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# Iron-Mediated Induction of the SOS Responses by Hydrogen Peroxide

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In the living cells there are three kinds of active oxygen species (AOS), arising during mono- or bivalent reduction of molecular oxygen, which are hydrogen peroxide ($H_2O_2$), superoxide radical anion, ($O_2^{\cdot-}$), and hydroxyl radical ($OH^{\cdot}$). Although their normal concentrations there are kept very low by anti-oxidant enzymes and other molecules, these concentrations may increase many times under special circumstances. There have been many experiments proving that AOS may be involved in mutation (Moody and Hassan 1982) and carcinogenesis (Weitzman et al. 1985), and their molecular target is DNA (Larramendy et al. 1987). It has been suggested that $OH^{\cdot}$ radicals, formed via the Haber-Weiss reaction catalyzed by iron:

$$H_2O_2 + Fe^{++} \text{ ------> } OH^- + Fe^{+++} + OH^{\cdot}$$
$$Fe^{+++} + O_2^{\cdot-} \text{ ------> } Fe^{++} + O_2$$
$$\text{---------- net: } H_2O_2 + O_2^{\cdot-} \text{ -------> } O_2 + OH^- + OH^{\cdot}$$

are the species ultimately responsible for DNA lesions (Halliwell 1978). It may be beneficial to extend these studies with the SOS chromotest (Quillardet and Hofnung 1985) to determine if the SOS responses can be initiated by DNA lesions via the same mechanism.

In Escherichia coli some of the responses induced by DNA-damaging agents involve a set of functions known as the SOS responses as well as "damage-inducible" genes (Kenyon and Walker 1980). The SOS responses can be detected with the SOS chromotest in which the tester strain E. coli PQ37 is used. This strain carries a sfiA::lacZ fusion placing lacZ, the structural gene for beta-galactosidase, under control of the sfiA gene which is one of the "damage-inducible" genes, and has a deletion for the normal lac region so that beta-galactosidase activity is strictly dependent on sfiA expression and can be taken as an indicator of DNA-damaging agents.

## MATERIALS AND METHODS

The tester strain E.coli PQ37 was obtained from the Second Mili-

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tary Medical College of the Chinese People's Liberation Army, Shanghai, China. The critical genetic markers of PQ37 and the procedures of the SOS chromotest were described by Quillardet and Hofnung (1985). Briefly, samples of an exponentially growing culture of the tester strain, freshly diluted in rich medium (1% tryptone, 0.5% yeast extract, 1% NaCl), were incubated for 2 h at 37°C with $H_2O_2$ and/or catalase, superoxide dismutase (SOD), iron-complexing agent o-phenanthroline, and $OH^{\cdot}$ radical scavenger thiourea respectively. After incubation, beta-galactosidase activity, in conventional units, was assayed as an indication of the SOS responses induction, and alkaline phosphatase activity, also in conventional units, was assayed as a toxicity indication. The inducing activity was expressed by the ratio R(C)=G/P, where C is the concentration of $H_2O_2$ tested, G, the beta-galactosidase activity, and P, the alkaline phosphatase activity. The induction factor of $H_2O_2$ at concentration C is defined as I(C)=R(C)/R(0), where 0 is the zero concentration of $H_2O_2$ (Quillardet and Hofnung 1985).

## RESULTS AND DISCUSSION

The results obtained from different concentrations of $H_2O_2$ with the SOS chromotest are presented in Table 1. At the concentration ranging from 20 μM to 200 μM, $H_2O_2$ induced significant increases in beta-galactosidase activity without significant decreases in alkaline phosphatase activity. At the concentration of 500 μM, $H_2O_2$ showed toxicity by reducing significantly alkaline phosphatase activity. The maximum value of the induction factor I(C) was 5.50. This means $H_2O_2$ is a strong SOS responses inducing agent. Because the genes responsible for the SOS responses are "damage-inducible" genes (Kenyon and walker 1980), it is reasonable to suppose that $H_2O_2$ is a kind of DNA-damaging agent. This conclusion is consistent with the previous experiments (Kaneko et al. 1984; Mello-Filho and Meneghini 1984; Larramendy et al. 1987)

Table 1. The SOS responses inducing effect of $H_2O_2$ on E.coli PQ37. G, P, R(C), and I(C) are beta-galactosidase activity, alkaline phosphatase activity, the inducing activity and the induction factor respectively. Each dose was tested at least in triplicate.

| $H_2O_2$ (μM) | G (mean ± SEM) (in conventional units) | P (mean ± SEM) (in conventional units) | R(C) | I(C) |
|---|---|---|---|---|
| 0 | 2.6 ± 0.5 | 18.6 ± 0.9 | 0.14 | 1.00 |
| 5 | 2.7 ± 0.6 | 18.4 ± 1.1 | 0.15 | 1.07 |
| 10 | 3.3 ± 0.5 | 18.7 ± 0.8 | 0.18 | 1.28 |
| 20 | 5.6 ± 0.7 | 18.3 ± 0.7 | 0.31 | 2.21 |
| 50 | 7.8 ± 0.4 | 17.6 ± 1.1 | 0.44 | 3.14 |
| 100 | 8.7 ± 0.8 | 14.5 ± 1.5 | 0.60 | 4.29 |
| 200 | 10.9 ± 0.7 | 14.2 ± 1.2 | 0.77 | 5.50 |
| 500 | 4.6 ± 0.3 | 7.3 ± 0.7 | 0.63 | 4.57 |

Catalase (0.1-0.2 mg/mL, 5000 units/mg), SOD (0.1-0.2 mg/mL, 3000 units/mg), iron-complexing agent o-phenanthroline (2-20 μM), and $OH^{\cdot}$ radical scavenger thiourea (20-200 μM) had no SOS responses inducing effect. This can be seen in Table 2 in which the corresponding induction factor I(C) was about 1 equal to the control.

Table 2. The SOS responses inducing results of catalase, SOD, o-phenanthroline, and thiourea in E.coli PQ37. G, P, R(C),and I(C) are beta-galactosidase activity, alkaline phosphatase activity, the inducing activity, and the induction factor respectively. Each dose was tested at least in triplicate.

| Enzymes,compounds (concentration) | G (in conventional units, mean ± SEM ) | P | R(C) | I(C) |
|---|---|---|---|---|
| Water (control) | 2.5 ± 0.3 | 18.5 ± 0.7 | 0.14 | 1.00 |
| Catalase(0.2 mg/mL) | 2.6 ± 0.4 | 18.6 ± 0.7 | 0.14 | 1.00 |
| Catalase(0.4 mg/mL) | 2.7 ± 0.4 | 18.5 ± 0.8 | 0.15 | 1.07 |
| SOD (0.2 mg/mL) | 2.8 ± 0.6 | 18.8 ± 0.5 | 0.15 | 1.07 |
| SOD (0.4 mg/mL) | 2.8 ± 0.5 | 18.6 ± 0.5 | 0.15 | 1.07 |
| o-P* ( 2 μM ) | 2.6 ± 0.6 | 18.3 ± 0.9 | 0.14 | 1.00 |
| o-P* ( 20 μM ) | 2.4 ± 0.7 | 18.5 ± 1.0 | 0.14 | 1.00 |
| Thiourea( 20 μM ) | 2.3 ± 0.4 | 18.2 ± 0.8 | 0.13 | 0.93 |
| Thiourea( 200 μM ) | 2.5 ± 0.6 | 17.9 ± 0.7 | 0.14 | 1.00 |

*o-P: o-Phenanthroline.

Reducing the induction factor I(C) from about 2.2 to about 1.0, the presence of each of the two enzymes (1-2 mg/mL) and the two compounds (o-phenanthroline:2-20 μM, thiourea:20-200 μM) totally stopped the SOS responses induced by $H_2O_2$ (20 μM) in E.coli PQ37. These results are shown in Table 3. But the enzymes and compounds have certainly different mechanisms to eliminate the SOS responses induced by $H_2O_2$ because of their different functions. Catalase is able to destroy $H_2O_2$ via the reaction: $2\ H_2O_2 \rightarrow O_2 + 2\ H_2O$ catalyzed by itself to eliminate the SOS responses induced by $H_2O_2$. Though not having the same function as catalase does, SOD is able to destroy $O_2^{\cdot-}$ radicals by catalyzing the reaction: $2\ O_2^{\cdot-} + 2\ H^+ \rightarrow O_2 + H_2O_2$ to eliminate the same induction. These facts mean that $H_2O_2$ and $O_2^{\cdot-}$ radicals are involved in the SOS responses inducing process, and that $H_2O_2$ is not the ultimate agent that directly induces the SOS responses, and $O_2^{\cdot-}$ radicals may exist originally in the cells or be generated via some reaction related to $H_2O_2$. Agent o-phenanthroline, a strong iron-complexing agent which is unable to destroy $H_2O_2$, $OH^{\cdot}$ radicals or $O_2^{\cdot-}$ radicals (Larramendy at al. 1987), could also eliminate the SOS responses induced by $H_2O_2$. This means that the Haber-Weiss reaction mediated by iron may be responsible for the SOS responses induced by $H_2O_2$ in E.coli PQ37 and $OH^{\cdot}$ radicals are likely the ultimate species that induce the SOS responses. The fact that the $OH^{\cdot}$ radical scavenger thiourea which is unable to destroy $H_2O_2$ or $O_2^{\cdot-}$ radicals (Mello-Filho and

Meneghini 1984) could also eliminate the SOS responses induced by $H_2O_2$ further supports the idea that OH· radicals are the ultimate species that induce the SOS responses.

Table 3. Protective effects of catalase, SOD, o-phenanthroline, and thiourea on the SOS responses induced by $H_2O_2$ in E.coli PQ37. G, P, R(C), and I(C) are beta-galactosidase activity, alkaline phosphatase activity, the inducing activity and the induction factor respectively. Each dose was tested at least in triplicate.

| Enzymes, compounds (concentration) with 20 μM $H_2O_2$ | G (in conventional units, mean ± SEM) | P (in conventional units, mean ± SEM) | R(C) | I(C) |
|---|---|---|---|---|
| Control (no $H_2O_2$) | 2.6 ± 0.5 | 18.6 ± 0.9 | 0.14 | 1.00 |
| Water | 5.6 ± 0.7 | 18.3 ± 0.7 | 0.31 | 2.21 |
| Catalase(0.1 mg/mL) | 2.6 ± 0.4 | 18.3 ± 0.8 | 0.14 | 1.00 |
| Catalase(0.2 mg/mL) | 2.7 ± 0.6 | 18.5 ± 0.8 | 0.15 | 1.07 |
| SOD (0.1 mg/mL) | 2.8 ± 0.7 | 17.9 ± 0.8 | 0.14 | 1.00 |
| SOD (0.2 mg/mL) | 2.5 ± 0.5 | 18.2 ± 0.6 | 0.15 | 1.07 |
| o-P* ( 2 μM ) | 2.7 ± 0.9 | 18.2 ± 1.2 | 0.15 | 1.07 |
| o-P* ( 20 μM ) | 2.5 ± 0.8 | 18.2 ± 1.0 | 0.14 | 1.00 |
| Thiourea( 20 μM ) | 2.9 ± 0.7 | 17.8 ± 0.9 | 0.16 | 1.15 |
| Thiourea( 200 μM ) | 2.5 ± 0.4 | 17.9 ± 0.8 | 0.14 | 1.00 |

*o-P: o-Phenanthroline.

According to the mechanism of the SOS responses ("damage-inducible" genes), OH· radicals may be the ultimate species damaging DNA that induce the SOS responses. Since OH· radicals are very reactive and probably have a mean path of less than 1 nm, the DNA-damaging OH· radicals might be produced in the vinicity of DNA (Kaneko et al. 1984). The iron mediating the Haber-Weiss reaction might originally be in the cells (Mello-Filho and Meneghini 1984).

One might suppose that the enzymes and compounds used in this experiment might block the expression of the induced SOS expression (beta-galactosidase activity) and not by the elimination of the active oxygen species to eliminate the SOS responses. This is not the case as can be seen in Table 4 in which the data show that 4-nitroquinoline 1-oxide (4NQO), a well known inducer of the SOS responses, had the same effect, irrespective of whether the treatments were carried out in the presence or absence of each of the four substances.

Accordingly, we suppose the following mechanism of $H_2O_2$ inducing the SOS responses. Extracellular $H_2O_2$ diffuses into the cells, carrying with it a damaging potential. In the vicinity of DNA, $H_2O_2$ and $O_2^{\cdot-}$ radicals which might be generated normally in the cells or via some reaction related to $H_2O_2$ give rise to OH· radicals via the Haber-Weiss reaction mediated by the iron originally in the

cells. The $OH^{\cdot}$ radicals thus formed are the ultimate DNA-damaging species that induce the SOS responses. Although other possible mechanisms can not be ruled out, the iron-mediated induction of the SOS responses by hydrogern peroxide is certainly conceivable.

Table 4. Effects of catalase, SOD, o-phenanthroline and thiourea on the SOS responses induced by 4NQO in E.coli PQ37. G, P, R(C), and I(C) are beta-galactosidase activity, alkaline phosphatase activity, the inducing activity, and the induction factor respectively. Each dose was tested at least in triplicate.

| Enzymes, compounds (concentration) with 100 μM 4NQO | G (in conventional units, mean ± SEM) | P | R(C) | I(C) |
|---|---|---|---|---|
| Control (no 4NQO) | 3.1 ± 0.4 | 27.4 ± 1.3 | 0.11 | 1.00 |
| Water | 8.0 ± 0.7 | 27.4 ± 0.9 | 0.29 | 2.64 |
| Catalase(0.2 mg/mL) | 8.1 ± 1.1 | 27.2 ± 1.2 | 0.30 | 2.73 |
| SOD (0.2 mg/mL) | 7.9 ± 0.8 | 26.7 ± 0.9 | 0.30 | 2.73 |
| o-P* ( 20 μM ) | 8.2 ± 1.4 | 29.3 ± 1.5 | 0.28 | 2.54 |
| Thiourea( 200 μM ) | 8.4 ± 0.9 | 27.6 ± 1.2 | 0.30 | 2.73 |

*o-P: o-Phenanthroline

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