acid to form intermediates, enzyme-bound-high-valent iron porphyrins (Compounds I and II).[1] It has also been known that active oxygens including hydroperoxide decompose metalloproteins both in vivo and in vitro.[2] As model reactions, a number of studies of the redox reactions of metal porphyrins have been carried out.[3–5]

Recently, we reported[6] on the reactions of a system composed of chloro(5,10,15,20-tetraphenylporphyrinato)cobalt(III), [$\mathrm{Co^{III}}$(Cl)(tpp)], and its π cation radical, [$\mathrm{Co^{III}(tpp)^{\dagger}}$](Cl)$_2$, with several phenols. The phenols were oxidized to phenoxyl radicals. Concomitantly, the cobalt complexes were reduced in different manners, depending on the oxidation potentials of the phenols. The phenols investigated were classified into two groups after taking into account the differences in their reaction activities and their redox potentials: the phenols mainly reacting with [$\mathrm{Co^{III}(tpp)^{\dagger}}$](Cl)$_2$, and those reacting with both [$\mathrm{Co^{III}(tpp)^{\dagger}}$](Cl)$_2$ and [$\mathrm{Co^{III}}$(Cl)(tpp)]. The reaction schemes of cobalt porphyrins [$\mathrm{Co^{II}}$(tpp)], [$\mathrm{Co^{III}}$(Cl)(tpp)], and [$\mathrm{Co^{III}(tpp)^{\dagger}}$](Cl)$_2$ with phenols showed a clear correspondence to the oxidation reactions of horseradish peroxidase (HRP).

In this study, we will investigate the inverse reactions of cobalt porphyrins ([$\mathrm{Co^{II}}$(tpp)] and [$\mathrm{Co^{III}}$(Cl)(tpp)]) with organic peroxides and unsaturated fatty acids in aerated benzene solutions by using ESR and optical absorption techniques. As the results, the reaction mechanisms between the cobalt porphyrins and the peroxide radicals will be presented. Finally, the reaction mechanisms of cobalt porphyrins with the peroxide radicals will be discussed in comparison with the formation mechanism of Compound I, and Compound II of HRP.[7]

Experimental

Materials. The chloro(5,10,15,20-tetraphenylporphyrinato)cobalt(III), [$\mathrm{Co^{III}}$(Cl)(tpp)], and (5,10,15,20-tetraphenylporphyrinato)cobalt(II), [$\mathrm{Co^{II}}$(tpp)],[8] were provided by Dr. Kiyoko Yamamoto of the Institute of Physical and Chemical Research.

The dibenzoyl peroxide (DBPO) and di-*t*-butyl peroxide (DBuPO) were supplied by Nakarai Chemical Industries. 99%-pure oleic acid (OA), linoleic acid (LA), linolenic acid (LNA), and arachidonic acid (AA) were supplied from the Sigma Chemical Co., Ltd., and were used without further purification. The nitrosodurene, a spin-trapping reagent, was supplied by Mr. Naohiro Takahashi of the Sumitomo Chemical Co., Ltd.

Preparation of Sample Solutions. Benzene was used as the solvent throughout this study.

The [$\mathrm{Co^{II}}$(tpp)] and [$\mathrm{Co^{III}}$(Cl)(tpp)] were dissolved in 2×10^{-3} mol dm^{-3} in the cases of reactions with all peroxide radicals except DBuPO. The solution of [$\mathrm{Co^{III}}$(Cl)(tpp)] contains 2.7×10^{-5} mol dm^{-3} of the π cation radical, [$\mathrm{Co^{III}(tpp)^{\dagger}}$](Cl)$_2$.[6] In the case of the reaction with DBuPO, [$\mathrm{Co^{II}}$(tpp)] and [$\mathrm{Co^{III}}$(Cl)(tpp)] were dissolved in 2×10^{-4} mol dm^{-3}. DBPO, DBuPO, OA, LA, LNA, and AA were dissolved just before use (DBPO, 2×10^{-3} mol dm^{-3}; DBuPO, 1×10^{-1} mol dm^{-3}; OA, LA, LNA, and AA, 10%).

Measurements. The ESR spectra were measured by means of a JEOL FE-2XG spectrometer equipped with a Takeda Riken TR-5211 microwave counter. The ESR spectra of the π cation radical, [$\mathrm{Co^{III}(tpp)^{\dagger}}$](x)(y), measured in this work show the pair of the maximum and the minimum separated by a wide plateau. Such spectra are due to eight unresolved, hyperfine lines. In this work, therefore, the *g* values were determined from the midpoint of the maximum and the minimum of the spectra. The spacings between maximum and minimum points, ΔH, are the measure of the hyperfine line of cobalt:

$$\Delta H \simeq 7 \times a_{\mathrm{Co^{III}}} + \Delta H_{\mathrm{msl}}, \tag{1}$$

where ΔH_{msl} is the intrinsic line width. Hereafter, we will adopt these values as the ESR parameters.

For a quantitative analysis of the π cation radicals, a computer system of JES ES-PRIT23 was used. The optical absorption spectra were measured by means of a Hitachi 330 spectrophotometer using quartz cells with optical path lengths of 1 and 0.1 cm.

Simultaneous measurements of the ESR and the optical absorption spectra were carried out by using an ESRET system combining a Spectro Multi-Channel Photo Detector MCPD-100 (Otsuka Electronics) with a JES FE-2XG apparatus equipped with a transmission cavity, ES-MCX-3B.[9] By the use of the ESRET, one can measure simultaneously both the ESR and the optical spectra of a sample solution under the same conditions. This apparatus is very useful for mesurement of a solution containing an unstable sample or a small amount of a sample. A quartz cell with an optical path length of 0.3 mm, 10 mm in width, and 40 mm in height, was used for the ESRET measurements. To 0.1 cm^3 of a [Co^{II}(tpp)] or [Co^{III}(Cl)(tpp)] solution was added 0.1 cm^3 of a peroxide or fatty-acid solution. The ESR and absorption spectra of the mixed solution were simultaneously measured at intervals of 1 min after mixing. The absorption spectra obtained from the ESRET were checked with those obtained by means of a Hitachi 330 spectrophotometer. The time dependent ESR spectra were measured using a rapid detection system.[6] 0.2 cm^3 of neat unsaturated fatty acids were added to 0.2 cm^3 of a [Co^{III}(Cl)(tpp)] solution, which was bubbled in by dry nitrogen gas.

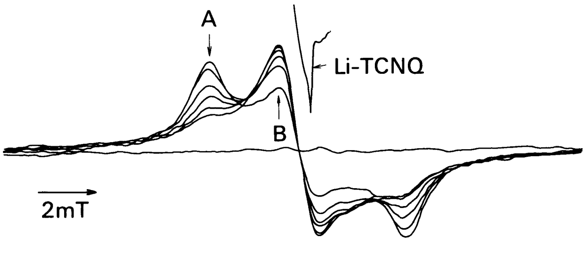


Fig. 1a. ESR spectra measured before and after (the interval of 1 min) addition of DBPO to [Co^{II}(tpp)] in a benzene solution. A: [Co^{III}(tpp)‡](bpox)$_2$, B: bpox·. Li-TCNQ is internal standard, g=2.0026.

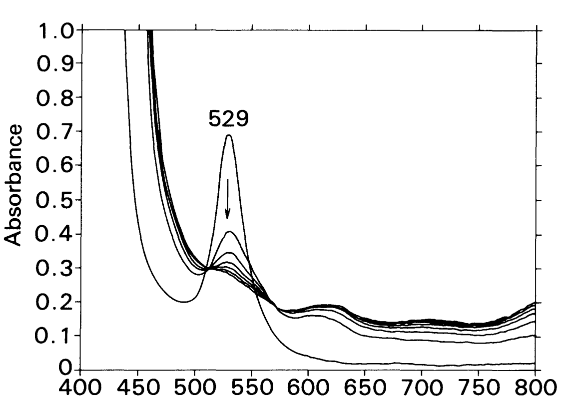


Fig. 1b. Absorption spectra measured before and after (the interval of 1 min) addition of DBPO to [Co^{II}(tpp)] in a benzene solution.

Results and Discussion

Reaction of [Co^{II}(tpp)] with DBPO or DBuPO. To a benzene solution of [Co^{II}(tpp)] were added the same amount of a DBPO solution; the ESR and the optical absorption spectra of the mixed solution were then measured by using the ESRET apparatus at intervals of 1 min after adding DBPO to the [Co^{II}(tpp)]. The ESR spectra thus measured are shown in Fig. 1a. The solution of [Co^{II}(tpp)] gave no ESR signals at room temperature because of the short relaxation time. One minute after the addition of DBPO, two signals, A and B, appeared. The ESR parameters of the A signal are g=2.0048 and ΔH=6.89 mT. This signal was assigned to a π cation radical, [Co^{III}(tpp)‡](bpox)$_2$ on the basis of the ESR parameters.[10,11] The ESR parameters of the B signal are g=2.0082 and ΔH=0.65 mT. It is well-known that DBPO decomposes easily to produce the peroxide radical under these ambient conditions: natural light, air, and room temperature. In order to confirm the B signal, DBPO in an aerated benzene solution was irradiated by using a mercury lamp as a light source without a filter; the ESR spectrum was then measured at 5 °C. An ESR signal of g=2.0155($\pm$0.0002) and ΔH=0.75 mT was obtained. The g value is typical of peroxide radicals,[12,13] so the signal was identified as a peroxede radical of DBPO (bpox·: $C_6H_5(CO)OO\cdot$). The same signal was observed for a deaerated benzene solution of irradiated DBPO. The g value of the B signal is somewhat smaller than that of bpox· obtained in neat benzene by irradiation. This might be because of an interaction between the radical and the tpp ligand of [Co^{II}(tpp)]. With an increase in the time, the intensity of the A signal decreased, while that of the B signal increased. This suggests that bpox· successively generated oxidizes [Co^{II}(tpp)] to produce [Co^{III}(tpp)‡](bpox)$_2$ and then further decomposes the π cation radical. The corresponding optical absorption spectra are shown in Fig. 1b. With an increase in the time, the characteristic absorption band at 529 nm of [Co^{II}(tpp)] decreased in intensity, while weak and broad bands appeared at longer wavelengths. The bands in the near infrared-region are interpreted as being due to the formation of isoporphyrin,[14–16] because the optical-absorption spectrum measured for the solution by means of the Hitachi 330 spectrophotometer showed absorption maxima at 700 and 800 nm. The absorption bands in the visible region may be due to those of [Co^{III}(tpp)]$^+$ and the π cation radical of Co^{III}(tpp). From both the ESR and the optical-absorption-spectral changes, it is concluded that bpox· oxidizes [Co^{II}(tpp)] to produce [Co^{III}(tpp)]$^+$, then the π cation radical, and finally isoporphyrin.

The optical-absorption spectra observed for the reaction mixture of [Co^{II}(tpp)] and DBuPO by using the

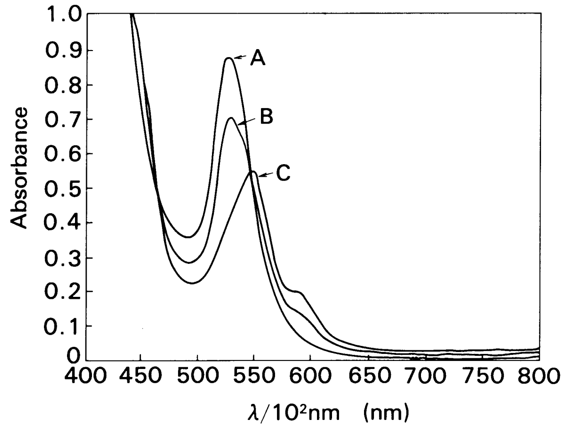


Fig. 2. Absorption spectra measured beore (A) and after (B: 7 min, C: 15 min) addition of DBuPO to [Co$^{\mathrm{II}}$(tpp)] in a benzene solution.

ESRET apparatus are shown in Fig. 2. The reaction mixture gave no ESR spectrum of the π cation radical. Upon the addition of DBuPO to [Co$^{\mathrm{II}}$(tpp)] in a benzene solution, the intensity of the absorption band of [Co$^{\mathrm{II}}$(tpp)] at 529 nm (A) decreased and the absorption maximum shifted towards longer wavelengths, showing one set of isosbestic points at 465 and 545 nm, and new bands appeared at 550 and 590 nm. The absorption spectrum represents the characteristic absorption of the six-coordinate [Co$^{\mathrm{III}}$(tpp)]$^{+}$ complex.[17] The spectrum was an unchanged with the increase in time. It is also well-known that DBuPO decomposes under ambient conditions. DBuPO in an aerated benzene solution was irradiated by using a mercury lamp as a light source without a filter, and the ESR spectrum was measured at 5 °C. The ESR signal of g=2.0155(±0.0002) and ΔH=0.3 mT appeared; it was identified as a peroxide radical, bupox·($\mathrm{C_4H_9OO\cdot}$), on the basis of the g value. From the results of the optical-absorption-spectral change and the lack of any ESR spectra, it is concluded that [Co$^{\mathrm{II}}$(tpp)] is oxidized by bupox· to produce five-coordinate [Co$^{\mathrm{III}}$(bupox)(tpp)]; then excess DBuPO coordinates as the 6th ligand to form a six-coordinate complex, [Co$^{\mathrm{III}}$(bupox)(tpp)(dbupo)].

The reactions of [Co$^{\mathrm{II}}$(tpp)] with unsaturated fatty acids, OA, LA, LNA, and AA, in aerated benzene solutions gave no ESR or absorption-spectral changes.

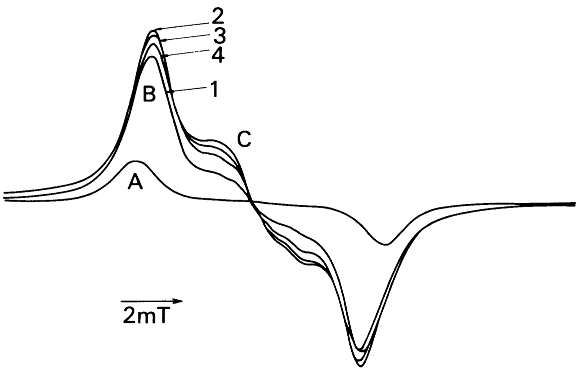


Fig. 3a. ESR spectra measured beore (A) and after (B: 1, 2, 3, 4) addition of BPO to [Co$^{\mathrm{III}}$(Cl)(tpp)] in a benzene solution at the interval of 1 min.
A: [Co$^{\mathrm{III}}$(tpp$^{\dot{+}}$](Cl)$_2$, B: [Co$^{\mathrm{III}}$(tpp)$^{\dot{+}}$](Cl)(bpox$_2$), C: bpox·.

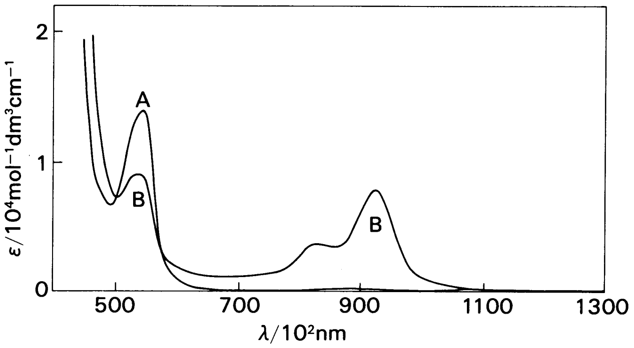


Fig. 3b. Absorption spectra measured beore (A) and after (B) addition of BPO to [Co$^{\mathrm{III}}$(Cl)(tpp)] in a benzene solution.

Reaction of [Co$^{\mathrm{III}}$(Cl)(tpp)] with DBPO, DBuPO, OA, LA, LNA, and AA. The ESR spectra measured for a mixed solution of [Co$^{\mathrm{III}}$(Cl)(tpp)] and DBPO are shown in Fig. 3a. Signal A in Fig. 3a represents the signal of [Co$^{\mathrm{III}}$(tpp)$^{\dot{+}}$](Cl)$_2$ (g=2.0047 and ΔH=8.98 mT) that is contained in the solid [Co$^{\mathrm{III}}$(Cl)(tpp)].[18] In the ESR spectrum obtained after the addition of DBPO, the signal of [Co$^{\mathrm{III}}$(tpp)$^{\dot{+}}$](Cl)$_2$ disappeared, and new two signals, B and C, appeared. The ESR parameters of the B signal are g=2.0062 and ΔH=6.37 mT. This showed the species to be a π cation radical, [Co$^{\mathrm{III}}$(tpp)$^{\dot{+}}$](Cl)(bpox), on the basis of the ESR parameters. The C signal represents the signal of bpox·. When an increase in the time, the signal intensity of B decrease, while that of C increases, as in the case of the reaction of [Co$^{\mathrm{II}}$(tpp)] with DBPO.

The optical-absorption spectra observed before and after the addition of DBPO, (A) and (B), respectively, are shown in Fig. 3b. The characteristic absorption band of [Co$^{\mathrm{III}}$(Cl)(tpp)] at 540 nm decrease in intensity upon the addition of DBPO, and new bands appeared at 815 and 915 nm. The bands in the near-infrared region are attributed to isoporphyrin. From the results of the ESR and the absorption spectra, it is concluded that bpox· oxidizes [Co$^{\mathrm{III}}$(Cl)(tpp)] to [Co$^{\mathrm{III}}$(tpp)$^{\dot{+}}$](Cl)(bpox) and then to isoporphyrin, while [Co$^{\mathrm{III}}$(tpp)$^{\dot{+}}$](Cl)$_2$ is oxidized to isoporphyrin.

The ESR spectra and optical absorption spectra of a reaction mixture of [Co$^{\mathrm{III}}$(Cl)(tpp)] and DBuPO were measured as in the case of the reaction with DBPO. The ESR spectra showed that the signal of [Co$^{\mathrm{III}}$(tpp)$^{\dot{+}}$](Cl)$_2$ disappeared and a new signal appeared. Based on the ESR parameters obtained from the spectrum, the B signal, g=2.0062 and ΔH=7.78 mT, can be interpreted as being to the formation of a π cation

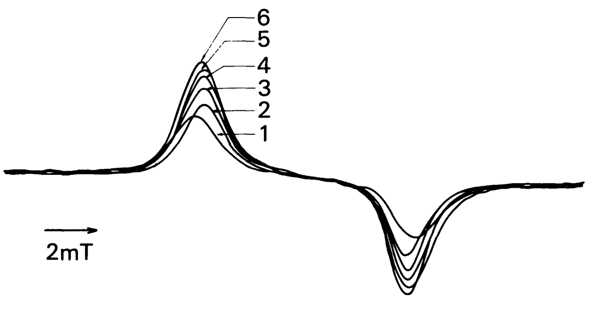


Fig. 4a. ESR spectra measured after addition of OA (1: 0%, 2: 2%, 3: 4%, 4: 6%, 5: 8%, and 6: 10%) to $[\mathrm{Co^{III}(Cl)(tpp)}]$ in a benzene solution.

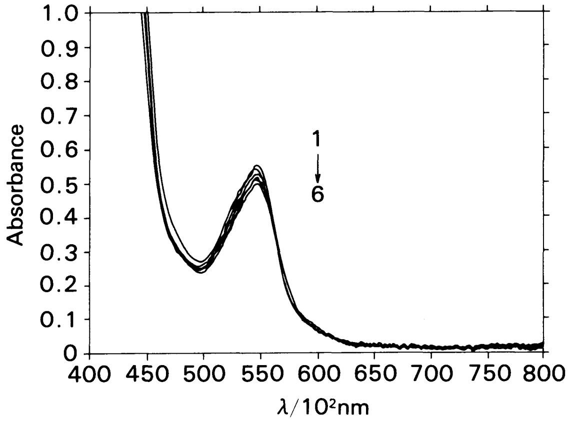


Fig. 4b. Absorption spectra measured after addition of OA (1: 0%, 2: 2%, 3: 4%, 4: 6%, 5: 8%, and 6: 10%) to $[\mathrm{Co^{III}(Cl)(tpp)}]$ in a benzene solution.

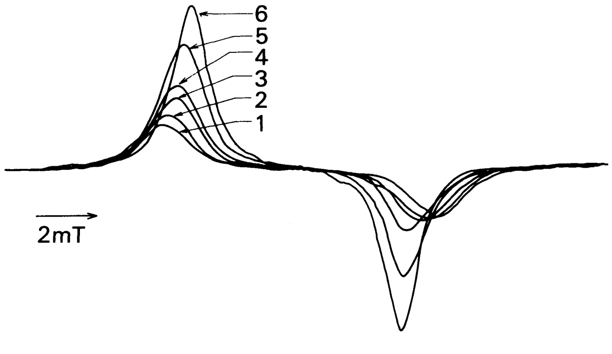


Fig. 5a. ESR spectra measured after addition of LA (1: 0%, 2: 1.25%, 3: 2.5%, 4: 5%, 5: 10%, and 6: 20%) to $[\mathrm{Co^{III}(Cl)(tpp)}]$ in a benzene solution.

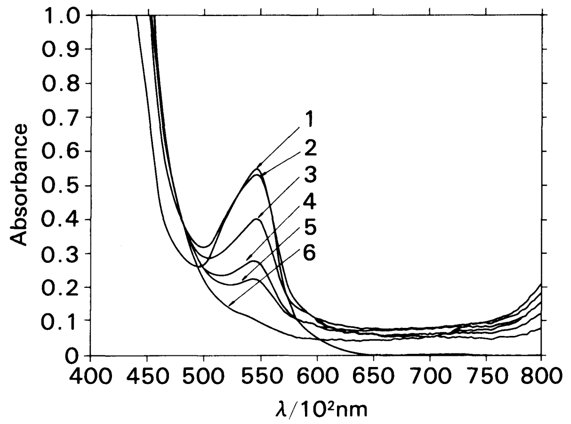


Fig. 5b. Absorption spectra measured after addition of LA (1: 0%, 2: 1.25%, 3: 2.5%, 4: 5%, 5: 10%, and 6: 20%) to $[\mathrm{Co^{III}(Cl)(tpp)}]$ in a benzene solution.

radical, $[\mathrm{Co^{III}(tpp)^{+\cdot}](Cl)(bupox)}$. The corresponding optical-absorption spectra showed that, with an increase in the time, the intensities of the characteristic absorption bands of $[\mathrm{Co^{III}(Cl)(tpp)}]$ at 406 and 540 nm decrease and a new shoulder appears at 436 nm which may be attributable to the formation of a π cation radical.19) The ESR and optical-absorption-spectral changes suggest that $[\mathrm{Co^{III}(Cl)(tpp)}]$ reacts with DBuPO to form a π cation radical, $[\mathrm{Co^{III}(tpp)^{+\cdot}](Cl)(bupox)}$.

The ESR spectra measured for aerated benzene solutions of $[\mathrm{Co^{III}(Cl)(tpp)}]$ with various amounts of OA (0, 2, 4, 6, 8, and 10%) are shown in Fig. 4a. Upon the addition of OA, the signal (1 in the figure) due to $[\mathrm{Co^{III}(tpp)^{+\cdot}](Cl)_2}$ disappeared and a new signal (2—6) appeared. The new signal is identified as a π cation radical, $[\mathrm{Co^{III}(tpp)^{+\cdot}](Cl)(oapox)}$, due to the ESR parameters obtained from the spectrum. With the increase in the amount of OA, the intensity of the signal increased. The concentration of $[\mathrm{Co^{III}(tpp)^{+\cdot}](Cl)(oapox)}$ produced by the addition of 10% of OA is $8\times10^{-5}\ \mathrm{mol\ dm^{-3}}$. The corresponding optical absorption spectra showed only a slight decrease in the absorption intensity of $[\mathrm{Co^{III}(Cl)(tpp)}]$ at 540 nm (Fig. 4b). The ESR and optical absorption spectral changes suggest that $[\mathrm{Co^{III}(Cl)(tpp)}]$ is oxidized by a peroxide radical (oapox·) generated from OA under the ambient conditions (to be described below) to produce $[\mathrm{Co^{III}(tpp)^{+\cdot}](Cl)(oapox)}$. The reactions of $[\mathrm{Co^{III}(Cl)(tpp)}]$ with LNA and AA also gave rise to the formation of π cation radicals, $[\mathrm{Co^{III}(tpp)^{+\cdot}](Cl)(lnapox)}$ and $[\mathrm{Co^{III}(tpp)^{+\cdot}](Cl)(aapox)}$ respectively.

The ESR and optical-absorption spectra of aerated benzene solutions of $[\mathrm{Co^{III}(Cl)(tpp)}]$ with various amounts of LA (0, 1.25, 2.5, 5, 10, and 20%) were also measured. The spectra are shown in Fig. 5a. Upon the addition of LA, the signal (1 in the figure) of $[\mathrm{Co^{III}(tpp)^{+\cdot}](Cl)_2}$ disappeared and a new signal (2—6) appeared. The new signal is identified as a π cation radical, $[\mathrm{Co^{III}(tpp)^{+\cdot}](Cl)(lapox)}$, on the basis of the ESR parameters obtained from the spectrum. With the increase in the amount of LA, the intensity of the signal increased. The concentration of $[\mathrm{Co^{III}(tpp)^{+\cdot}](Cl)(lapox)}$ produced by the addition of 10% of OA is ca. $6\times10^{-5}\ \mathrm{mol\ dm^{-3}}$. The corresponding optical-absorption spectra (Fig. 5b) obtained by using ESRET apparatus showed that the intensity of the absorption band of $[\mathrm{Co^{III}(Cl)(tpp)}]$ at 540 nm rapidly decreased and the new bands appeared at 825 and 912 nm attributable to the formation of isoporphyrin. The ESR and optical-absorption-spectral changes lidicate that the peroxide radical of lapox· oxidizes [Co^{III}(Cl)(tpp)] to [Co^{III}(tpp)$^{\dot{+}}$](Cl)(lapox) and then afterward to isoporphyrin.

The ESR parameters of the π cation radicals obtained are summarized in Table 1.

Reaction Rates. In the reactions of [Co^{III}(Cl)(tpp)] (2×10^{-3} mol dm^{-3}) with unsaturated fatty acids (10^{-1} mol dm^{-3}) in aerated benzene solutions, the formation rate of the π cation radical, [Co^{III}(tpp)$^{\dot{+}}$](Cl)(lpox), was estimated by using a rapid-detection system.[6)] In Fig. 6, the concentrations of the π cation radicals formed are plotted against the reaction time. In the cases of the reactions with OA, LNA, and AA, the amounts of the π cation radicals, [Co^{III}(tpp)$^{\dot{+}}$](Cl)(oapox), [Co^{III}(tpp)$^{\dot{+}}$](Cl)(lnapox), and [Co^{III}(tpp)$^{\dot{+}}$](Cl)(aapox), increase with the time in the early stage of the reaction. However, in the case of the reaction with LA, the amount of [Co^{III}(tpp)$^{\dot{+}}$](Cl)(lapox) is the largest, though it decreases rapidly. The reaction rates were estimated from the linear portion in Fig. 6, the reaction rates were obtained as 3.5×10^{-5}, 2.5×10^{-5}, and 3×10^{-6} mol s^{-1} for OA, LNA, and AA respectively. The rate for LA was larger than 2.4×10^{-4} mol s^{-1}. The decrease in the π cation radicals which may be seen in Fig. 6 may show that lpox· oxidizes the π cation radicals further to isoporphyrin and also that the oxidation activity of lapox· is distinctly large among lpox·. It should also be noted here that the formation rates of the π cation radical, [Co^{III}(tpp)$^{\dot{+}}$](Cl)(lpox), are not ralated to the autooxidation rates of unsaturated fatty acids (OA<LA<LNA<AA),[21)] but rather with the oxidation potentials of unsaturated fatty acids, as in the reactions between cobalt porphyrins and phenols.[6)]

Table 1. The ESR Parameters of the π Cation Radicals Obtained, [Co^{III}(tpp)$^{\dot{+}}$](x)(y), in Benzene

x	y	g (±0.0002)	ΔH[a)] mT
Cl	Cl	2.0047	8.98
Cl	bpox	2.0062	6.37
Cl	bupox	2.0058	7.78
Cl	oapox	2.0054	7.63
Cl	lapox	2.0063	6.93
Cl	lnapox	2.0047	7.73
Cl	aapox	2.0047	7.72
bpox	bpox	2.0082	6.89

a) Peak-to-peak width.

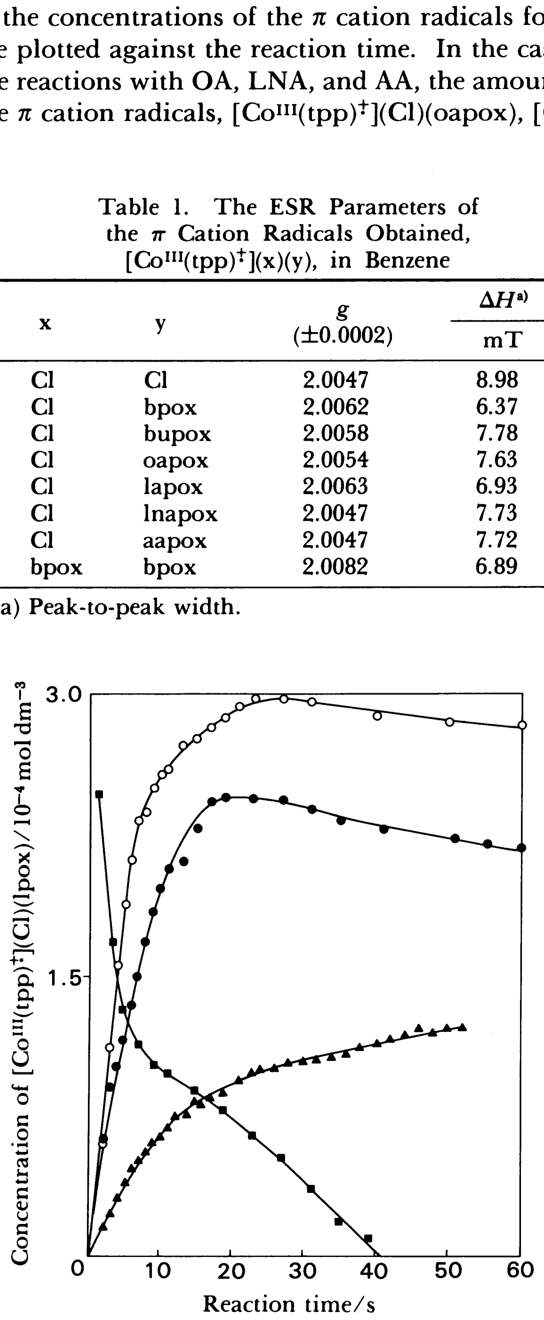


Fig. 6. Concentration decay of [Co^{III}(tpp$^{\dot{+}}$](Cl)(lpox) generated from the reaction of [Co^{III}(Cl)(tpp)] with unsaturated fatty acids (○: OA, ■: LA, ●: LNA, and ▲: AA) against reaction time.

Detection of Peroxide Radicals Generated from Unsaturated Fatty Acids under Ambient Conditions. It has been well-known that unsaturated fatty acids, when subjected to autooxidation in air, produce peroxide radicals. The active species generated from unsaturated fatty acids were investigated by using the spin-trapping method. Nitrosodurene (3 mg) was added to neat OA (and LA), after which the solution was irradiated under degassed conditions by using a mercury lamp as a light source; then the ESR spectrum was measured. A signal with an anisotropic pattern in the high magnetic field was obtained, as is shown in Fig. 7. The ESR parameters obtained from the spectrum are a_N=1.36 mT and a_H=0.64 mT. These parameters coincide with the values of the adduct with the alkyl radicals (R·).[20)] In order to examine the effect of air, nitrosodurene dissolved in OA was irradiated under aerated condition and the ESR spectrum was measured. The spectrum showed the same radical; however, the amount of spin adducts observed was smaller than that observed in degassed conditions, as is shown in Fig. 8. This is interpreted as showing

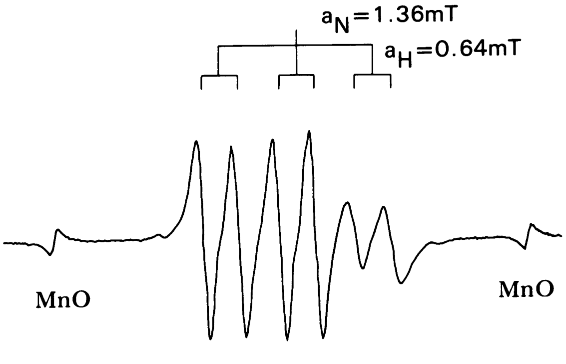


Fig. 7. ESR spectrum of spin adduct of nitrosodurene with alkyl radical of OA. MnO is internal standard signal.

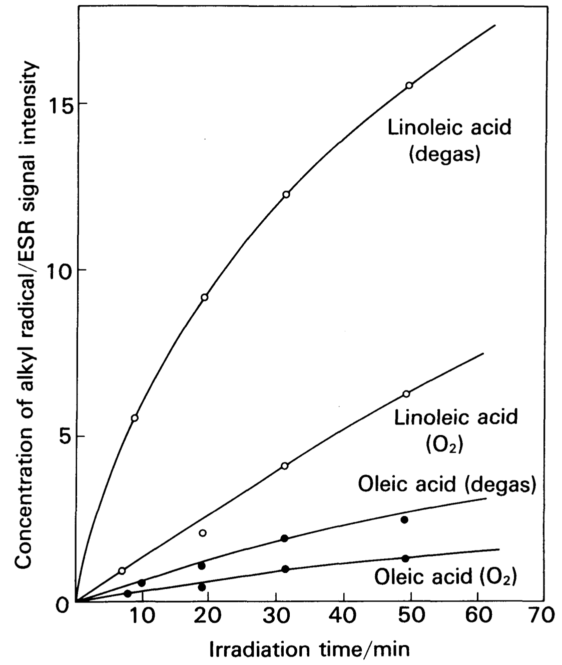


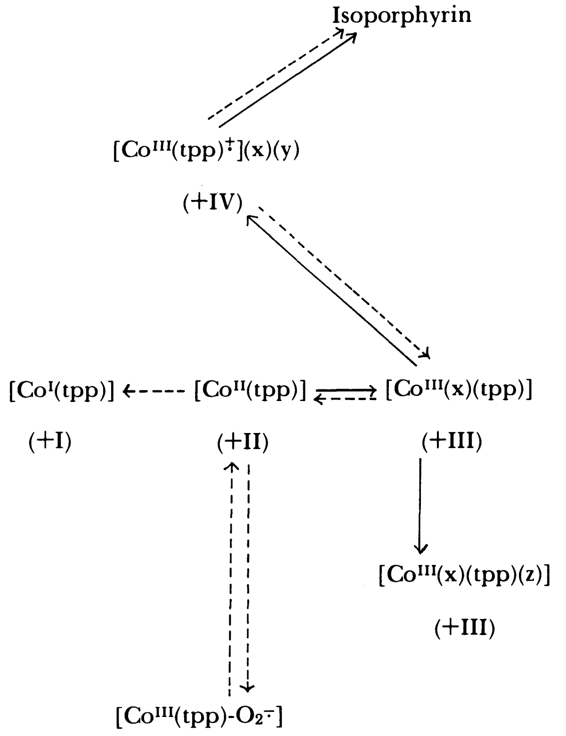
Fig. 8. Effect of oxygen molecule on concentration of formed spin adducts of nitrosodurene with alkyl radical of OA or LA in aerated and deaerated conditions.

that the alkyl radicals generated from unsaturated fatty acids combine with oxygen molecules to form stable peroxide radicals (lpox·).

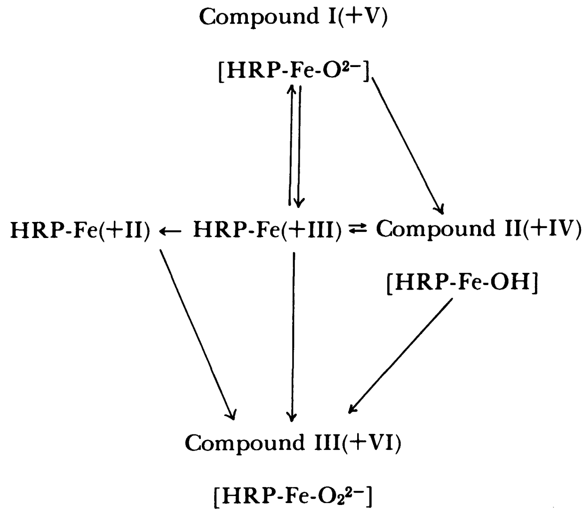
The present reactions may be summarized as follows: (1) the bpox· oxidizes both $[\mathrm{Co^{II}(tpp)}]$ and $[\mathrm{Co^{III}(Cl)(tpp)}]$ to π cation radicals; (2) the bupox· oxidizes $[\mathrm{Co^{II}(tpp)}]$ to $[\mathrm{Co^{III}(bupox)(tpp)(dbupo)}]$, while the cobalt(III) species is not subjected to any further oxidation of the bupox·; however, $[\mathrm{Co^{III}(Cl)(tpp)}]$ is oxizided to form π cation radicals; (3) the lpox· (oapox·, lapox·, lnapox·, and aapox·) oxidizes $[\mathrm{Co^{III}(Cl)(tpp)}]$, but not $[\mathrm{Co^{II}(tpp)}]$, to π cation radicals and (4) the bpox· and the lapox· further react with π cation radicals to give isoporphyrins. These results indicate that the oxidation reactions proceed depending on the oxidation potentials of both cobalt porphyrins and peroxide radicals.

Cobalt tpp and Horseradish Peroxidase(HRP)

We will now compare our results with the reaction of a typical enzyme, HRP. The function of cobalt porphyrins, $[\mathrm{Co^{II}(tpp)}]$ and $[\mathrm{Co^{III}(Cl)(tpp)}]$, and the enzymatic reaction of HRP are written in Schemes 1 and 2. The numbers in parentheses in these schemes represent the apparent oxidation states estimated on the basis of data from earlier studies.[6,7] $[\mathrm{Co^{III}(tpp)^{\dagger}}]^{2+}$ and Compound I of HRP are both π cation radicals. Here, we assume a correspondence (1) of $[\mathrm{Co^{II}(tpp)}]$ to native HRP, (2) of $[\mathrm{Co^{III}(tpp)}]^{+}$ to Compound II, and (3) of $[\mathrm{Co^{III}(tpp)^{\dagger}}]^{2+}$ to Compound I.



Scheme 1. Redox system of cobalt porphyrins. —: present oxidation process, -----: reduction and oxidation process as previously reported.[6,22]



Scheme 2. Redox system of HRP.[7]

In the HRP system, it has been known that native HRP is subjected to two-electron oxidation to produce Compound I, and also that Compound I reacts with such substrates as phenol derivatives and is reduced to native HRP through Compound II. In the present study, we found that peroxide radicals oxidize $[\mathrm{Co^{II}(tpp)}]$ to produce, successively, $[\mathrm{Co^{III}(tpp)}]^{+}$, $[\mathrm{Co^{III}(tpp)^{\dagger}}]^{2+}$, and isoporphyrin. This fact suggests that, in the system of HRP and alkyl hydroperoxide, Compound I is formed by the successive oxidation of native HRP with the peroxide radicals (·OOR).

Furthermore, Compound II has been known to be [HRP–Fe–OH] which is coordinated with the OH generated from hydroperoxide or alkyl hydroperoxide. Taking into account the fact that [Co^{II}(tpp)] is oxidized by a peroxide radical (bpox·) to produce [Co^{III}(bpox)(tpp)], it is considered that the Compound II formed in the system of HRP and alkyl hydroperoxide may be [HRP–Fe–OOR]. The formation of isoporphyrins in the reactions suggests that the degradation of HRP is caused by the peroxide radicals formed in the process of the autooxidation of lipids.

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