KOR. JOUR. MICROBIOL April, 1994 p. 139~146 Vol. 32, No. 2

Functional Characteristics of Cytoplasmic and Periplasmic *Photobacterium leiognathi* CuZnSOD (PSOD) in *Escherichia coli* SOD Double Mutants

Kim, Young Gon* and Mi Kyung Yang

Department of Biology, College of Natural Sciences, Chosun University, Kwangju 501-759, Korea

Protective effects on subcellular localization of *Photobacterium leiognathi* CuZnSOD (PSOD) were examined in *Escherichia coli* SOD mutant cells on the treatment of paraquat, heat shock (37℃→42℃), hydrogen peroxide and copper sulfate, respectively. The physiological characteristics of the periplasmic and cytoplasmic PSOD localized differently are dependent on the conditions in this experiment. Cells expressing SOD periplasmically in the treatments of paraquat and H_2O_2 respectively were somewhat better protective effects cells expressing SOD cytoplasmically at comparable level and SOD expression level showed, the most consistently important variable. However, this was reversed in the treatments of heat shock and $CuSO_4$, respectively.

KEY WORDS □ superoxide dismutase, subcellular localization

Superoxide dismutases (SODs: E.C.1.15.1.1) are metalloenzymes which catalyze the reaction: $O_2^- + O_2^- + 2H^+ \rightarrow H_2O_2 + O_2$. Thus SODs are considered as the first line of defense against oxygen free radicals (14). They are classified into three forms according to the metal of their active sites: Cu/Zn, Fe and Mn. SODs of the erythrocuprein type (CuZn) are characteristically found in the cytosols of eukaryotes (15, 16, 17). MnSODs are found in prokaryotes and organelles (17, 18, 20) and FeSODs are found primarily in prokaryotes. Several exceptions to these generalizations are known (9, 33, 38, 41, 43). The idea of SOD specialization is also supported by two recently described SOD types. First, it has been observed that the CuZnSODs of *P. leiognathi* (44) and *Caulobacter crescentus* (45) possess a signal sequence, absent from eukaryotic CuZnSODs, which provides the major component of total SOD activity. Second, it is now well documented that diverse animals possess a CuZnSOD associated with the extracellular matrix (ECSOD) (31) that is related to, but quite different from cytosolic SOD (21, 32). The existence of these enzymes challenges the once popular assumption that SOD activity was necessary to counteract only intracellular superoxide. Recently, by expressing periplasmically the *P. leiognathi* gene in SOD-deficient *E. coli* cells, we have been able to address general questions concerning extracellular superoxide and superoxide dismutase, independent of any special conditions that occur in the periplasm of *P. leiognathi* and *C. crescentus* or in the extracellular matrices of animal tissues (31). MnSOD and FeSOD have been assigned distinct subcellular localizations and physiological functions. Thus in *E. coli* system, both enzymes are found in the cytoplasm and this distinct localization was thought to increase levels of SODs resistance against endogeneously produced superoxide radicals (4). The subcellular distribution of SODs in plants is a matter of debate. However, it has been suggested that cyanide-sensitive CuZnSOD was found to be localized in the cytosol (13), chloroplasts (41), and mitochondrial inner membrane space (42). In blue green algae, the FeSOD is localized in the cytosol, while the MnSOD is distributed in the thylakoid (37). The significance of SOD distributions has been suggested by complementing SOD deficient *E. coli* mutants with genetically engineered plasmids, pYK2 and pYK4 which express PSOD periplasmically or cytoplasmically with or without signal sequences, respectively (23, 24).

Recently, Steinman (44) described the cytoplasmic location of the *P. leiognathi* CuZnSOD. However, no specific cellular localization of the SODs has been clearly demonstrated and there is no evidence that they have different biological roles. Thus, we report here that functional

differences of cytoplasmically or periplasmically expressed *Photobacterium leiognathi* CuZnSOD in bacterial system exist and their functions are expressed during various superoxide-mediated stresses such as heat shock, paraquat treatment, H_2O_2 and metal ion treatment.

MATERIALS AND METHODS

E. coli strains and plasmids

The *E. coli* K12 sodA sodB mutants strain (QC 774, GC4468 φ (sodA::MudPR13) 25, Cm^r φ (sodB-Kan)-Δ2Km^r) and wild type strain GC4468 (F ΔlacU169 rspL) were described previously (5, 7, 34).

To explore the functional differences between cytoplasmic and periplasmic PSOD, plasmids pYK2 and pYK4 (23) were employed, respectively.

Culture conditions for oxidative-mediated stress

To transform the plasmids pYK2 and pYK4 into *E. coli* sodA sodB mutants, cells were made competent and transformed by the procedure of Maniatis *et al.* (30). To assess the effects of oxidative stress agents such as paraquat (PQ), hydrogen peroxide, copper sulfate and heat shock on SOD localization, plasmid carrying QC774 mutant cells were grown overnight with vigorous aeration (250 rpm) at 37℃ in LB broth supplemented with ampicillin (40 μg/ml).

Overnight cultures were diluted 1:100 into 50 ml of fresh LB in 250 ml Erlenmyer flasks, and were monitored for optical density at 600 nm. When cells were grown (250 rpm) to $OD_{600}=0.1$ at 37℃, 0.1 mM PQ, 5 mM H_2O_2 and 0.1 mM copper sulfate were added, respectively. To determine the effects of PQ, cells were grown in LB medium supplemented with 25, 50, 100, 150 μM PQ for 6 hours at 37℃. To investigate the effect of heat shock, cells were transferred from 37℃ to 42℃, and were grown for 6 hours. Cells were grown for 2 hours at 42℃, and then transferred into 37℃ and were grown for 4 hours. The sensitivity of growth rates were measured by OD_{600} every hour.

SOD assay

SOD measurement was carried out under the same growth condition as described in the previous report (25). Transformants QC774 (pYK4 or pYK2), were grown in rich medium(LB) under various conditions for 6 hours, harvested at 4℃ by centrifugation at 10,000×*g* for 3 min, washed in 0.1 M potassium phosphate (pH 7.0), and suspended in a 1:24 volume of 10^{-2} M phosphate buffer (pH 7.8). Cytoplasmic or periplasmic fractions for SOD crude extracts were obtained using the osmotic shock method of Neu and Heppel (36). Cells were then disrupted by five cycles of freezing and thawing and by vortexing successively at 4℃. Cell debris was removed by 15min centrifugation at 15,000×*g*. Crude extracts were kept frozen at −20℃. SOD assays were done according to Beauchamp and Fridovich (3) with slight modifications as described by Kim (23). Protein was estimated by method of Lowry *et al.* (29). One unit of SOD activity is defined as the quantity which decreases the absorbance by 50%.

RESULTS

Different biological effects of PSOD on cellular localization

Effect of paraquat: The growth of cells encoded cytoplasmic or periplasmic PSOD gene were both affected by 0.1 mM paraquat treatment. Their sensitivities were directly proportional to increasing concentrations of paraquat (Fig. 1, Table 1). Growth rates of both cells were also dramatically inhibited at >0.5 mM paraquat (Fig. 1, 2). SOD double mutants carrying plasmid pYK2 that produces the periplasmic PSOD were more sensitive to oxidative stress than that of pYK4. At 0.1 mM paraquat-treated medium, the growth rates of cells were up to twice those of the

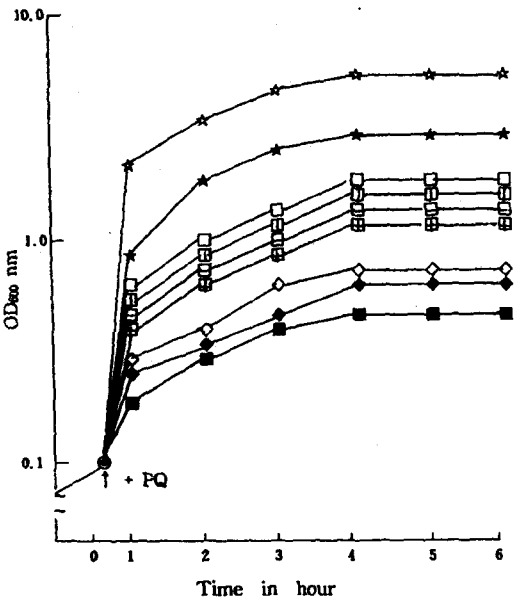

Fig. 1. *The survival rates on increasing of paraquat concentration in pYK2 (QC774) cells.*
☆, GC4468; ★, GC4468+PQ (100 μM); □, pYK2 (QC774); ◫, pYK2 (QC774)+PQ (25 μM); ⊟, pYK2 (QC774)+PQ (50 μM); ⊞, pYK 2 (QC774)+PQ (100 μM); ■, pYK2 (QC774) +PQ (150 μM); ◇, QC774; ◆, QC774+PQ (100 μM). The values given are the average of two independent determinations.

Table 1. *Response of E. coli SOD mutants (QC774) carrying periplasmic and cytoplasmic plasmids to plating on LB agar and to PQ diffusion assays under various conditions.*

E. coli SOD mutants carrying plasmid	Various conditions	Relative zone of inhibition (PQ disk assays)
GC4468	+PQ	0.40
QC774	+PQ	1.00
pYK2(QC774)	+PQ	0.72/0.75
pYK4(QC774)	+Heat shock	0.63/0.54
	$+H_2O_2$	0.60/0.80
	$+CuSO_4$	0.52/0.60

A 100 μl sample from cell culture on LB liquid medium for 6 hr was spreaded onto LB agar medium. After 30 minutes, a Whatman #1 filter disk with 20 μl of 100 μM PQ was placed in the center of the plate. Sensitivity to PQ was measured as the diameter of the zone of inhibition measured after 48 hr. The values given are the average of three independent determinations.

controls, respectively, and stationary levels were remarkably inhibited by over 60% (Fig. 1, 2). PSOD activity was slightly induced with periplasmically expressed SOD in the treatment of PQ.

Effect of heat shock: When early logarithmic cultures of *E. coli* sodA sodB cells (QC774) encoding plasmids pYK2 and pYK4, growing aerobically in LB medium at 37℃, were exposed for 2 hrs to 42℃, the induction rates of cells carrying pYK2 and pYK4 were enhanced by 1.3 fold and 0.9 fold compared to control, respectively (Fig. 3, Table 2). Nevertheless the growth rates of cells were significantly inhibited and irreversibly by heat shock for 2 hrs at 42℃ compared with controls. When again cells were transferred from 42℃ into 37℃, the growth rates were recovered by 56% after 4 hrs. Inhibition was greater for periplasmic SOD than for cytoplasmic (Fig. 4). The results also showed that SOD activities were slightly enhanced on both controls at stationary phase after 6 hrs.

Effect of hydrogen peroxide: Cells carrying plasmids were strongly inhibited by pretreatment with H_2O_2 (5 mM). The inhibition of transport by oxidative stress was diminished after approximately 3 hrs to approximately 56% compared to control cells encoding cytoplasmic PSOD. The inhibition was further enhanced on cells containing periplasmic SOD than that of cytoplasmic (Fig. 5). Cells carrying plasmids showed induced SOD activities, respectively, and survival rates were consistent with controls for SOD induction. This suggests that cells can acquire the ability to overcome the inhibitory effects of

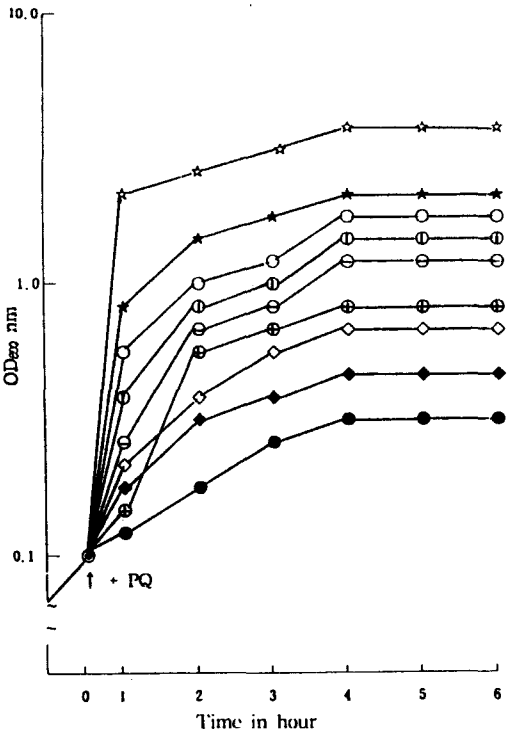

Fig. 2. *The survival rates on increasing of paraquat concentration in pYK4 (QC774) cells.*
☆, GC4468; ★, GC4468+PQ (100 μM); ○, pYK4 (QC774); ◐, pYK4 (QC774)+PQ (25 μM); ⊖, pYK4 (QC774)+PQ (50 μM); ⊕, pYK 4 (QC774)+PQ (100 μM); ●, pYK4 (QC774) +PQ (150 μM); ◇, QC774; ◆, QC774+PQ (100 μM). The given values are the average of two independent determinations.

oxidative stress. Increasing the hydrogen peroxide concentration to 10 mM (data not shown) enhanced significantly the sensitivity. Significant levels of SOD induction with H_2O_2 were shown to be 1.4 fold for periplasmic SOD (Fig. 4).

Effects of copper sulfate: The results show that cells expressing SOD cytoplasmically show greater survival than did the periplasmically expressed SOD at comparable levels on LB liquid medium supplemented with 0.1 mM $CuSO_4$. Survival rates were also significantly increased with copper sulfate compared to controls (Fig. 6). PSOD activity was also consistently parallel to growth rates with the cytoplasmically expressed SOD activity 2.2 fold higher than that of periplasmically expressed SOD (Fig. 4).

DISCUSSION

We have previously reported that *E. coli* SOD double mutants carrying pYK2 produce the

Table 2. *SOD activity in E. coli mutant cells (QC774) carrying pYK2 and pYK4, respectively.*

Location	Plasmid	SOD specific activity (U/mg)				
		LB (control)	PQ	Heat shock 42℃	H_2O_2	$CuSO_4$
Periplasm	pYK2	24	26	28	33	40
Cytoplasm	pYK4	20	21	35	23	86

SOD activities were determined from cells grown 6 hrs on LB liquid medium or LB liquid medium with PQ (0.1 mM), H_2O_2 (0.1 mM) and $CuSO_4$ (0.1 mM) at 37℃. The values given are the average of three independent determinations. The other details were described in growth conditions.

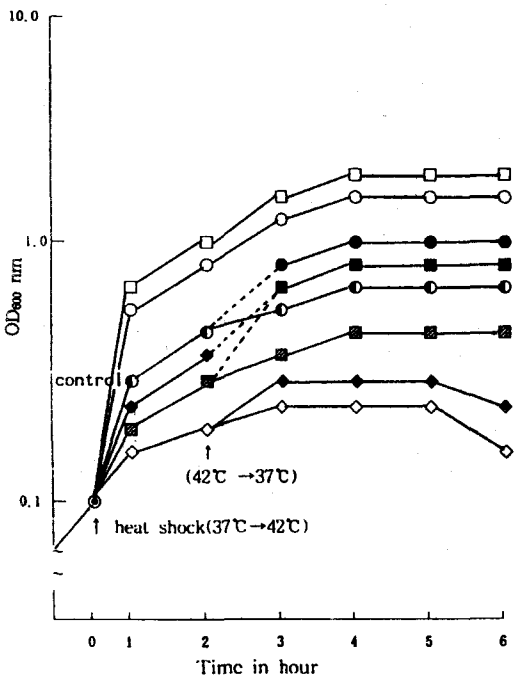

Fig. 3. *The effect of heat shock for 6 hrs on pYK2 (QC774) and pYK4 (QC774) cells.*
□, pYK2 (QC774); ▨, pYK2 (QC774)+Heat shock (37℃→42℃); ■, pYK2 (QC774)+Heat shock (37℃→42℃→37℃); ○, pYK4 (QC774); ◑, pYK4 (QC774)+Heat shock (37℃→42℃); ●, pYK4 (QC774)+Heat shock (37℃→42℃→37℃); ◇, QC774; ◈, QC774+Heat shock (37℃→42℃); ◆, QC774+Heat shock (37℃→42℃→37℃). The given values are the average of two independent determinations.

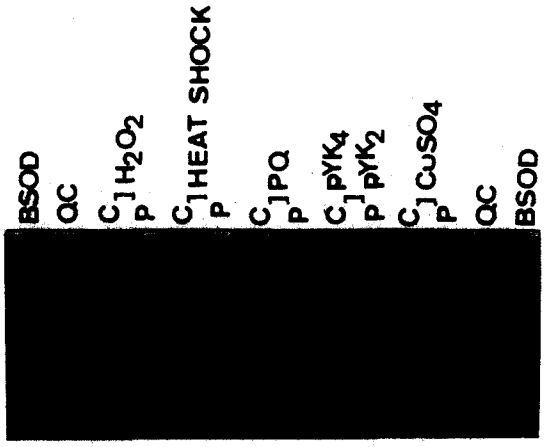

Fig. 4. *Cytoplasmic and periplasmic expression of P. leiognathi CuZnSODs.*
Each lane received 50 μg of total cell protein from *E. coli* sodA sodB cells carrying the plasmids, pYK2 and pYK4. P, periplasmic fraction of pYK2 (QC774); C, cytoplasmic fraction of pYK4 (QC774). BSOD indicates bovine CuZnSOD as a comparable standard and PSOD indicates *P. leiognathi* CuZnSOD, respectively.

periplasmic PSOD, while cells carrying pYK4 generate cytoplasmic PSOD (23). This result has been further confirmed by the observation of periplasmic CuZnSOD in *Caulobacter crescentus* (45). This unique subcellular localization of SOD challenges the once popular assumption that SOD activity was necessary to counteract only intracellular oxygen radicals and thereby function only endogeneously. A previous report demonstrated that *E. coli* possesses FeSOD and MnSOD and these enzymes are located in cytoplasm (4). Hassan and Fridovich (19) suggested that paraquat could diffuse across the cell envelope and react with oxygen, thus resulting in the intracellular production of superoxide radicals at levels sufficient for cell damage.

They further argued superoxide anion is unable to transport across the cell envelope. Their interpretation about the mechanism of extracellular superoxide radicals may not be appropriate because exogeneous oxygen radicals may also damage cell surfaces and thereby effect the oxygen radicals flux into the periplasm. To answer this question, the toxicity of paraquat was

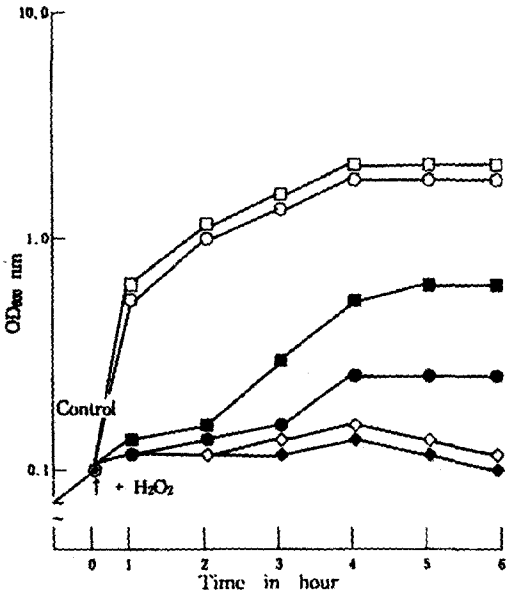

Fig. 5. *The effect of H_2O_2 treatment on pYK2 (QC774) and pYK4 (QC774) cells.*
□, pYK2 (QC774); ■, pYK2 (QC774)+H_2O_2; ○, pYK4 (QC774); ●, pYK4 (QC774)+H_2O_2; ◇, QC774; ◆, QC774+H_2O_2. The given values are the average of two independent determinations.

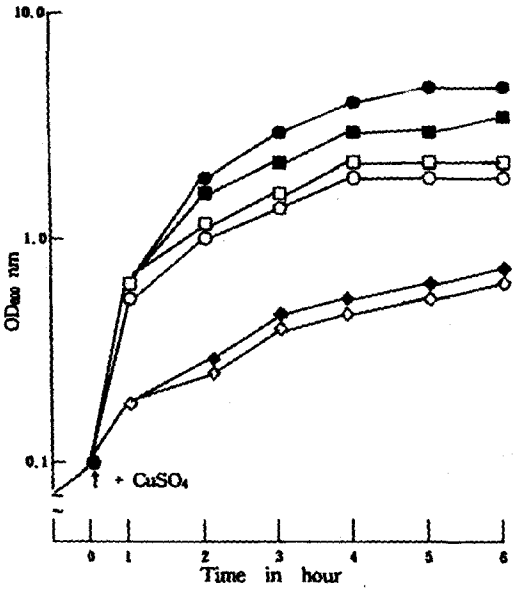

Fig. 6. *The effect of $CuSO_4$ on pYK2 (QC774) and pYK4 (QC774) cells.*
□, pYK2 (QC774); ■, pYK2 (QC774)+$CuSO_4$; ○, pYK4 (QC774); ●, pYK4 (QC774)+$CuSO_4$; ◇, QC774; ◆, QC774+$CuSO_4$. The given values are the average of two independent determinations.

investigated based on subcellular localization of SOD because elevated levels of SOD can enhance the resistance towards paraquat toxicity. This is very important in understanding oxygen radical biology and its mechanism in the cell. The result showed that a relatively high level of protection was conferred on *E. coli* SOD mutants by periplasmic CuZnSOD and reversed by the treatment of copper sulfate and heat shock.

Therefore, there is at least circumstantial evidence from this experiment that O_2^- is of biological consequence. This extracellular SOD function suggests that intracellular oxygen radicals produced from paraquat can transverse the cellular membrane and periplasmic SOD can primarily protect from the extracellular oxygen radical influx.

On the other hand, *E. coli* responds to enhanced temperature by increasing the rate of synthesis of heat shock proteins (35). This cellular response may also be part of a general adaption to oxidative stress. When cells carrying PSOD were treated from 37℃ to 42℃ for 2 hours, cells with cytoplasmically expressed PSOD resisted stress better than periplasmically expressed PSOD. This suggests that several other stimuli including oxidants can cause the induction of heat shock proteins (26, 27). Cytoplasmic PSOD was also stimulated or induced by this variety of species. This result was also suggested by the observation of SOD activity measured quantitatively (Fig. 3, Table 2). The induction of cytoplasmically expressed PSOD was 1.3 fold and 1.2 fold of periplasmically expressed PSOD. This appears to be a response to increased intracellular production of oxygen radicals during 2 hrs of heat shock when the temperature was raised from 37℃ to 42℃. Therefore, it is possible that heat shock increases oxygen radicals in *E. coli* cells by destroying the electron transport chains of the plasma membrane. Therefore, the elevated oxygen radicals can increase PSOD biosynthesis. These results provide further evidence suggesting a relationship between heat shock and oxidative stress. However, this proposal should be further studied for the inter-relationship between intracellular heat shock proteins and oxidative stress. The survival growth rate did not significantly increase following three hours exposure to hydrogen peroxide but did allow rapid recovery from inhibiton.

This suggests that cells may need the ability to overcome the deleterious effects of oxidative stress. This assumption can be further studied on the loss of transport by pretreatment of transportable substances such as lactose or uracil.

Pretreated cells with H_2O_2 can remove the inhibitory effects of H_2O_2 and repair the oxidative damage responsible for the loss of glucose transport (12). Previous reports suggest that *oxyR* and *katG* gene products are required for induced rapid recovery of transport (6, 10). Although this proposal is not straightforward in defense against oxidative stress, recent work by Imlay and Linn (22) suggested that catalase encoded by *katG* is the only gene regulated by *oxyR* that is necessary for induced resistance to killing by H_2O_2. It has been known that oxidative disruption of membrane integrity by H_2O_2 is a general phenomenon. Consequently, this results in membrane damage also and thereby cells must protect from oxidative stress by increasing antioxidants such as SOD or catalase. There is also evidence that conditions of increased O_2^- and H_2O_2 exposure induce distinct DNA repair responses (8, 11). However, the nature of the membrane damage resulting in the loss of membrane function and the mechanism for DNA damage by H_2O_2 are not known. Our results show that cells periplasmically expressing periplasmic SOD was more protective than cells expressing cytoplasmic SOD. This means that periplasmic SOD defenses primarily against oxidative stress were caused by outer membrane damage and thereby such DNA damage can be repaired after 3 hrs by rapid recovery of membrane damage or other complicating factors. This recovery systems to oxidative stress remain to be determined.

Cells expressing SOD periplasmically in the absence of supplemental copper were somewhat better than cells expressing SOD cytoplasmically at comparable level (Table 2, Fig. 5). However, when cells were grown in L broth liquid medium supplemented with 0.1 mM $CuSO_4$, the cytoplasmically-expressed SOD gave greater cell survival than did periplasmically-expressed SOD. This remarkable effect was more enhanced than that of the control in the absence of supplemental copper during log phase (Fig. 5). This can be interpreted by the conventional mechanism (2):

$$E\ Cu(II) + O_2^- \longrightarrow E\ Cu(I) + O_2$$
$$E\ Cu(I) + O_2^- + 2H^+ \longrightarrow E\ Cu(II) + H_2O_2$$

In this mechanism, copper produces oxygen with detoxifying the oxygen radicals, and in turn it results in H_2O_2 which is also toxic to the cell. Therefore, copper ions may function in the reduction of oxygen radicals early on but may lead to inhibition. It has been also shown that the conventional mechanism can be reversed under particular conditions such as high pH and scavenging of oxygen radicals (39, 46).

Nevertheless, in the present study, SOD activity shows significantly high levels in both sites. Induction was increased by about 4 fold in the cytoplasm as compared to the control, while PSOD induction was increased by approximately 2 fold. It is reasonable to assume the possibility of the low efficiency of a periplasmically expressing SOD. Because if copper ions were transported into cells, first the periplasm would have the high ionic strength and thereby cause the inhibition of enzyme activity (40). Several investigators reported that copper led to induction of the CuZnSOD activity in bacterial (23, 34) and eukaryotic systems (28). However, this enhanced CuZnSOD activity might accelerate lipid peroxidation without an adaptive increase in H_2O_2 elimination. On the other hand, Balevska *et al.* (1) reported that copper deficiency in rats causes increased levels of lipid peroxidation by 2 fold in liver mitochondria and microsomes. If this is true, induced CuZnSOD might play the role in the dismutation of the oxygen radicals. Our results indicate that increased CuZnSOD could effect the inhibition of the lipid peroxidation as shown in the growth survival curves.

Finally, it is important to acknowledge that physiological characteristics of the periplasmic and cytoplasmic SOD localized differently are dependent on the conditions in this experiment. Moreover, the interaction between oxygen radicals and the other antioxidants could directly effect the SOD function under oxidative stress. Further studies on catalase activity with these experimental conditions may provide clues as to whether catalase can play important defense role in regards to SOD distribution.

ACKNOWLEDGEMENT

This work was supported by the Chosun University research grant. The authors are very grateful for the supports. We wish to thank Dr. Tom Magee of Lab. of Structural biology & Molecular biology at UCLA in USA for review and comment.

REFERENCES

1. **Balevska, P.S., E.M. Russanov, and T.A. Kassabova,** 1981. Studies on lipid peroxidation in rat liver by copper deficiency. *Int. J. Biochem.* **13**, 489-493.
2. **Bannister, J.V. and G. Rotilio,** 1987. Aspects of the structure, function and applications of superoxide dismutase. *CRC* **22**, 111-173.
3. **Beauchamp, C. and I. Fridovich,** 1971. Superoxide dismutase: Improved assays and an assay applicable to acrylamide gels. *Anal. Biochem.* **44**, 276-287.
4. **Britton, L. and I. Fridovich,** 1977. Intracellular localization of the superoxide dismutase of *Escherichia coli*: Intracellular localization and function. *J. Bacteriol.* **131**, 815-820.

5. **Carlioz, A. and D. Touati,** 1986. Isolation of superoxide dismutase mutants in *Escherichia coli*: Is superoxide dismutase necessary for aerobic life? *EMBO* **5**, 623-630.
6. **Chan, E. and B. Weiss,** 1987. Endonuclease IV of *Escherichia coli* is induced by paraquat. *Proc. Natl. Acad. Sci. USA* **84**, 3189-3194.
7. **D'Ari, R., J. George, and O. Huisman,** 1979. Suppression of tif-mediated induction of SOS functions in *Escherichia coli* by an altered dnaB protein. *J. Bacteriol.* **140**, 381-387.
8. **Demple, B. and J. Halbrook,** 1983. Inducible repair of oxidative DNA damage in *Escherichia coli*. *Nature* (London) **304**, 446-448.
9. **Duke, M.V. and M.L. Salin,** 1985. Purification and characterization of an iron containing superoxide dismutase from eucaryote. *Ginkgo biloba*. *Arch. Biochem. Biophys.* **243**, 305-314.
10. **Eisenstark, A. and G. Perrot,** 1987. Catalase has only a minor role in protection against near UV radiation damage in bacteria. *Mol. Gen. Genet.* **207**, 68-72.
11. **Farr, S.B., D.O. Natvig, and T. Kogoma,** 1985. Toxicity and mutagenicity of plumbagin and the induction of a possible new DNA repair pathway in *Escherichia coli*. *J. Bacteriol.* **164**, 1309-1316.
12. **Farr, S.B., D. Touati, and T. Kogoma,** 1988. Effects of oxygen stress on membrane functions in *Escherichia coli*: Role of HPI catalase. *J. Bacteriol.* **170**, 1837-1842.
13. **Foster, J.K. and G.E. Edwards,** 1980. Localization of superoxide dismutase in leaves of C_3 and C_4 plants. *Plant. Cell. Physiol.* **21**, 895-906.
14. **Fridovich, I.,** 1978. The biology of oxygen radicals. *Science* **201**, 875.
15. **Fridovich, I.,** 1979a. Superoxide and superoxide dismutases. *Adv. Inorg. Biochem.* **1**, 67-90.
16. **Fridovich, I.,** 1979b. Oxygen free radicals and tissue damage. Ciba Foundation, New York.
17. **Fridovich, I.,** 1986. Superoxide dismutases. *Adv. Enzymol.* **58**, 61-97.
18. **Gregory, E.M., F.J. Yost, and I. Fridovich,** 1973. Superoxide dismutases and oxygen toxicity of *Escherichia coli*: Intracellular localization and functions. *J. Bacteriol.* **115**, 987-991.
19. **Hassan, H.M. and I. Fridovich,** 1979. Paraquat and *Escherichia coli*: Mechanism of production of extracellular superoxide radical. *J. Biol. Chem.* **254**, 10846-10856.
20. **Hewitt, J. and Morris,** 1975. Superoxide dismutase in some obligately anaerobic bacteria. *FEBS Lett.* **50**, 315-318.
21. **Hjalmarsson, K., S.L. Marklund, A. Engstrom, and T. Edlund,** 1987. Isolation and sequence of complementary DNA encoding human extracellular superoxide dismutase. *Proc. Natl. Acad. Sci. USA* **94**, 6340-6344.
22. **Imlay, J. and S. Linn,** 1987. Mutagenesis and stress responses induced in *Escherichia coli* by hydrogen peroxide. *J. Bacteriol.* **169**, 2967-2976.
23. **Kim, Y.G.,** 1990. Superoxide dismutase: Gene replacement studies. Ph.D. Dissertation, University of New Mexico.
24. **Kim, Y.G.,** 1991. Localization of superoxide dismutase (SOD) as a possible exogeneous function. *Kor. J. Gerontol.* **1**, 1-12.
25. **Kim, Y.G.,** 1992. Effects of gene expression of *Photobacterium leiognathi* CuZn superoxide dismutase (PSOD) by lacZ promoter control under oxidative stress. *Kor. J. Microbiol.* **31**, 460-465.
26. **Krueger, J.H. and G. Walker,** 1984. *groEL* and *dnaK* genes of *Escherichia coli* are induced by UV irradiation and nalidixic acid in and $htpR^+$-dependent fusion. *Proc. Natl. Acad. Sci. USA* **81**, 1499-1503.
27. **Lee, P.C., B.R. Bochner, and B.N. Ames,** 1983. ApppA, heat shock stress, and cell oxidation. *Proc. Natl. Acad. Sci. USA* **80**, 7496-7500.
28. **Ljutakova, S.G., E.M. Russanov, and S.I. Liochev,** 1984. Copper increases superoxide dismutase activity in rat liver. *Arch. Biochem. Biophy.* **235**, 636-643.
29. **Lowry, O.H., N.J. Rosebrough, A.L. Farr, and R.J. Randall,** 1951. Protein measurement with the Folin phenol reagent. *J. Biol. Chem.* **193**, 265-275.
30. **Maniatis, T., E.F. Fritsch, and J. Sambrook,** 1982. Molecular cloning. Cold Spring Harbor Laboratory Press, Cold Spring Harbor.
31. **Marklund, S.L.,** 1984. Extracellular superoxide dismutase and other superoxide dismutase isoenzyme in tissues from nine mammalian species. *Biochem. J.* **222**, 649-655.
32. **Marklund, S.L., E. Holme, and L. Hellner,** 1982. Superoxide dismutase in extracellular fluids. *Clin. Chem. Acta.* **126**, 41-51.
33. **Natvig, D.O.,** 1982. Comparative biochemistry of oxygen toxicity in latic acid forming aquatic fungi. *Arch. Microbiology*. **132**, 107-114.
34. **Natvig, D.O., K. Imlay, D. Touati, and H.A. Hallewell,** 1987. Human copper-zinc superoxide dismutase deficient *Escherichia coli* mutants. *J. Biol. Chem.* **262**, 14697-14701.
35. **Neidhardt, F.C., R.A. VanBogelen, and V. Vaughn,** 1984. The genetics and regulation of heat shock proteins. *Annu. Rev. Genet.* **18**, 295-329.
36. **Neu, H.C. and L.A. Heppel,** 1965. The release of enzymes from *Escherichia coli* by osmotic shock and during the formation of spheroplasts. *J. Biol. Chem.* **240**, 3685-3692.
37. **Okada, S., S. Kanematsu, and K. Asada,** 1979. Intracellular distribution of manganic and ferric superoxide dismutase in blue-green algae. *FEBS Lett.* **103**, 106-110.
38. **Puget, K. and A.M. Michelson,** 1974. Isolation of a new copper containing superoxide dismutase bacteriocuprein. *Biochem. Res. Commun.* **58**, 830-838.
39. **Rigo, A., P. Viglino, and G. Rotilio,** 1986.

Reduction and oxidation of bovine superoxide dismutase by H_2O_2, in superoxide and superoxide dismutase in chemistry, biology and medicine. Elsevier, Amsterdam.
40. **Rigo, A., P. Viglino, G. Rotilio, and R. Tomat,** 1975. Effect of ionic strength on the activity of bovine superoxide dismutase. *FEBS Lett.* **50**, 86.
41. **Salin, M.L. and S.M. Bridges,** 1980. Localization of superoxide dismutase in chloroplasts from *Brassica campestris*. *Z. Pflanzenphysiol.* **99**, 37.
42. **Salin, M.L. and S.M. Bridges,** 1981. Absence of the iron-containing superoxide dismutase in mitochondria from mustard (*Brassica campestris*). *Biochem. J.* **195**, 229-233.
43. **Steinman, H.M.,** 1982. Copper-zinc superoxide dismutase from *Caulobacter crescentus* CB15: A novel bacteriocuprein form of the enzyme. *J. Biol. Chem.* **257**, 10283-10293.
44. **Steinman, H.M.,** 1987. Bacteriocuprein superoxide dismutase of *Photobacterium leiognathi*. *J. Biol. Chem.* **262**, 1882-1887.
45. **Steinman, H.M. and B. Ely,** 1990. Copperzinc superoxide dismutase of *Caulobacter crescentus*: Cloning, sequencing and mapping of the gene and periplasmic location of the enzyme. *J. Bacteriol.* **172**, 2901-2910.
46. **Viglino, P., M. Scarpa, F. Coin, G. Rotilio, and A. Rigo,** 1986. Oxidation of reduced Cu, Zn superoxide dismutase by molecular oxygen: A kinetic study. *Biochem. J.* **237**, 305.

(Received December 8, 1993)
(Accepted February 24, 1994)

초 록: *Escherichia coli* SOD 이중 돌연변이체내에서 세포질과 Periplasm에 분포하는 *Photobacterium leiognathi* CuZnSOD(PSOD)의 기능적 특성

김영곤* · 양미경 (조선대학교 자연과학대학 생물학과)

Photobacterium leiognathi CuZnSOD(PSOD)의 부세포성 분포에 따른 방어효과가 paraquat, 열 쇼크, 과산화수소 그리고 $CuSO_4$의 처리시 각각 조사되었다. 서로 달리 periplasm내에 분포하는 PSOD와 세포질에 분포하는 PSOD 사이의 생리적 특성은 본 연구에서는 조건에 따라 차이를 보였다. Paraquat나 H_2O_2 처리시에는 periplasm에 SOD를 발현하는 세포에서 세포질내에 SOD를 분포하는 경우보다 약간 방호효과가 나았으며 SOD 발현 정도는 이와 일관되게 아주 중요한 변화를 보였다. 그러나 열 쇼크와 $CuSO_4$의 처리시는 각각 이런 현상이 역으로 나타났다.