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Oxidation of Sesamol Dimer by Active Species produced in the Interaction of Peroxide and Hemoglobin

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Sesamol dimer (I) was converted into the quinone (II) having an absorption maximum at 550 nm by treatment with hydrogen peroxide, linoleic acid hydroperoxide or *tert*-butyl hydroperoxide in the presence of hemoglobin. The order of the color-forming activity was linoleic acid hydroperoxide≫hydrogen peroxide>*tert*-butyl hydroperoxide. Methemoglobin was much more effecive than oxyhemoglobin for coloration.

Hydrogen peroxide and methemoglobin produced a complex which generated singlet oxygen and hydroxyl radical, the latter being effective for coloration of I. The reaction of linoleic acid hydroperoxide with methemoglobin produced a complex which generated peroxy free radical and then the keto acid and singlet oxygen; the peroxy free radical was found to be the active species for oxidation of I. Sesamol dimer (I) may be useful for chromogenic assay of hydroxyl and peroxy free radicals.

Keywords——sesamol dimer; hemoglobin; oxyhemoglobin; methemoglobin; hydrogen peroxide; linoleic acid hydroperoxide; *tert*-butyl hydroperoxide; hydroxyl radical; singlet oxygen; peroxy free radical

Hydrogen peroxide and lipid hydroperoxides are physiologically important peroxides that may be derived by ordinary metabolism or lipid peroxidation. It has been demonstrated that hydrogen peroxide is converted into some kinds of active oxygen by *in vitro* interaction with hemoglobin, and these species can oxidize organic amines such as benzidine,[1-3] and phenolics such as gum guaiac[4] to produce characteristic blue- or violet-colored products. These *in vitro* reactions have been frequently applied for chromogenic detection of blood in the fields of clinical chemistry and forensic science. These reactions suggest that the interaction of peroxides with hemoglobin may produce other molecules with oxidative activity *in vivo* when they are produced in red cells. It has not, however, been clarified what kinds of oxygen are produced in the interaction of hydrogen peroxide with hemoglobin and participate in the oxidation of these substrates.

Most of the organic amines used in the chromogenic assay have been demonstrated to be carcinogenic,[2] and gum guaiac, which contains many phenolics besides guaiaconic acid as color-forming species, is less sensitive to the coloration.[4] In the previous papers,[5,6] we demonstrated that sesamol dimer (I), a phenolic compound obtained from sesame oil, was converted into a violet-colored quinone (II) by treatment with hydrogen peroxide and horseradish peroxidase, and this safe compound was found to be useful for the colorimetric assay of hydrogen peroxide. In the present work compound (I) was found to be colored by hydrogen peroxide–hemoglobin and linoleic acid hydroperoxide–hemoglobin systems. This paper describes the characteristics of the coloration of sesamol dimer (I) by these peroxide–hemoglobin systems, and what kinds of active species were produced during the interactions and participated in the coloration of sesamol dimer (I). We also discuss the relationships between the transformation of hemoglobin by these peroxides[7] and the formation of active species for coloration of sesamol dimer (I).

Experimental

Commercial reagent-grade 31% H_2O_2 was estimated to be 9.73M by iodometrc titration. *tert*-Butyl

hydroperoxide (BHPO), 70% (Nakarai Chemicals, Ltd.) was dissolved in dimethylsulfoxide for use.[7] 2,5-Diphenylfuran, nitro blue tetrazolium, D-mannose, D-mannitol, 1,1-dimethoxyethane and 1,2-dimethoxyethane were guaranteed-grade reagents obtained from Kanto Chemical Company, Ltd. 2,6-Di-*tert*-butyl-4-methylphenol (BHT) obtained from Nikki-Universal Company, Ltd. was used after recrystallization from ethanol. *cis*-Dibenzoylethylene was prepared according to the method of Lutz *et al.*[8] Catalase (bovine liver, 2000 U/mg solid) was obtained from Sigma Chemical Company, Ltd. Bovine serum albumin was a product of Tokyo Kasei Kogyo, Ltd. Sesamol dimer (I) was obtained from sesamol as described previously.[5,6] Linoleic acid hydroperoxide (LAHPO) was prepared as described.[7]

Human normal oxyhemoglobin (HbO_2) and methemoglobin (MetHb) were prepared according to the previously reported methods, and concentrations of these hemoglobins were determined on a heme basis as described.[7]

Absorption spectra were recorded with a Shimadzu UV-200S double beam spectrophotometer equipped with a thermostatic control apparatus. Thin-layer chromatography was performed on Wakogel B-5F (Wako Pure Chemical Industris, Ltd.).

Formation of the Quinone (II) from Sesamol Dimer (I) in Peroxide–Hemoglobin System——A mixture of 0.5 ml of 2.5 mM I in CH_3CN, 0.5 ml of H_2O_2, LAHPO sodium salt, BHPO solution, 0.5 ml of H_2O (or 10 mM KCN in the case of systems containing HbO_2), and 3.0 ml of 0.1 M phosphate buffer (pH 7.0) was preincubated for 5 min, then 0.5 ml of HbO_2 or MetHb solution was added. The mixtures were incubated at 37°C (H_2O_2 and LAHPO) or at 30°C (BHPO) for 10 min in the dark, then diluted with 50 ml of H_2O and extracted with 5 ml of $CHCl_3$. The chloroform layer was dried with filter paper and subjected to spectrophotometric assay at 550 nm.

Singlet Oxygen Generation in Peroxide–Hemoglobin System——A mixture of 0.5 ml of 0.1 mM diphenylfuran in CH_3CN, 0.5 ml of 50 mM H_2O_2 or 0.5 mM LAHPO sodium salt solution, 0.5 ml of H_2O and 3.0 ml of 0.1 M phosphate buffer (pH 7.0) was preincubated at 37°C for 5 min, then 0.5 ml of 5 μM MetHb solution or H_2O was added, and the decrease in absorbance at 324 nm was monitored.

Superoxide Anion Generation in Peroxide–Hemoglobin System——A mixture of 0.5 ml of 0.5 mM nitro blue tetrazolium solution, 0.5 ml of ethanol, 0.5 ml of 50 mM H_2O_2 or 0.5 mM LAHPO sodium salt solution and 3.0 ml of 0.1 M phosphate buffer (pH 7.0) was preincubated at 37°C for 5 min, then 0.5 ml of 5 μM MetHb solution was added, and the increase in absorbance at 560 nm was monitored.

Effect of Hydroxyl Radical Scavengers on the Color Formation from I——A mixture of 0.5 ml of 2.5 mM I in CH_3CN, 0.5 ml of a solution of a hydroxyl radical scavenger in H_2O, 0.5 ml of 50 mM H_2O_2 (or 2 mM LAHPO) and 3.0 ml of 0.1 M phosphate buffer (pH 7.0) was preincubated at 37°C for 5 min, then 0.5 ml of 25 μM MetHb solution was added, and the mixture was incubated at 37°C for 10 min. The solution was extracted with 5 ml of $CHCl_3$ for measuremnt of absorbance at 550 nm.

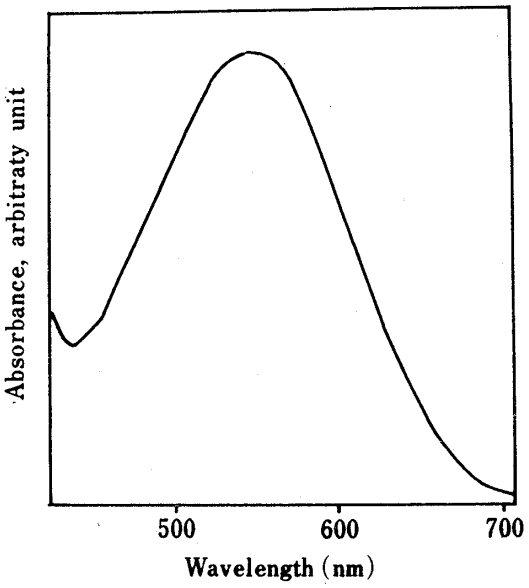

Fig. 1. Absorption Spectrum of the Chloroform Extract of the Reaction Mixture of Sesamol Dimer (I) and H_2O_2–HbO_2

A 5 ml mixture of 0.25 mM I, 0.1 M H_2O_2 and 12 μM HbO_2 in 10% EtOH–0.06 M phosphate buffer (pH 7.0) was treated at room temperature for 3 min, then the mixture was extracted with 5 ml of $CHCl_3$ for measurement of the absorption spectrum.

Results

When 0.25 mM sesamol dimer (I) was treated with 0.1 M hydrogen peroxide (H_2O_2)–12 μM oxyhemoglobin (HbO_2) system at pH 7.0 and room temperature for about 3 min, the chloroform extract of the mixture showed a violet color. The absorption spectrum of the extract exhibited the absorption maximum at 550 nm (Fig. 1). The characteristic profile of the absorption spectrum coincided with that of the quinoid compound (II) produced by reaction of I with the H_2O_2–horseradish peroxidase system.[5] When 0.25 mM I was treated

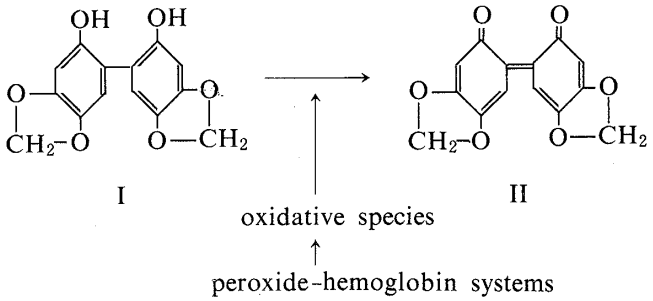

Chart 1

with various peroxides such as H_2O_2, *tert*-butyl hydroperoxide (BHPO) and linoleic acid hydroperoxide (LAHPO) in the presence of 48 μM hemoglobin [HbO_2 or methemoglobin(MetHb)], the same violet-colored product (II) was produced. It was clear that some species with oxidative activity toward I were generated by interaction of H_2O_2, BHPO or LAHPO with HbO_2 or MetHb, since these peroxides alone could not oxidize I.

In order to characterize the formation of II in each of the peroxide-hemoglobin systems, 0.25 mM I was treated with the following systems in 10% acetonitile at pH 7.0: 5 mM H_2O_2–HbO_2, 5 mM H_2O_2–MetHb, 0.2 mM LAHPO–HbO_2, 0.2 mM LAHPO–MetHb, 3.7 mM BHPO–HbO_2 and 3.7 mM BHPO–MetHb, with various amounts of hemoglobin (Fig. 2). It has been shown in the previous paper[7] that treatment of HbO_2 with these peroxides produced MetHb,

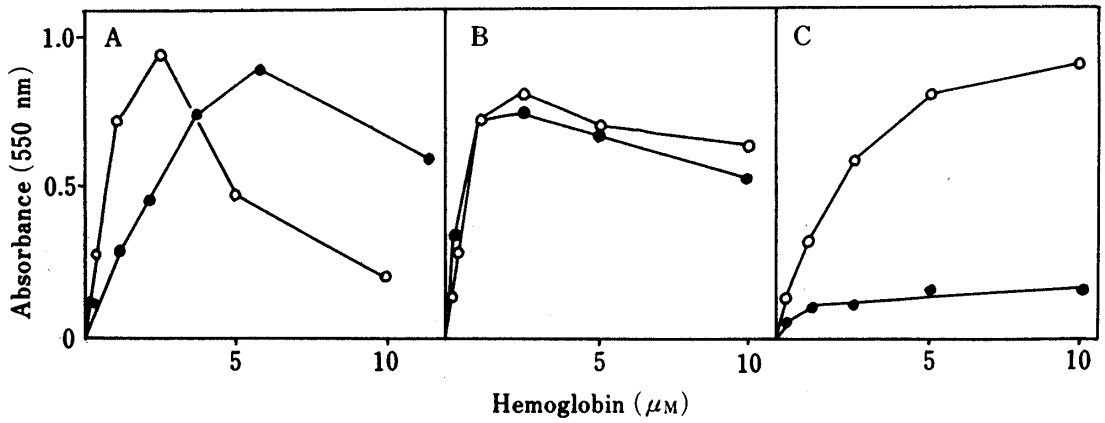

Fig. 2. Color Formation from Sesamol Dimer (I) by the Peroxide-Hemoglobin System

A 5 ml mixture of 0.25 mM I, peroxide and hemoglobin in 10% CH_3CN–0.06 M phosphate buffer (pH 7.0) was incubated at 37°C (A and B) or at 30°C (C) for 10 min, then extracted with 5 ml of $CHCl_3$ for measurement of absorbance.
A: 5 mM H_2O_2+HbO_2+1 mM KCN (●), 5 mM H_2O_2+MetHb (○).
B: 0.2 mM LAHPO+HbO_2+1 mM KCN (●), 0.2 mM LAHPO+MetHb (○).
C: 3.7 mM BHPO+HbO_2+1 mM KCN (●), 3.7 mM BHPO+MetHb (○).

which was in turn transformed into the complexes by reaction with the peroxides, and this transformation of MetHb was prevented by potassium cyanide. Potassium cyanide was added to the reaction mixtures of HbO_2 to prevent MetHb transformation. All the reactions were terminated after 10 min at 37 or 30°C, and in each case there was no further increase in absorbance on prolongation of the incubation time. The absorbance increased as the concentration of HbO_2 or MetHb increased in all the systems, but the absorbance decreased at higher concentrations of hemoglobin in the H_2O_2 and LAHPO systems. In the case of the reactions of H_2O_2, the optimal concentrations of HbO_2 and MetHb were 5.8 and 2.5 μM, respectively (Fig. 2A), and the species with oxidative activity was more effectively produced by MetHb than by HbO_2. In the case of the reactions with LAHPO, the optimal concentrations of HbO_2 and MetHb were both close to 2.5 μM (Fig. 2B), and the active species were equally well generated in these systems. The decreases in the absorbance at higher concentrations of hemoglobin indicated that the active species might be consumed by a large amount of apoprotein of hemoglobin or that the excess amount of the active species produced during the reaction destroyed the violet-colored quinone (II). In the case of BHPO systems, the color yields increased as the concentration of hemoglobin increased, and were much higher with MetHb than with HbO_2 (Fig. 2C), and the absorbances did not decrease at the higher concentrations of hemoglobin. These results suggest that the mechanisms of the color formation from I in each system were different, and that different kinds of species with oxidative activity were generated in these systems.

When 0.25 mM I was treated with various amounts of peroxides in the presence of 2.5 μM MetHb, formation of II increased as the concentration of each peroxide increased (Fig.

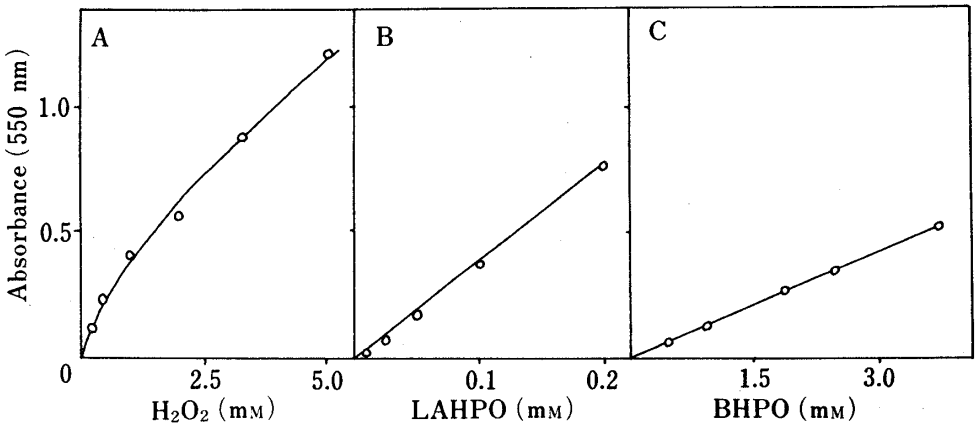

Fig. 3. Color Formation from Sesamol Dimer (I) by the Peroxide–MetHb System

A 5 ml mixture of 0.25 mM I, peroxide and 2.5 μM MetHb in 10% CH_3CN–0.06 M phosphate buffer (pH 7.0) was incubated at 37°C (A and B) or at 30°C (C) for 10 min, then extracted with 5 ml of $CHCl_3$ for measurement of absorbance.
A: H_2O_2, B: LAHPO, and C: BHPO.

3). To obtain an absorbance of 0.50, 1.4 mM, 0.14 and 3.5 mM concentrations of the peroxide were required for H_2O_2, LAHPO and BHPO, respectively. Thus, the order of the potency of color formation from I was LAHPO$\gg H_2O_2 >$BHPO in the reactions. The color yield from I in the H_2O_2–MetHb system was much less than that with H_2O_2–horseradish peroxidase;[5,6] only 70—80 μM H_2O_2 was required for coloration with an absorbance of 0.50 in the 0.3 mM I–1 μM horseradish peroxidase system.[6] The color yield in the LAHPO–MetHb system was much higher than that in the H_2O_2–MetHb system, and the plots of absorbance against the concentration of LAHPO were linear.

In order to investigate the relevance of some active species which may be released by the interaction of peroxide and hemoglobin to the coloration of I, production of active species from two selected systems, H_2O_2–MetHb and LAHPO–MetHb, was investigated. Since there is much evidence demonstrating that H_2O_2 produces various kinds of active oxygen species such as hydroxyl radical ($\cdot OH$), singlet oxygen ($^1O_2$) and superoxide anion (O_2^-) under certain conditions,[9-12] the oxidative species generated in the interaction of H_2O_2 and hemoglobin was considered to be one or more of these active oxygen species. If singlet oxygen was generated in the system, 3,5-diphenylfuran having an absorption maximum at 324 nm could be converted into *cis*-dibenzoylethylene.[3] Treatment of 10μM diphenylfuran with 5 mM H_2O_2–0.5 mM MetHb or 50 μM LAHPO–0.5 μM MetHb at pH 7.0 and at 37°C resulted in a gradual decrease of the absorbance due to diphenylfuran (Fig. 4), indicating that both systems generated singlet oxygen. The existence of superoxide anion in these systems was studied by the use of nitro

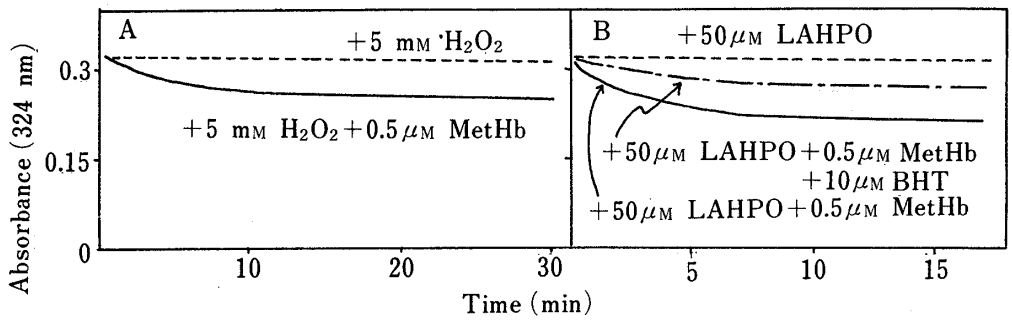

Fig. 4. Oxidation of Diphenylfuran with the H_2O_2–MetHb and LAHPO–MetHb Systems

A mixture of 10 μM diphenylfuran with one of these systems in 10% CH_3CN–0.06 M phosphate buffer (pH 7.0) was incubated at 37°C.

blue tetrazolium, which is converted by superoxide anion to the reduced form of nitro blue tetrazolium having an absorption maximum at 560 nm.[14] No increase in absorbance was observed in either system, so it is likely that superoxide anion is not produced in these systems.

These experiments showed that the H_2O_2–MetHb and LAHPO–MetHb systems generated singlet oxygen. The formation of the violet-colored quinone (II) from I in the H_2O_2–MetHb system was not, however, inhibited by singlet oxygen scavengers, diphenylfuran and L-methionine.[15] Thus, when a mixture of 0.25 mM I and 5 mM H_2O_2–2.5 μM MetHb in 10—20% acetonitrile–phosphate buffer (pH 7.0) was incubated in the presence and absence of 50 μM diphenylfuran or 10 mM L-methionine, the absorbances at 550 nm of the chloroform extract due to II were the same. Diphenylfuran was found to have been completely converted into *cis*-dibenzoylethylene, as determined by thin-layer chromatography. No participation of singlet oxygen in the coloration of I was found in other experiments. Thus, treatment of 0.25 mM I in the presence of 0.1 mM erythrosine under light for 30 min did not produce any violet-colored products, though the dye is known to catalyze the production of singlet oxygen under light.[16] It was concluded that singlet oxygen which was produced in H_2O_2–MetHb and LAHPO–MetHb systems was unable to oxidize sesamol dimer (I).

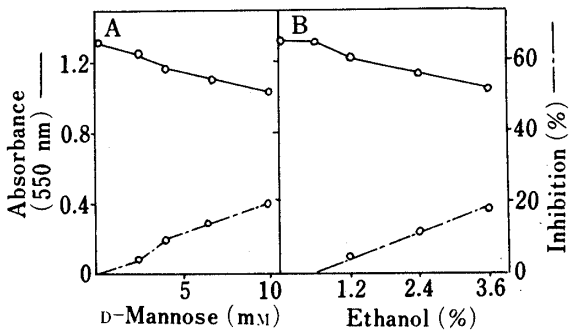

Fig. 5. Inhibition of Color Formation from Sesamol Dimer (I) by Hydroxyl Radical Scavengers

A 5 ml mixture of 0.25 mM I, 5 mM H_2O_2 and 2.5 μM MetHb in the presence of D-mannose or ethanol in 10% CH_3CN–0.06 M phosphate buffer (pH 7.0) was treated at 37°C for 10 min, then extracted with 5 ml of $CHCl_3$ for measurement of absorbance.

When hydroxyl radical scavengers such as ethanol and D-mannose[15] were added to the I–H_2O_2–MetHb system, formation of the violet-colored quinone (II) was suppressed (Fig. 5). The inhibitory effect was dependent on the concentration of the scavengers and the inhibition of quinone formation in the 0.25 mM I–5 mM H_2O_2–2.5 μM MetHb system was 20% in the presence of 3.6% ethanol or 10 mM D-mannose.

Other hydroxyl radical scavengers such as D-mannitol, formic acid, 1,1-dimethoxyethane and 1,2-dimethoxyethane[17] also inhibited the reaction (Table I). These results demonstrate that the oxidative species toward I in the H_2O_2–MetHb system may be hydroxyl radical.

TABLE I. Effect of Hydroxyl Radical Scavengers on Color Formation from Sesamol Dimer (I)

Scavenger	Concentration	Absorbance at 550 nm	Inhibition(%)
None		0.510	0
D-mannitol	10 mM	0.240	53
	1 mM	0.340	33
Formic acid	10 mM	0.320	37
	1 mM	0.367	28
1, 1-Dimethoxyethane	2.8 %	0.347	32
1, 2-Dimethoxyethane	2.8 %	0.330	35

The system containing I–LAHPO–MetHb was tested in the presence of catalase or hydroxyl radical scavengers, but no significant inhibitory effects of catalase or hydroxyl radical scavengers on the coloration were observed. Thus, the color yield from a mixture of 0.2 mM I–0.2 mM LAHPO–2.5 μM MetHb–3.6% ethanol(or 10 mM D-mannose, or1000 units of catalase) was the same as that from the mixture in the absence of these hydroxyl scavengers or catalase.

As described above (Fig. 4B), the LAHPO–MetHb system consumed diphenylfuran, and diphenylfuran was converted into *cis*-dibenzoylethylene as determined by thin-layer

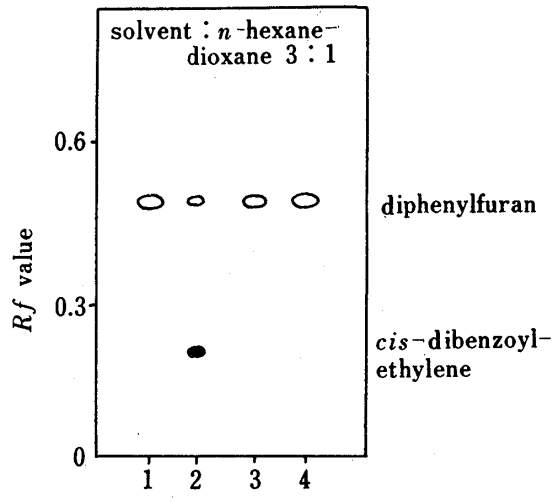

Fig. 6. Thin-Layer Chromatography of the Chloroform Extracts of the Reaction Mixtures of Diphenylfuran-LAHPO-MetHb

Diphenylfuran (50 μM) was treated with (1) 0.2 mM LAHPO, (2) 0.2 mM LAHPO and 2.5 μM MetHb, (3) 0.2 mM LAHPO, 2.5 μM MetHb, and 0.25 mM I, (4) 0.2 mM LAHPO, 2.5 μM MetHb and 0.1 mM BHT, in 20% CH_3CN–0.06 M phosphate buffer (pH 7.0) at 37°C for 40 min. ●, UV-absorbing spot irradiated at 254 nm; ○, fluorescent spot irradiated at 365 nm.

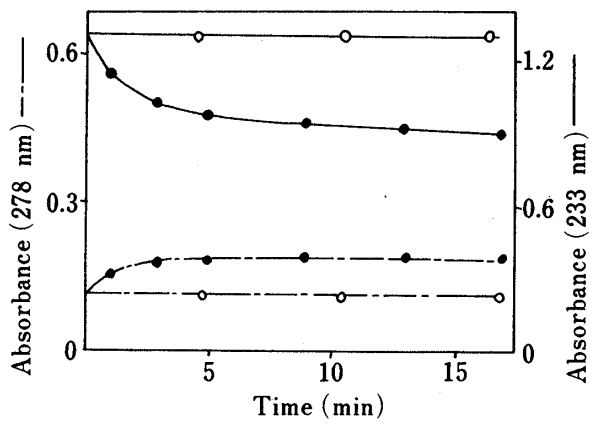

Fig. 7. Decomposition of LAHPO by MetHb

LAHPO (50 μM) was treated without (○) or with 0.5 μM MetHb (●) in 10% CH_3CN–0.06 M phosphate buffer (pH 7.0) at 37°C.

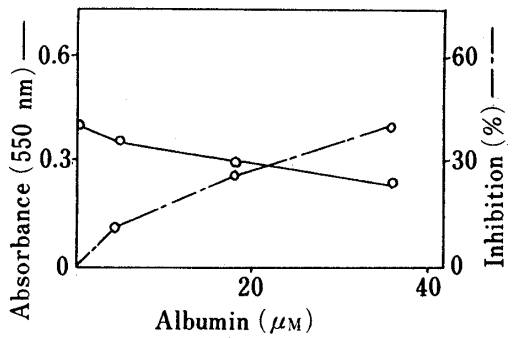

Fig. 8. Inhibition of Color Formation of Sesamol Dimer (I) by Albumin

I, 0.25 mM, was treated with 0.2 mM LAHPO and 2.5 μM MetHb in the presence of albumin in 5.0 ml of 10% CH_3CN–0.06 M phosphate buffer (pH 7.0) at 37°C for 10 min and the mixtures were extracted with $CHCl_3$ for measurement of absorbance.

chromatography (Fig. 6, lane 2). The addition of I or di-*tert*-butyl-methylphenol (BHT) prevented the transformation of diphenylfuran (Fig. 6, lanes 3 and 4), indicating that I and BHT suppressed the generation of singlet oxygen. Thus, inhibition of the generation of singlet oxygen by I in the LAHPO-MetHb system may be significant, in contrast to the observation that I did not prevent the generation of singlet oxygen in the H_2O_2–MetHb system.

Sesamol dimer (I) and BHT seem to scavenge some species with oxidative activity being transformed into oxidized products, and thus inhibit the generation of singlet oxygen.

In order to examine how LAHPO was derivatized by MetHb under these conditions, the time courses of the absorbance at 233 nm due to LAHPO (LOOH)[18] and that at 278 nm due to the keto form (LO)[19] were followed (Fig. 7). LAHPO (LOOH) gradually decreased and the keto form (LO) gradually increased in the presence of MetHb. Thus, it seemed that LAHPO(LOOH) was decomposed by MetHb into the keto form (LO) with the generation of singlet oxygen. It has been generally recognized that BHT reacts with peroxy free radical and inhibits the propagation of olefin and lipid peroxidation.[20-22] Sesamol dimer (I) and BHT might scavenge the peroxy free radical (LOO·) and thus inhibit the formation of singlet oxygen, as has been demonstrated in earlier studies,[19,23-26] during which I and BHT may be converted to the oxidized form.

When albumin was added to the reaction system of I-LAHPO-MetHb, the protein effectively prevented the color formation from I (Fig. 8). In the previous paper,[7] it was suggested that the interaction of LAHPO with MetHb first produced the complex which regenerated MetHb and produced LOO· peroxy free radical, a denaturant of hemoglobin, and albumin inhibited the formation of the complex and thus LOO· peroxy free radical. Inhibition of oxidation of I by albumin in the LAHPO-MetHb system could thus be

explained by the inhibition of formation of the complex and consequently LOO· peroxy free radical. These results demonstrate that LAHPO (LOOH) first produced LOO· peroxy free radical, which can oxidize I and which transforms into the keto form (LO) and singlet oxygen in the absence of I.

Discussion

Coloration Characteristics of Sesamol Dimer (I) in Peroxide–Hemoglobin Systems

It has previously been shown that sesamol dimer (I) produces a violet-colored quinone (II) on treatment with H_2O_2–horseradish peroxidase.[5,6] The dimer (I) is very sensitive to this treatment and was found to be useful for chromogenic assay of H_2O_2 in foodstuffs.[6] In the present experiments, we characterized the coloration of I in the H_2O_2–, LAHPO– and BHPO–hemoglobin systems. The absorption spectra of the product revealed that the quinone (II) was also produced in these systems. The color-forming activity of MetHb was much larger than that of HbO_2 in the H_2O_2– and BHPO–systems, and the order of the potency was LAHPO$\gg H_2O_2>$BHPO in MetHb systems. The color yield from I in the H_2O_2–MetHb system was much less than that in the H_2O_2–horseradish peroxidase system,[5] but that in the LAHPO–MetHb system was much greater than that in the H_2O_2–MetHb system.

Generation of Oxidative Species in H_2O_2– and LAHPO–MetHb Systems

Previously it was demonstrated that H_2O_2 converted MetHb into a complex which readily regenerated MetHb.[7] The present experiments showed that the H_2O_2–MetHb system generates both singlet oxygen and hydroxyl radical. MetHb was transformed into the complex by H_2O_2, which in turn regenerated MetHb and produced singlet oxygen and hydroxyl radical; the latter may be involved in the oxidation of I into the violet-colored quinone (II). The interaction of H_2O_2 with MetHb may be rationalized as shown in the following chart:

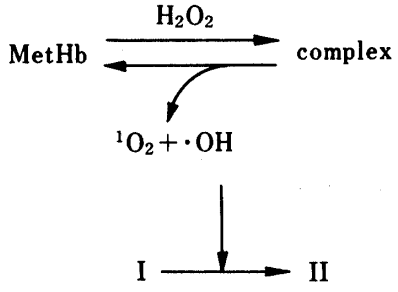

Chart 2

In the conversion of HbO_2 into MetHb by H_2O_2,[7] the violet-colored quinone (II) was also formed from I, although its efficiency of formation was much lower than in the case of MetHb. A species with oxidative activity, such as hydroxyl radical, may also be produced during the interaction of H_2O_2 with HbO_2, but generation of the species did not seem to involve the further derivation of MetHb, since MetHb was trapped by potassium cyanide.

HbO_2 and DeoxyHb treated with LAHPO produced MetHb, which was in turn converted into the complex and then into precipitates; the formation of precipitates was explained by the production of active species such as LOO· peroxy free radical.[7] Earlier experiments[19,23–26] suggested that the reaction of LAHPO with MetHb produces LOO· peroxy free radical, which yields the keto acid (LO), the alcohol (LOH) and singlet oxygen. The present experiments demonstrated that the reaction of LAHPO with MetHb produces a complex[7] whose degradation involves the formation of some oxidative species such as LOO· peroxy free radical and then the keto acid and singlet oxygen. Production of singlet oxygen was prevented by phenolic antioxidants such as sesamol dimer (I) and BHT, probably because the phenolics

donate hydrogen to the LOO· peroxy free radical to form LAHPO (LOOH). The inhibition by albumin of the color formation from I in the LAHPO–MetHb system may be explained by the inhibition of formation of the complex and thus of generation of LOO· peroxy free radical.[7] The species with oxidative activity toward I was concluded to be LOO· peroxy free radical, and the reaction scheme for the coloration of I in the LAHPO–MetHb system may be as shown in the following chart:

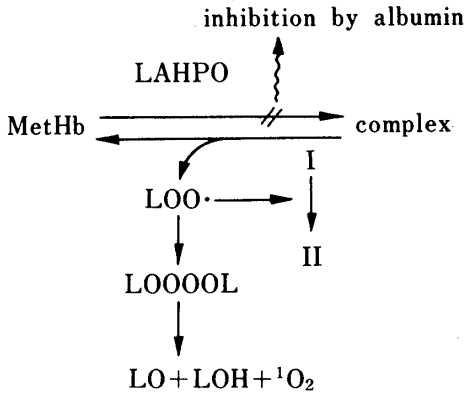

Chart 3

In the LAHPO–HbO_2 system, a potent active species was also generated, but the mechanisms of production of the active species seemed not to involve MetHb, since conversion of MetHb into the complex was blocked by potassium cyanide.

Sesamol Dimer (I) as a Hydroxyl and Peroxy Free Radical Detector

Sesamol dimer (I) was found to be oxidized into the violet-colored quinone (II) by hydroxyl and peroxy free radicals and is effective for the chromogenic detection of these radicals. Gum guaiac is well known to be colored blue by these oxidative species produced by the interaction of H_2O_2–hemoglobin or H_2O_2–peroxidase. The substrate in gum guaiac was elucidated to be α-guaiaconic acid, which is converted into guaiacum blue, having a highly conjugated bismethylene quinoid structure.[4] However, gum guaiac is less sensitive to coloration, probably because it contains many phenolics other than the substrate which can reduce the colored quinone. The high sensitivity of sesamol dimer (I) to these active species seems to be due to its purity. Sesamol dimer (I) is derived from the natural oil[15] and is considered to be safe. This sensitive and apparently innocuous phenolic may be useful for chromogenic assay of hydroxyl and peroxy free radicals. This compound was found to be superior to organic amines such as benzidine[1-3] for the safe chromogenic assay of H_2O_2, hemoglobin and peroxidase.

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