# Toxicity of Singlet Oxygen Generated Thermolytically in *Escherichia coli*

Tetsuo NAGANO, Takako TANAKA, Hitomo MIZUKI, and Masaaki HIROBE*

*Faculty of Pharmaceutical Sciences, University of Tokyo, Hongo, Bunkyo-ku, Tokyo 113, Japan.*
Received September 1, 1993; accepted December 11, 1993

**Three water-soluble derivatives of naphthalene endoperoxide were prepared to examine the toxicity of singlet oxygen towards *Escherichia coli*. On incubation without irradiation, these endoperoxides produce singlet oxygen thermolytically in a dose-dependent manner. The amount of singlet oxygen produced per unit time can be controlled by varying both the incubation temperature and the number of methyl substituents of the naphthalene endoperoxide derivatives. 3-(1,4-Dihydro-1,4-epidioxy-4-methyl-1-naphthyl)propionic acid (EP-1), one of the derivatives, inhibited *E. coli* growth dose- and incubation temperature-dependently and caused *E. coli* lethality. Furthermore, EP-1 did not induce superoxide dismutase or catalase in *E. coli* even when the cells were incubated in nutritionally rich medium containing trypticase/soy/yeast extract. Singlet oxygen, one of the reactive oxygen species, did not act as a signal for induction of superoxide dismutase and catalase, in contrast to the actions of superoxide and hydrogen peroxide.**

**Keywords** naphthalene endoperoxide; singlet oxygen; *Escherichia coli*; superoxide dismutase; thermolysis

Much interest has been focused on the reactivities of singlet oxygen ($^1O_2$) not only in the field of organic chemistry, but also in that of biological chemistry.[1] Some attempts have been made to characterize the toxic action of $^1O_2$ on living cells, generally by using a class of dyes known as "photodynamic sensitizers."[2-4] Human porphyrias,[5] which are caused by defects in the metabolism of blood porphyrins, are associated with photosensitivity and $^1O_2$ is believed to be the active damaging species, formed through energy transfer from photoexcited protoporphyrin to the ground state of dioxygen ($^3O_2$).

Some $^1O_2$-generating reactions are known, such as NaOCl–$H_2O_2$ and photosensitizer–light–$O_2$,[6] but these reaction systems often generate other reactive species whose toxicities may be difficult to distinguish from those of $^1O_2$ in living cells. Photosensitizers often form complexes with biological components, and then react with $^1O_2$. In photodynamic systems it is difficult to establish to what extent an effect can be ascribed to $^1O_2$ itself.[7-10] Thus, results in a photosensitizer–light–$O_2$ system should be interpreted with care.[11,12] Also, the NaOCl–$H_2O_2$ system may not be appropriate for accurate evaluation of toxicity of $^1O_2$ towards living cells, because $H_2O_2$ itself is a reactive oxygen species that does severe damage to cells.[13-15]

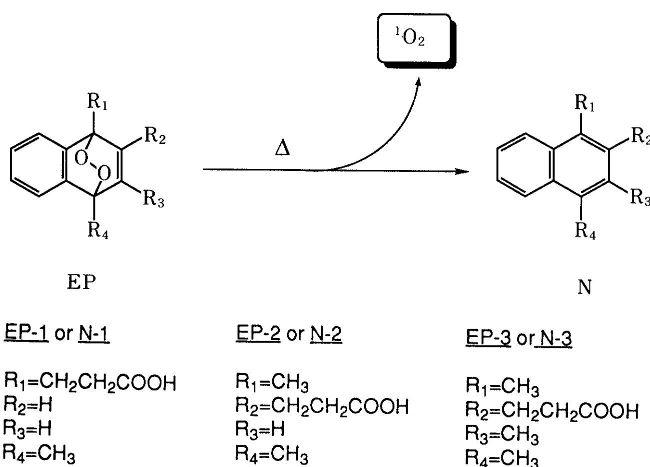


Chart 1. Water-Soluble Singlet Oxygen ($^1O_2$)-Generating System

Saito *et al.*[16] have synthesized a novel water-soluble naphthalene endoperoxide (EP-1), which produces $^1O_2$ thermolytically, not photochemically. The reaction scheme is shown in Chart 1. The amounts of $^1O_2$ production per unit time can be controlled by varying the incubation temperature and methyl substitution. The $^1O_2$-generating system using EPs appears to provide significant advantages for elucidation of $^1O_2$ toxicity. Our interest was focused on the toxicity of $^1O_2$ generated from EPs to *Escherichia coli* in terms of growth and survival, compared with that of superoxide.[17]

In this paper, we report that serious damage was done to *E. coli* cells by $^1O_2$ generated from various naphthalene endoperoxides (EPs) without induction of superoxide dismutase, in contrast to the case of superoxide.[17-26] Furthermore, the toxicity was well correlated with the amount of $^1O_2$ produced.

## Experimental

**Materials** *Escherichia coli* B $B_{12}^-$ (ATCC 29682) was used throughout. Bactotryptone, bactoagar, bacto yeast extract and bacto tryptic soy broth were purchased from Difco. Superoxide dismutase (SOD), catalase (CAT), alkaline phosphate, xanthine and xanthine oxidase were obtained from Sigma Chemical Co. Other chemicals were obtained from Tokyo Chemical Co.

**Preparation of EP-1** The water-soluble naphthalene endoperoxide EP-1 was prepared from the corresponding naphthalenes N-1 by a modification of Saito's method. The purity of EP-1 (92%) was measured by KI titration.

**Preparation of N-2 and N-3** The compounds N-2 and N-3 were prepared according to Fieser's method. N-2: pale yellow needle (recrystallized from benzene), mp 125—126 °C. $^1$H-NMR ($CDCl_3$/DMSO) δ: 2.56 (t, $J$=7.8 Hz, 2H, –$CH_2$–), 2.60 (s, 6H, Me), 3.07 (t, $J$=7.8 Hz, 2H, –$CH_2$–), 7.15 (s, 1H, aromatic H), 7.42—7.50 (m, 2H, aromatic H), 7.96 (s, 2H, aromatic H). *Anal.* Calcd for $C_{15}H_{16}O_2$: C, 78.92; H, 7.06. Found: C, 78.73; H, 7.10. MS $m/z$: 228 ($M^+$). N-3: yellow powder (recrystallized from benzene), mp 118—120 °C. $^1$H-NMR ($CDCl_3$/DMSO) δ: 2.43 (s, 3H, Me), 2.55 (m, 5H, –$CH_2$–, Me), 2.60 (s, 3H, Me), 2.70 (t, $J$=7.8 Hz, 2H, –$CH_2$–), 7.25—7.35 (m, 2H, aromatic H), 7.88—7.92 (m, 2H, aromatic H). *Anal.* Calcd for $C_{16}H_{18}O_2$: C, 79.31; H, 7.49. Found: C, 79.07; H, 7.46. MS $m/z$: 242 ($M^+$).

**Preparation of EP-2** A solution of 3-(1,4-dimethyl-2-naphthyl)propionic acid (N-1) (500 mg, 2.2 mmol) in the presence of methylene blue (2.5 mg, 8 μmol) in dichloromethane (30 ml) was irradiated with two

© 1994 Pharmaceutical Society of Japan

NII-Electronic Library Service

110 W tungsten–bromine lamps at 0 °C under dioxygen bubbling for 3 h. After removal of the solvent at 0 °C, the residue was purified by rapid column chromatography (silica gel, precooled $CH_2Cl_2$) to give a pale yellow solid which contained EP-2 and a trace amount of N-2 (less than 1%). KI titration 99% <. Yield, 96%. $^1$H-NMR ($CDCl_3$) $\delta$: 1.85 (s, 6H, Me), 2.55 (t, $J$=7.8 Hz, 2H, $-CH_2-$), 2.70 (t, $J$=7.8 Hz, 2H, $-CH_2-$), 6.7 (s, 1H, $-C=CH-$), 7.2—7.4 (m, 4H, aromatic H), 9.2 (br s, 1H, COOH).

**Preparation of EP-3** EP-3 was synthesized by the same method as EP-2, except using N-3 in place of N-2. Yield, 95%. $^1$H-NMR ($CDCl_3$) $\delta$: 1.85 (s, 9H, Me), 2.4—2.6 (m, 4H, $-CH_2CH_2-$), 7.1—7.2 (m, 4H, aromatic H), 9.2 (br s, 1H, COOH).

**Half-Lives of EPs by Thermolysis** EPs were prepared from the corresponding naphthalenes (Ns) by irradiation in the presence of methylene blue[27]; the reactions could be monitored by following the disappearance of the absorption at 289 nm owing to the formation of the endoperoxide groups. Warming the solution to 37—70 °C regenerated the absorption of the naphthalene chromophore at 289 nm. The half-lives of EPs were obtained from the spectral change.

**Reaction of EPs with Potassium Rubrenetetracarboxylate (RTC)** $^1O_2$ produced from EP-1 reacted with RTC to form the stable endoperoxide, RTCEP. A typical procedure was as follows. EP-1 (4.8 mmol) was added to a 50 mM potassium phosphate buffer (pH 7.4) solution of RTC (0.24 mmol)[28] in a cuvette at room temperature, which was placed in a spectrophotometer to follow the reaction at 537 nm. The relative rate of $^1O_2$ production could be estimated from the spectral change owing to this reaction. The $^1O_2$ scavenger, $NaN_3$, was added at various concentrations together with EP-1 to the buffer solution containing RTC. When the effect of $D_2O$ was to be examined, pD was adjusted to 7.0.

**Growth of *E. coli*** *E. coli* was cultured at 37 or 25 °C in a water-bath shaker at 100 rpm with a ratio of flask volume of 5 : 1. Glucose minimal (GM) medium contained, per liter, $MgSO_4 \cdot 7H_2O$, 0.2 g; citric acid $\cdot H_2O$, 2.0 g; $K_2HPO_4$, 10.0 g; $NaNH_4HPO_4 \cdot 4H_2O$, 3.5 g; vitamin $B_{12}$, 1.0 mg; and glucose, 5.0 g. Growth was monitored in the presence or in the absence of EPs or Ns at the concentrations indicated in Fig. 1 in terms of changes in turbidity at 600 nm using a test tube (diameter = 15 mm) attached to the flask with a Coleman Junior II spectrophotometer.

*E. coli* was grown at 37 °C with or without EP-1 or N-1 in trypticase/soy/yeast extract (TSY) medium to examine the specific activities of SOD and CAT in *E. coli*. TSY medium contained 3% trypticase soy broth and 0.5% yeast extract. Cultures were grown for 22 h, harvested at 20000 *g* for 5 min at 4 °C, washed three times with 50 mM potassium phosphate, 0.1 mM EDTA (pH 7.8), and then suspended in 0.5—2.0 ml of this buffer. Washed cells were lysed by two passages through a French pressure cell at 20000 lb/in$^2$. Lysates were clarified at 13000 *g* for 10 min at 4 °C.

SOD and CAT activities were assayed as previously described.[29,30] Protein was estimated by the Lowry procedure[31] with bovine serum albumin (Sigma Chemical Co.) as a standard.

**Lethality of *E. coli*** *E. coli* was grown in medium containing $KH_2PO_4$, 0.7%; $K_2HPO_4$, 0.3%; sodium citrate, 0.5%; $(NH_4)_2SO_4$, 0.1%; $MgSO_4 \cdot 7H_2O$, 0.1%; and glycerol as a carbon source, 1% at pH 7.0. The washing was carried out by centrifugation for 5 min at 0 °C using a total volume of 5 ml/wash. The washed cells were suspended in phosphate buffer (1 mM, pH 7.4) containing glucose (0.5% (w/v)) and $MgSO_4$ (1 mM) to a density of $1 \times 10^9$ cells/ml. Lethality of cells was determined according to the following procedure: the total volume of each system was 1 ml, containing $1 \times 10^7$ cells. Samples from the reaction mixture including EP-1 or N-1 were taken at various times and were diluted with a phosphate buffer (1 mM pH 7.4). The samples were additionally diluted in this manner two more times to reach a dilution factor of $10^3$—$10^6$. Viable cells were enumerated by spreading appropriate dilutions in quadruplicate on 1% bactotrytone (Difco)/0.5% NaCl medium solidified with 2% agar (Difco) and counting colonies after 24 h at 37 °C.

## Results

**Effect of EPs on *E. coli* Growth** *E. coli* was exposed to a wide concentration range of various endoperoxides. As shown in Fig. 1a, EP-1 at 0.5—2 mM completely inhibited the growth in GM medium, and at 0.1 mM it affected the postlag growth rate. Though EP-1 produced a dose-dependent inactivation, the parent compound, the corresponding naphthalene derivative (N-1), failed to inhibit *E. coli* growth. EP-2 was less effective than EP-1 (Fig. 1b) and EP-3 was completely ineffective even at 2 mM (Fig. 1c).

EPs were converted to the corresponding naphthalenes (Ns) without side reactions by warming the solution and the half-lives at each temperature were obtained from the progressive UV spectral change. Figure 2 shows the spectral change in the case of EP-2 at 45 °C; the final UV spectrum was identical with that of N-2.

The order of the growth-inhibiting effect of EPs correlated with that of the half-lives of EPs (Table I). From a comparison of Fig. 3 with Fig. 1a, EP-1 was less toxic when *E. coli* was incubated at 25 °C than at 37 °C. This can be explained in terms of the half-lives for thermolysis of EP-1 at 25 and 37 °C. The half-life of EP-1 at 25 °C was about five times as long as that at 37 °C, which indicates that $^1O_2$ production per minute at 25 °C is much less than at 37 °C.

We estimated the amounts of $^1O_2$ produced per unit time from the oxidation of RTC to the endoperoxide (RTCEP) by EP-1 at various temperatures (Chart 2). The reaction was followed in terms of diminution of the visible

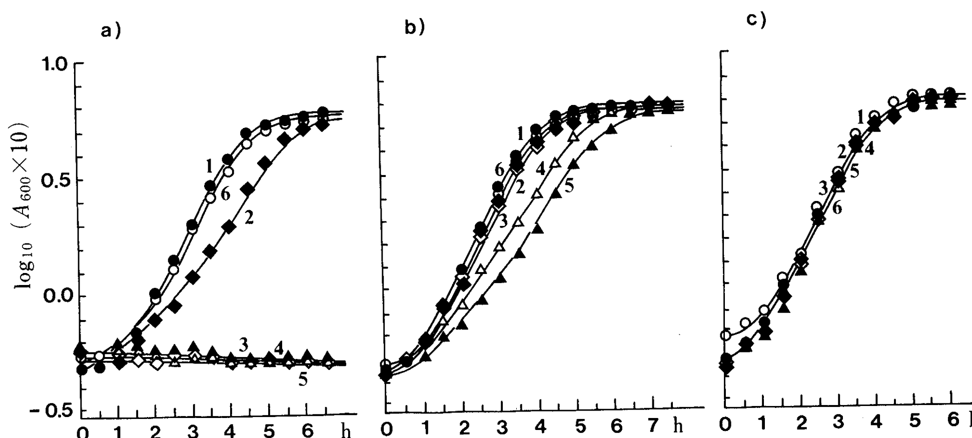


Fig. 1. Effect of EPs and Ns on Growth of *E. coli*

Growth was monitored in terms of absorbance at 600 nm over a period of 6 or 7 h at 37 °C. a) Line 1, no additive; line 2, 0.1 mM EP-1; line 3, 0.5 mM EP-1; line 4, 1 mM EP-1; line 5, 2 mM EP-1; line 6, 2 mM N-1. b) Line 1, no additive; line 2, 0.1 mM EP-2; line 3, 0.5 mM EP-2; line 4, 1 mM EP-2; line 5, 2 mM EP-2; line 6, 2 mM N-2. c) Line 1, no additive; line 2, 0.1 mM EP-3; line 3, 0.5 mM EP-3; line 4, 1 mM EP-3; line 5, 2 mM EP-3; line 6, 2 mM N-3.

NII-Electronic Library Service

absorption at 537 nm, which was inhibited by $NaN_3$ as an $^1O_2$ scavenger and enhanced by using $D_2O$ instead of $H_2O$ (Table II). $^1O_2$ is known to have a longer life-time in $D_2O$ than in $H_2O$.[32]

It was found using this system that EP-1 produced about eight times as much $^1O_2$ at 37 °C as at 25 °C. This supports the conclusion that the toxicity of EPs to *E. coli* is due to $^1O_2$.

**Effect of EPs on Specific Activities of SOD and CAT in *E. coli*** Hassan and Fridovich[33] have reported that paraquat induces SOD and CAT in *E. coli* grown in nutritionally rich medium. Paraquat is one-electron-reduced to the cation radical by NAD(P)H enzymatically in living cells. The cation radical reacts rapidly with dioxygen to form superoxide, which is an inducer of SOD and CAT. In view of the possible relation of $^1O_2$ with SOD, it is of interest to determine its SOD-inducing ability. Table III shows the level of specific activities of SOD and CAT in *E. coli* grown in TSY medium over a range of concentrations of EP-1 and with N-1 at 2 mM. Neither EP-1 nor N-1 caused an increase of specific activities of SOD and CAT in the cells. Thus, $^1O_2$ was shown not to act as a signal for induction of SOD and CAT, being different in this respect from superoxide.

**Lethality of *E. coli* Induced by EP-1** Figures 4 and 5 illustrate the effect of EP-1 on survival of *E. coli*. EP-1 had a lethal effect on *E. coli* in a dose-dependent manner, while N-1 had no such effect. EP-2 and EP-3, which

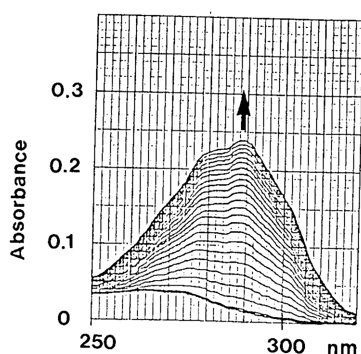


Fig. 2. Progressive UV Spectral Change during the Thermolysis of EP-2 at 45 °C

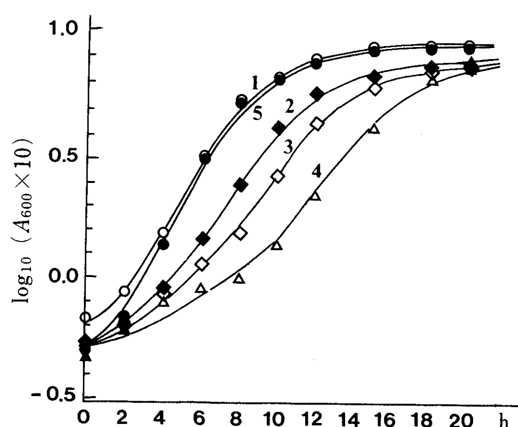


Fig. 3. Effect of Incubation Temperature on EP-1 Toxicity

Growth was monitored in terms of absorbance at 600 nm over a period of 20 h at 25 °C. Line 1, no additive; line 2, 0.1 mM EP-1; line 3, 0.5 mM EP-1; line 4, 1 mM EP-1; line 5, 2 mM N-1.

TABLE I. Half-Lives for Thermolysis of Naphthalene Endoperoxides (EPs)[a] ($T_{1/2}$, min)

| Compd. | 25 °C | 37 °C | 45 °C | 55 °C | 70 °C |
|---|---|---|---|---|---|
| EP-1 | 290 | 58 | 20 | 8 | n.d. |
| EP-2 | n.d. | 480 | 165 | 45 | n.d. |
| EP-3 | n.d. | n.d. | n.d. | 3000 | 420 |

*a*) Determined from appearance of absorbance ($A_{289}$) due to naphthalene formation. n.d.: not determined.

TABLE II. Effect of Sodium Azide ($NaN_3$) and $D_2O$ on Bleaching of Rubrenetetracarboxylate (RTC)

| Run | EP-1 (mM) | Solvent | Additive | Relative rate (%)[a] |
|---|---|---|---|---|
| 1 | 1.6 | $H_2O$ | None | 100 |
| 2 | 1.6 | $H_2O$ | 0.5 mM $NaN_3$ | 32 |
| 3 | 1.6 | $H_2O$ | 1.0 mM $NaN_3$ | 29 |
| 4 | 1.6 | $H_2O$ | 10.0 mM $NaN_3$ | 0 |
| 5 | 1.6 | $D_2O$ | None | 211 |

*a*) 100% corresponds to the formation of 0.26 mM/min of RTC.

TABLE III. Effect of EP-1 on Superoxide Dismutase (SOD) and Catalase (CAT) Specific Activities in *E. coli*

| | EP-1 (mM) | | | | | N-1 (mM) |
|---|---|---|---|---|---|---|
| | 0 | 0.1 | 0.5 | 1.0 | 2.0 | 2.0 |
| SOD (U/mg protein) | 52 | 51 | 49 | 45 | 48 | 42 |
| CAT (U/mg protein) | 40 | 30 | 29 | 52 | 54 | 48 |

Cells were grown for 6 h at 37 °C in TSY medium at each level of EP-1 and with 2 mM N-1 prior to assay for SOD and CAT.

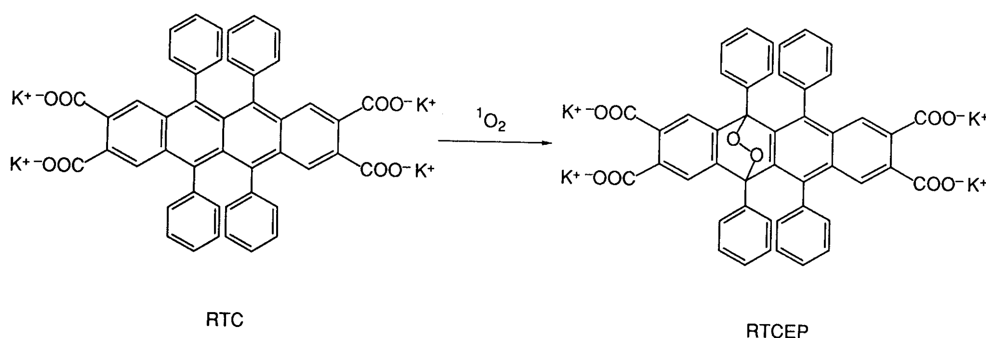


Chart 2. Reaction of Potassium Rubrenetetracarboxylate (RTC) with Singlet Oxygen

NII-Electronic Library Service

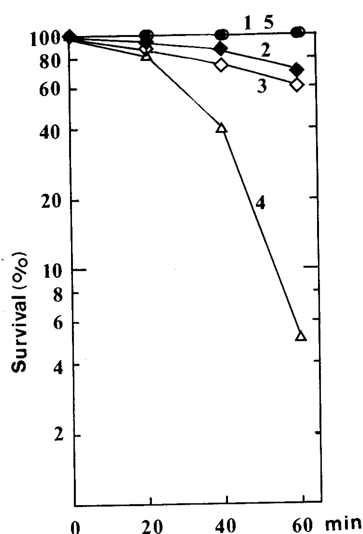


Fig. 4. *E. coli* Survival Curves on Treatment with Buffer Containing EP-1 or N-1

Line 1, no additive; line 2, 0.5 mM EP-1; line 3, 0.75 mM EP-1; line 4, 1.0 mM; line 5, 1.0 mM N-1.

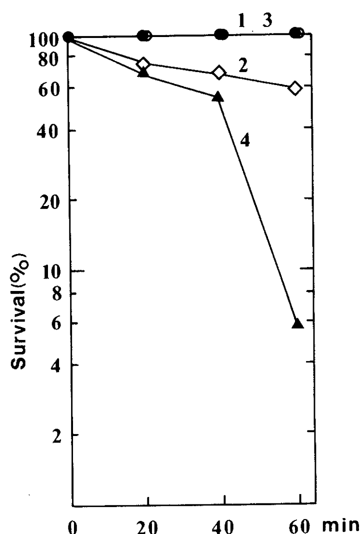


Fig. 5. Acceleration of Inactivation of *E. coli* in Deuterated Water ($D_2O$)

Line 1, no additive in $H_2O$; line 2, 0.75 mM EP-1 in $H_2O$; line 3, no additive in $D_2O$; line 4, 0.75 mM EP-1 in $D_2O$.

produce little $^1O_2$ at 37 °C, had no lethal effect at 1 mM (data not shown).

Furthermore, we examined the effect of $D_2O$ on lethality of EP-1 to *E. coli*. $D_2O$ markedly enhanced the toxicity of EP-1 (Fig. 5), while in the absence of EP-1, $D_2O$ had no toxic effect on *E. coli*. These results also support the view that EP-1 toxicity to *E. coli* is caused by $^1O_2$ produced thermolytically.

## Discussion

$^1O_2$ is one of the reactive oxygen species, which include superoxide, hydrogen peroxide, hydroxyl radical, metal-oxygen complex, *etc*. The reactivity of $^1O_2$ is very different from those of other reactive oxygen species and $^1O_2$ may cause oxidative damage to biological components such as nucleic acids, lipid, amino acids and hydrocarbons regio- and/or stereo-selectively, not at random. Nieuwint *et al.*[34,35] found, using EP-1, that $^1O_2$ is capable of causing not strand breakage of plasmid DNA from *E. coli* but damage to the base or sugar moieties of DNA. On the other hand, Sies *et al.*[36] have reported that thermolytically generated $^1O_2$ causes bacteriophage DNA strand breakage. They explained this difference as possibly due to a difference in the initial level of the open circular form in the DNA preparation employed. Raetz *et al.*[37,38] have reported that plasmalogens, a unique class of glycerophospholipids, can react selectively with $^1O_2$. There is much interest in the reactions of $^1O_2$ itself with biological components under physiological conditions.

In this paper, we showed that $^1O_2$ does serious damage to *E. coli* cells dose-dependently, though the damaged site has not yet been identified. Further studies on the nature of the toxicity caused by $^1O_2$ itself are in progress, though lipid membranes are probably not the target because *E. coli* membranes contain mainly phosphatidylethanolamines, which are not readily oxidized.

Furthermore, $^1O_2$ production by some biological systems has recently been reported[39,40] and EPs may be useful for elucidation of the possible biological action of $^1O_2$ as a signal, *e.g.*, *via* production of nitric oxide.

**Acknowledgement** This work was supported by a Grant-in-Aid for Scientific Research on Priority Areas (No. 04225107) from the Ministry of Education, Science and Culture, Japan.

### References and Notes

1) C. S. Foote, "Pathology of Oxygen," Academic Press, London, 1982, p. 21 and references cited therein.
2) R. C. Straight, J. D. Spikes, "Singlet $O_2$," CRC Press, Boca Raton, FL, 1985, p. 91.
3) T. Ito, *Photochem. Photobiol.*, **25**, 47 (1977).
4) T. Ito, K. Kobayashi, *Photochem. Photobiol.*, **25**, 399 (1977).
5) T. M. A. R. Dubbelman, A. F. P. De Goeij, J. V. Steveninck, *Biochim. Biophys. Acta*, **511**, 141 (1978).
6) I. Rosenthal, "Singlet $O_2$," CRC Press, Boca Raton, FL, 1985, p. 13.
7) K. Kobayashi, T. Ito, *Photochem. Photobiol.*, **25**, 385 (1977).
8) T. Ito, *Photochem. Photobiol.*, **31**, 565 (1980).
9) A. Ito, T. Ito, *Photochem. Photobiol.*, **35**, 501 (1982).
10) T. Ito, A. Ito, *Photochem. Photobiol.*, **40**, 429 (1984).
11) T. A. Dahl, W. R. Midden, P. E. Hartman, *Photochem. Photobiol.*, **46**, 345 (1987).
12) T. Ito, *Photochem. Photobiol.*, **28**, 493 (1978).
13) J. A. Imlay, S. Linn, *Science*, **240**, 1302 (1988).
14) G. Brandi, F. Cattabeni, A. Albano, O. Cantoni, *Free Rad. Res. Comms.*, **6**, 47 (1989).
15) J. A. Imlay, S. M. Chin, S. Linn, *Science*, **240**, 640 (1988).
16) I. Saito, T. Matsuura, K. Inoue, *J. Am. Chem. Soc.*, **103**, 188 (1981).
17) H. M. Hassan, I. Fridovich, *J. Biol. Chem.*, **254**, 10846 (1979).
18) M. Takahashi, T. Nagano, M. Hirobe, *Arch. Biochem. Biophys.*, **268**, 137 (1989).
19) H. Yamamoto, T. Mashino, T. Nagano, M. Hirobe, *J. Am. Chem. Soc.*, **108**, 539 (1986).
20) H. Yamamoto, T. Nagano, M. Hirobe, *J. Biol. Chem.*, **263**, 12224 (1988).
21) T. Takabatake, M. Hasegawa, T. Nagano, M. Hirobe, *Chem. Pharm. Bull.*, **38**, 128 (1990).
22) T. Takabatake, M. Hasegawa, T. Nagano, M. Hirobe, *Chem. Pharm. Bull.*, **39**, 1352 (1991).
23) T. Nagano, M. Takahashi, M. Hirobe, *Chem. Pharm. Bull.*, **39**, 1914 (1991).
24) T. Takabatake, M. Hasegawa, T. Nagano, M. Hirobe, *J. Biol. Chem.*, **267**, 4613 (1992).
25) T. Takabatake, M. Hasegawa, T. Nagano, M. Hirobe, *Chem.*

NII-Electronic Library Service

*Pharm. Bull.*, **40**, 1644 (1992).
26) A. Mitsumoto, T. Nagano, M. Hirobe, *Arch. Biochem. Biophys.*, **296**, 482 (1992).
27) I. Saito, T. Matsuura, K. Inoue, *J. Am. Chem. Soc.*, **105**, 3200 (1983).
28) T. Nagano, I. Fridovich, *Photochem. Photobiol.*, **41**, 33 (1985).
29) J. M. McCord, I. Fridovich, *J. Biol. Chem.*, **244**, 6049 (1969).
30) R. F. Beers, I. W. Sizer, *J. Biol. Chem.*, **195**, 133 (1952).
31) O. H. Lowry, N. J. Rosebrough, A. L. Farr, R. J. Randall, *J. Biol. Chem.*, **193**, 265 (1951).
32) P. R. Ogilby, C. S. Foote, *J. Am. Chem. Soc.*, **104**, 2069 (1982).
33) H. M. Hassan, I. Fridovich, *J. Biol. Chem.*, **252**, 7667 (1977).
34) A. W. N. Nieuwint, J. M. Aubry, F. Arwert, H. Kortbeek, S. Herzberg, H. Joenje, *Free Rad. Res. Commun.*, **1**, 1 (1985).
35) M. V. M. Lafleur, A. W. M. Nieuwint, J. M. Aubry, H. Kortbeek, F. Artwert, H. Joenje, *Free Rad. Res. Commun.*, **2**, 343 (1986).
36) P. Di Mascio, H. Wefers, H. P. Do-Thi, M. V. M. Lafleur, H. Sies, *Biochim. Biophys. Acta*, **1007**, 151 (1989).
37) R. A. Zoeller, O. H. Morand, C. R. H. Raetz, *J. Biol. Chem.*, **263**, 11590 (1988).
38) O. H. Morand, R. A. Zoeller, C. R. H. Raetz, *J. Biol. Chem.*, **263**, 11597 (1988).
39) J. R. Kanofsky, *Chem.-Biol. Interact.*, **70**, 1 (1989).
40) J. R. Kanofsky, H. Hoogland, R. Wever, S. J. Weiss, *J. Biol. Chem.*, **263**, 9692 (1988).

NII-Electronic Library Service