The Activation of the Rat Copper/Zinc Superoxide Dismutase Gene by Hydrogen Peroxide through the Hydrogen Peroxide-responsive Element and by Paraquat and Heat Shock through the Same Heat Shock Element*

Hae Yong Yoo, Mun Seog Chang, and Hyune Mo Rho‡

From the Department of Molecular Biology and Research Center for Cell Differentiation, Seoul National University, Seoul 151-742, Korea

Copper/zinc superoxide dismutase (SOD1) protects cells against oxidative hazards by the dismutation of superoxide radicals. The promoter activity of the SOD1 gene was increased 3–5-fold by hydrogen peroxide, paraquat (PQ) and heat shock. Functional analyses of the regulatory region of the SOD1 gene by deletions, mutations, and heterologous promoter systems confirmed the induction of the SOD1 gene by H2O2 through the hydrogen peroxide-responsive element (HRE) (between nucleotides −533 and −520). Gel mobility shift assays showed that the existence of an H2O2-inducible protein bound to the oligonucleotide of the HRE. Similar analyses showed that the heat shock activated the SOD1 promoter through the heat shock element (HSE) (between nucleotides −185 and −171). A strong specific far-shifted complex with the oligonucleotide of the HSE was observed by the treatment of heat shock. When cells were treated with PQ, a strong far-shifted complex with the HSE was observed and was competed out by the cold HSE probe, indicating that PQ also activated the SOD1 promoter through the same HSE site. It is very interesting to note that chemical and physical stresses, such as PQ and heat shock, respectively, activated the SOD1 promoter through the same cis-element HSE. These results indicate that the SOD1 was inducible by H2O2 through the HRE and by PQ and heat shock through the same HSE to protect cells from oxidative hazards.

Copper/zinc superoxide dismutase (SOD1) is one of the major cellular defense enzymes that perform a vital role in protecting cells against the toxic effect of superoxide radicals. It catalyzes the dismutation of superoxide radicals (O2−) to oxygen and hydrogen peroxide (1). SOD not only prevents the Fenton reaction and DNA nicking in vitro but also protects against toxicity by H2O2 in vivo without O2− being directly involved in the generation of DNA damage (2). The production and/or removal of superoxides has been observed to play a significant role in a variety of critical homeostatic mechanisms both at the cellular and organismic levels. Because biological macromolecules are targets for the damaging action of abundant oxygen radicals, it is assumed that these increased superoxides should be initially eliminated by SOD. Therefore, the regulation and induction mechanism of the SOD1 gene would be of great interest (3). It has also been reported that SOD1 could prevent oncogenesis and tumor promotion (4), reduce the cytotoxic and cardiotoxic effects of anticancer drugs (5), and protect against reperfusion damage of ischemic tissue (6). A recent report suggested that overexpression of SOD1 and catalase could increase the average lifespan of the fly (7). Lutropin, Ca2+, and reactive oxygen seemed to induce SOD1 in rats (8–10).

Stress conditions (i.e. oxidative stresses, heat shock, osmotic stress, and toxic metals) are deleterious to normal cellular function. To survive those environmental and physiological stresses, all organisms possess specific defense systems to protect themselves from various stresses. Aerobic organisms are continuously exposed to oxygen, which renders them prone to damage generated by oxygen-derived free radicals. Oxidative stress is largely mediated by reactive oxygen species, including superoxide anion (O2−), H2O2, and hydroxyl radical (OH•), which are intermediates of oxygen reduction generated by metal ion catalyzed redox reactions, metabolism of chemicals, and normal physiological activities, including respiration and inflammatory responses to infection (11). The free radicals generated by these mechanisms cause severe damage to critical cellular macromolecules, including nucleic acids, proteins, and lipids (12). Oxidative damage has been strongly correlated with aging and a number of diseases, including Parkinson’s disease, Lou Gehrig’s disease (amyotrophic lateral sclerosis), rheumatoid arthritis, and cancer (11, 13). Therefore, free radical levels must be carefully monitored under both physiological conditions and when generated by environmental stress.

Cells employ a number of defense mechanisms to sense and respond appropriately to oxidative stress. Enzymes such as superoxide dismutases and catalases play critical roles in oxidative stress protection through catalyzing the conversion of reactive oxygen species to less harmful products (12, 14). As has been demonstrated in yeast, cells lacking a functional gene encoding SOD1 are highly sensitive to dioxygen and redox-cycling drugs, fail to grow on respiratory carbon sources, and exhibit increased spontaneous mutagenesis rates and a number of other phenotypes during aerobic growth (15). Free radical scavenging activities are also exacerbated by small antioxidant molecules, including glutathione, thioredoxin, and ascorbic acid (12). In addition to the prevention of oxidative damage, repair mechanisms are employed by cells to remove or repair damaged cellular components, including exo- and endo-
nucleases for DNA damage repair, proteolytic enzymes for degradation of severely damaged proteins, and phospholipases glutathione peroxide/transferase/reductase for degradation and repair of damaged lipids (16).

Superoxide dismutase plays a key role in protection from the damage caused by oxygen radicals. The SOD1 expression is highly induced during environmental stresses. However, little is known about how the environmental signal is transmitted to a transcriptional regulator in cells during oxidative stress such as hydrogen peroxide, paraquat (PQ) and heat shock. In this study, the induction of the SOD1 gene by hydrogen peroxide, PQ, and heat shock was investigated by the transfection of CAT expression vectors containing the rat SOD1 promoter. The deletion, mutation, and heterologous promoter systems were used to identify cis-elements that respond to hydrogen peroxide, PQ, and heat shock for minimizing hazardous effects. A specific interaction of the hydrogen peroxide-responsive element (HRE) binding protein and heat shock element (HSE) binding protein (heat shock factor) was also investigated by gel mobility shift assays. The results demonstrated that the SOD1 was inducible by H$_2$O$_2$ through the HRE and by PQ and heat shock through the same HSE to protect cells from oxidative hazards.

**EXPERIMENTAL PROCEDURES**

**Plasmid Constructions**—The plasmids, containing the upstream region of the rat SOD1 gene (17) and the chloramphenicol acetyltransferase structural gene (CAT), were constructed as described previously (3). For the construction of pHHrEk and pHSrEk, the oligonucleotide of the HRE (between nucleotides –533 and –520) and HSE (between nucleotides –185 and –171) sequence of the SOD1 promoter was cloned into the BamHI site of pBLCAT2, which is derived from pBLCAT2. The plasmid pBLCAT2 has a minimal region (∼80–to ∼51) of the herpes simplex virus thymidine kinase promoter (18). Three copies of the HRE and HSE oligonucleotides were introduced. The plasmid pHHrEk and pHSrEk are mutants of pHrEk and pHSrEk with three copies of the mutated site. Their sequences are as follows: SOD-HRE, 5′-CATGCGGTGGCTAGGAAGCGGATCC-CTCGGCTTTCCTCAG-3′; SOD-3HRE, 5′-GATCGCGGTTCAGGAAGCGGATCC-CTCGGCTTTCCTCAG-3′; SOD-HSE, 5′-CTGGATGGAGAATTCTCCCTT-CTGGATGGAGAATTCTCCCT-3′; SOD-3HSE, 5′-CTGGATGGAGAATTCTCCCTT-CTGGATGGAGAATTCTCCCTT-3′. The insertion of the HRE and HSE consensus and mutant sequences was confirmed by DNA sequencing.

**Cell Culture and Transfection**—Human HepG2 hepatoma cells were grown in Dulbecco’s modified Eagle’s medium/10% fetal calf serum/penicillin G 100 U/ml at 37 °C in humidified incubator (5% CO$_2$, 95% air) at 100 µg/ml and 1 ml/100 mm dish. Cells were seeded into 60-mm plastic dishes by the calcium phosphate DNA coprecipitation method (19). An equal amount (3.0 pmol) of the various constructs was transfected to the culture dishes (30–50% confluence) for 24 h prior to transfection. An amphotericin B at 250 ng/ml. Cells were seeded into 60-mm plastic dishes and maintained for appropriate times. The various chemicals were evaluated on the culture medium at 36 h after transfection, and the cells were thawing. After removing cell debris by centrifugation, cell extracts were prepared by a modified procedure of Andrews and Fuller (21).

**CAT Assay**—The CAT assay was performed as described previously (22). The transfected cells were washed twice with phosphate-buffered saline and harvested. The pellet cells were resuspended in 100 µl of 0.25 M Tris-Cl (pH 7.9) and lysed by three cycles of freezing and thawing. After removing cell debris by centrifugation, cell extracts were first assayed for β-galactosidase activity (23). Equal quantities of proteins were assayed for CAT activity on the basis of β-galactosidase activity. Extracts were incubated with 0.025 µCi of [14C]chloramphenicol, 0.25 M Tris-Cl (pH 7.6), 0.4 mM acetyl coenzyme A for 1 h at 37 °C. The enzyme assay was terminated by adding ethyl acetate. The organic layer was analyzed by TLC with chloroform/methanol (95:5). After autoradiography, both acetylated and unacylated forms of [14C]chloramphenicol were scraped from the plate, and the conversion of chloramphenicol to acetylated form was calculated by measuring radioactivities. The relative CAT activities were calculated from the percentage conversion. Results are the average of three independent experiments.

**Mobility Shift Assay**—The oligonucleotides used for the HRE and HSE sites were synthesized and labeled with [γ-32P]ATP and polynucleotide kinase (23). An equal amount (10 µg) of nuclear extract from each sample was mixed with labeled oligonucleotide for 20 min at 20 °C in a 15-μl solution containing 10 mM HEPES, 100 mM KCl, 5 mM MgCl$_2$, 1 mM dithiothreitol, 1 mM EDTA, 10% glycerol, and 2 µg of poly(dI-dC). The binding reaction mixtures were electrophoresed in 6% acrylamide gels in 0.5 TBE (44 mM Tris, 44 mM boric acid, and 1 mM EDTA). After electrophoresis, the gels were dried and exposed to X-ray film. For competition assays, a binding reaction was performed with an excess of cold probe or competitor DNA. In the supershift assay, the specific polyclonal antibody against Elk1 (Santa Cruz Biotech, Inc) was added to the reaction mixture after the binding reaction.

**RESULTS**

**Activation of the SOD1 Promoter by Hydrogen Peroxide, PQ, and Heat Shock**—SOD1 is one of the major cellular defense enzymes protecting cells against oxidative stress. It was hypothesized that hydrogen peroxide, PQ, and heat shock are involved in the activation of the SOD1 promoter. H$_2$O$_2$ was used as a membrane-permeable reagent, which allows studies of the effects of oxygen radicals in living cells. If hydrogen peroxide is insufficiently removed or if an excess is present, it can react with superoxide to generate the highly toxic hydroxyl radical. PQ is a redox-active compound that is photoreduced and subsequently reoxidized by transfer of its electrons to oxygen, generating superoxide (O$_2$). HepG2 cells were transfected with plasmid pRSP-1633, containing the rat SOD1 upstream region fused to the bacterial CAT. 36 h after transfection, hydrogen peroxide, PQ, and heat shock were separately applied to the transfected cells. The transfected cells were incubated in the presence of 200 µM H$_2$O$_2$, 50 µM PQ and at a temperature of 42 °C, respectively. After 2 h, the CAT activity of each transfected cell was determined (Fig. 1B). These treatments increased the expression of the SOD1 promoter about 3-fold. H$_2$O$_2$ and PQ at concentrations of up to 300 and 100 µM, respectively, did not affect cell viability. As shown in Fig. 2, the time-dependent activation of the SOD1 promoter by H$_2$O$_2$, PQ, and heat shock was observed. The level of CAT activity reached a maximum at 2 h when the cells transfected with pRSP-576 were treated with H$_2$O$_2$. Maximum activity appeared at 1 h with the treatment of PQ and heat shock. It may be possible that the peak of SOD1 activation occurred earlier than 1 h. When the cells transfected with pRSP-576 were treated with...
Induction of SOD1 by \( \text{H}_2\text{O}_2 \), PQ, and Heat Shock

\[ 2 \]

**Fig. 2. Time-dependent activation of the SOD1 promoter by \( \text{H}_2\text{O}_2 \), PQ, and heat shock.** Cells transfected with pRSP-576 were treated with \( \text{H}_2\text{O}_2 \) (A), PQ (B), and heat shock (HS; C) for 0–4 h, and then CAT assays were carried out. The results are the means of three experiments. Relative CAT activity is expressed as compared with CAT activity obtained in untreated cells.

\[ 3 \]

**Fig. 3. Effect of \( \text{H}_2\text{O}_2 \), PQ, and heat shock on the activation of the SOD1 promoter.** A, schematic diagram of the SOD1 promoter from nucleotides −576 to +85 (pRSP-576) and from nucleotides −412 to +85 (pRSP-412) attached to CAT. pRSP-576 contains the HRE site, but pRSP-412 does not. B, schematic diagram of the SOD1 promoter from nucleotides −305 to +85 (pRSP-305) and from nucleotides −55 to +85 (pRSP-55) attached to CAT. pRSP-305 contains the HSE site, but pRSP-55 does not. The transfected cells were treated with factors in the same conditions as Fig. 1B. The numbers in the names of plasmids represent the deletion points of SOD1 5′-flanking sequence. The results are the means of three experiments. Relative CAT activities with (+) and without (−) stress are indicated by the solid and open bars, respectively.

\[ 4 \]

**Fig. 4. Detection of the \( \text{H}_2\text{O}_2 \)-related cis-element.** A, schematic diagram of pRSP-576 and DNA fragment of PRE in the heterologous promoter (tk) attached to CAT (pPREtk). B, effect of \( \text{H}_2\text{O}_2 \) on the SOD1 promoter and PRE in the heterologous promoter. The results are the means of three experiments. Relative CAT activity is expressed as compared with CAT activity obtained in untreated cells.

\[ 5 \]

**Fig. 5. Effect of \( \text{H}_2\text{O}_2 \) on the HRE.** A, schematic diagram of pRSP-576 and DNA fragment of HRE in the heterologous promoter (tk) attached to CAT (pHREtk). B, effect of \( \text{H}_2\text{O}_2 \) on the SOD1 promoter and PRE in the heterologous promoter.

\[ 6 \]

H\( \text{O}_2 \), a 3-fold induction of CAT activity was observed. No induction was observed in cells bearing the plasmid pRSP-412 (Fig. 3C). These results mean that the activation of the SOD1 gene by \( \text{H}_2\text{O}_2 \) may be due to the existence of DNA elements between −576 and −412. The induction by PQ was observed in both transfection experiments with pRSP-576 and pRSP-412 (data not shown). It seems that the cis-element corresponding to induction by PQ was located downstream of −412 of the SOD1 gene. When the SOD-CAT construct (pRSP-305) was transiently expressed in HepG2 cells, 4- and 3-fold induction of CAT activity by heat shock (42 °C) and PQ was observed, respectively (Fig. 3D). No induction was observed in cells bearing the plasmid pRSP-55 (Fig. 3D). These results mean that the activation of the SOD1 promoter by heat shock and PQ may be due to the existence of DNA elements between −305 and −56.

### cis-Element Responsible for the Activation of SOD1 Promoter by \( \text{H}_2\text{O}_2 \)

When the plasmids pRSP-576 and pRSP-412 were transfected into HepG2 cells and exposed to \( \text{H}_2\text{O}_2 \), the induction with \( \text{H}_2\text{O}_2 \) was observed only in the case of transfection with pRSP-576 (Fig. 3C). However, the induction with PQ was observed in both cases (data not shown). These results might suggest that \( \text{H}_2\text{O}_2 \) and PQ regulate the SOD1 gene by different regulatory elements and factors. The region between −576 and −413 was previously identified as a positive regulatory element (PRE) (24). As an initial trial to identify the transcriptional enhancer element that was involved in \( \text{H}_2\text{O}_2 \) induction of the SOD1 gene, transient assays using CAT constructs were carried out. The plasmid pPREtk, which has the PRE linked to the CAT gene, and pRSP-55 as a positive control were transiently expressed in HepG2 cells, 4- and 3-fold induction of CAT activity was observed. No induction was observed in cells bearing the plasmid pRSP-55 (Fig. 3D). These results mean that the activation of the SOD1 promoter by heat shock and PQ was due to the DNA sequences between −305 and −56.

### Heat Shock and PQ Activation of the SOD1 Promoter through the Same HSE

The activation of the SOD1 gene by heat shock and PQ was due to the DNA sequences between −305 and −56 (Fig. 3D). From the sequence analysis of transcription factor binding sites in this region, the heat shock element (NGAAN) (27), was found to be located between −185 and −171 of the SOD1 gene (Fig. 1A). Heat shock treatment of murine macrophage resulted in an enhanced capacity to release superoxide anion (O\( _2^- \)) (28). Also, Omar and Pappolla (29) showed that heat shock protein synthesis was induced by high levels of superoxide anion. Therefore, it was assumed that the activation of the SOD1 promoter by heat shock and PQ may be mediated through the heat shock element. To confirm this possibility, the heat shock element was synthesized and ligated to the herpes simplex virus minimal thymidine kinase promoter proximal to the CAT gene (Fig. 5B). The CAT activity of this construct was analyzed after exposure of cells to either heat shock or PQ (Fig.
served. An HRE oligonucleotide-protein complex disappeared
when a32P-labeled SOD1 HRE probe, one prominent DNA-protein complex was ob-
erved in untreated cells. The probe corresponding to HRE was labeled at
its 5’ end. The DNA-protein complex disappeared by cold competitor
(lane 3) but did not disappear by mutated HRE (mHRE) and was
supershifted by anti-Elk1 antibody (lanes 5 and 6). B, increased binding activity of HRE binding protein in vivo. A gel mobility shift assay was performed using nuclear extracts prepared from
untreated HepG2 cells. The probe corresponding to HRE was labeled with
H2O2 treatment in the cell-free system (Fig. 6).

These results establish that the HSF play some kind of role in
activate gene transcription. These findings indicate that the HSE is sufficient to confer the
inducibility is mediated by HSF. These findings indicate that the HSE is sufficient to confer the
inducibility is mediated by HSF.

HRE Binding Protein and Increased Binding Activity Be-
cause of H2O2—To determine the nuclear factors that interact
with the HRE sequence, gel mobility shift assays were carried out by using a double-stranded oligonucleotide corresponding
to HRE as a probe (Fig. 6). When a nuclear extract prepared from HepG2 cultures was incubated with a32P-labeled SOD1
HRE probe, one prominent DNA-protein complex was ob-
served. An HRE oligonucleotide-protein complex disappeared
disappeared by self-competition (Fig. 7, lane 4). The formation of
the protein-DNA complex was not inhibited with a mutated HRE
oligonucleotide, indicating the specificity of this protein-DNA
interaction. The binding of Elk1 to the HRE was further con-
firmed by the result that the protein-DNA complex was supershifted by the addition of the anti-Elk1 antibody (Fig. 6A, lane 6). These results imply that Elk1 is the binding protein of HRE. When nuclear proteins prepared from the H2O2-treated HepG2
cells (2 h) were used, the intensity of the specific complex increased (Fig. 6B). The binding activity of the HRE-binding factor was also investigated in the cell-free system (Fig. 6C). Nuclear extract prepared from nontreated HepG2 cells was incubated with H2O2 (200 μM) to examine the variation of its
binding activity to the HRE site. The incubation of the nuclear extract with H2O2 resulted in increasing the intensity of the
retarded complex (Fig. 6C, lane 3) and disappearance by com-
petition with the cold probe (Fig. 6C, lane 4). Because the
intensity of the specific HRE-protein complex was increased by
H2O2 treatment in the cell-free system (Fig. 6C, lane 3), it was also assumed that hydrogen peroxide treatment increased the
binding activity of the HRE binding protein.

HSE Binding Protein (Heat Shock Factor) and Change of
Binding—Gel mobility shift assays were used to detect se-
quence-specific binding of factors to the SOD1 HSE sequence in
extracts from HepG2 cells. Nuclear extracts were prepared from
cells that had been heat treated at 42 °C for 1 h or
maintained at the control temperature of 37 °C. These extracts were incubated with a 5’ end-labeled oligonucleotide contain-
ing an HSE sequence (between sequence –185 and –171 of the
SOD1 gene). In reactions with extracts from the control cells, two sets of retarded bands were observed (Fig. 7). Bands pro-
duced by sequence-specific factor binding were identified by
competition assays with unlabeled DNA fragments. Unlabeled
HSE fragments competed efficiently with the formation of a
faster-migrating protein-DNA complex (Fig. 7, lanes 3 and 4). No such competition was observed with the mHSE oligonucleo-
tide (Fig. 7, lane 5). The formation of the more slowly migrating
complex was not in competition with the HRE fragment. Thus,
the latter complexes did not result from sequence-specific fac-
tor binding to the HSE sequences. When nuclear proteins pre-
pared from the heat-treated cells were used, a strong specific far-shifted complex was observed. This strong new complex
disappeared by self-competition (Fig. 7, lanes 9 and 10). The
HSF in eukaryotes acquire high affinity DNA binding activity
upon heat shock activation by conversion from a monomer to
homo trimer (30). Therefore, the new far-shifted complex may
be a homotrimer HSF-DNA complex. Analogous results were
obtained from experiments with nuclear extracts from cells
treated with PQ for 1 h (Fig. 8A). A far-shifted complex disap-
or H2O2 are produced in mitochondria production. Oxygen free radicals appeared with the cold probe. 

The DNA-protein complex disappeared by cold competitor (lanes 5 and 11) and nonspecific competitor (lanes 6 and 12). Using nuclear extracts prepared from untreated and PQ-treated HepG2 cells. The active oxygens generated in these locations may regulate certain cellular genes by inducing early response genes.

To mount an appropriate oxidative stress defense, cells must harbor oxidative stress sensors. A number of antioxidant responses in bacteria have been elegantly studied, and it has been established that reactive oxygen species are directly sensed by key regulatory molecules that activate the expression of genes encoding antioxidant proteins at the level of transcription (32-34). Distinct defense mechanisms are involved in H2O2 and O2- detoxification in Escherichia coli through the OxyR and SoxRS regulons, respectively. OxyR directly senses oxidative stress to activate the expression of H2O2-inducible genes, including those encoding catalase and alkyl hydroperoxide reductase (34). The SoxRS regulon is controlled in a two-stage process. First an iron-sulfur protein, SoxR, is activated by increases in intracellular superoxide anion levels and triggers transcription of the soxS gene. The SoxS protein in turn induces transcription of other genes of the regulon, including those encoding manganese superoxide dismutase, the DNA repair endonuclease IV, and glucose-6-phosphate dehydrogenase (32, 33, 35). The oxidative stress response and its relationship to heat shock phenomena are also being intensely investigated in eukaryotic systems. Unlike the bacterial systems described earlier, little is known about how eukaryotic cells co-ordinate gene expression in response to oxidative stress (34).

Heat shock proteins are known to protect cells from thermal and oxidative injuries as well as other types of injuries (36). In this study, we have found that the paraquat, O2- generating agent, could activate the SOD1 gene through the heat shock element. Heat shock may cause activation of the membrane-associated oxidase system directly or indirectly through HSP, resulting in increased O2- production. Oxygen free radicals induce heat shock protein synthesis in cultured human neuroblastoma cells (29). These findings suggest a common mechanism by which various forms of injury, such as hyperthermia, cause HSP induction, that is, via oxidative stress or increased production of oxygen free radicals.

The results presented in this study showed that the rat SOD1 gene was inducible by hydrogen peroxide, paraquat, and heat shock. It was shown that the HRE sequence is one of the targets of transcriptional activation by H2O2. Also, we have found that superoxides could activate the SOD1 gene. Because the steady state concentration of H2O2 in the cell is so low (1 × 10^-8 M) and specific enzymes (i.e. catalase, glutathione peroxidase, and myeloperoxidase) actively convert H2O2 to H2O in a fast speed, the resulting hydrogen peroxide by SOD1 is thought not to activate the SOD1 gene by the positive feedback mechanism. In this experiment, the promoter activation was observed at the concentration around 100 μM of H2O2. Active oxygens are suggested to be involved in inflammatory responses; the activations by H2O2 of c-fos, c-jun, and egr-1, which activate the expression of cellular genes, are probably essential for these responses. DNA damaging agents have been reported to activate c-fos and AP1-inducible genes such as those for collagenase and metallothionein (31). Active oxygens such as O2- or H2O2 are produced in mitochondria during oxidative electron transport and in the endoplasmic reticulum, peroxisomes, and nuclear and plasma membranes. The active oxygens generated in these locations may regulate certain cellular genes by inducing early response genes.

DISCUSSION

In transient expression experiments the activation of the SOD1 promoter by H2O2 was mediated through the HRE sequence. To analyze the target of transcriptional activation of the SOD1 gene, CAT plasmids were constructed that contain these elements in the upstream region of the thymidine kinase promoter. From these results, it was shown that the HRE...
sity of SOD1 inducers implies that there are multiple regulatory elements for the proper adjustment to various conditions.

REFERENCES

1. Fridovich, I. (1975) *Annu. Rev. Biochem.* **44**, 147–159
2. Imlay, J. A., and Linn, S. (1988) *Science* **240**, 1302–1309
3. Kim, Y. H., Park, K. H., and Rho, H. M. (1996) *J. Biol. Chem.* **271**, 24539–24543
4. McCord, J. M., Keele, B. B., Jr., and Fridovich, I. (1971) *Proc. Natl. Acad. Sci. U. S. A.* **68**, 1024–1027
5. Huber, W., and Menander, K. B. (1980) *Clin. Rheum. Dis.* **6**, 465–498
6. Fridovich, I. (1983) *Annu. Rev. Pharmacol. Toxicol.* **23**, 238–257
7. Orr, W. C., and Sohal, R. S. (1994) *Science* **263**, 1128–1130
8. Laloraya, M., Pradeep, K. G., and Laloraya, M. M. (1988) *Biochem. Biophys. Res. Commun.* **157**, 267–271
9. Brown, J. R. H. (1995) *Cell* **80**, 687–692
10. Beyer, W., Imlay, J., and Fridovich, I. (1991) *Prog. Nucleic Acids Res. Mol. Biol.* **40**, 221–253
11. Davies, K. J. A., Wiese, A. G., Sevanian, A., and Kim, E. H. (1990) *Molecular Biology of Aging*, pp. 123–141, Alan R. Liss, New York
12. Fridovich, I. (1975) *Annu. Rev. Biochem.* **44**, 147–159
13. Chen, C., and Okayama, H. (1988) *BioTechniques* **6**, 632–638
14. McCord, J. M., Keele, B. B., Jr., and Fridovich, I. (1971) *Proc. Natl. Acad. Sci. U. S. A.* **68**, 1024–1027
15. Huber, W., and Menander, K. B. (1980) *Clin. Rheum. Dis.* **6**, 465–498
16. Fridovich, I. (1983) *Annu. Rev. Pharmacol. Toxicol.* **23**, 238–257
17. Orr, W. C., and Sohal, R. S. (1994) *Science* **263**, 1128–1130
18. Laloraya, M., Pradeep, K. G., and Laloraya, M. M. (1988) *Biochem. Biophys. Res. Commun.* **157**, 267–271
19. Brown, J. R. H. (1995) *Cell* **80**, 687–692
20. Beyer, W., Imlay, J., and Fridovich, I. (1991) *Prog. Nucleic Acids Res. Mol. Biol.* **40**, 221–253
21. Davies, K. J. A., Wiese, A. G., Sevanian, A., and Kim, E. H. (1990) *Molecular Biology of Aging*, pp. 123–141, Alan R. Liss, New York
22. Fridovich, I. (1975) *Annu. Rev. Biochem.* **44**, 147–159
23. Fridovich, I. (1983) *Annu. Rev. Pharmacol. Toxicol.* **23**, 238–257
24. Fridovich, I. (1983) *Annu. Rev. Pharmacol. Toxicol.* **23**, 238–257
25. Fridovich, I. (1983) *Annu. Rev. Pharmacol. Toxicol.* **23**, 238–257
26. Halliwell, B., and Gutteridge, J. M. C. (1984) *Biochem. J.* **219**, 1–14
27. Halliwell, B. (1994) *Nutr. Rev.* **52**, 253–265
28. Halliwell, B. (1994) *Nutr. Rev.* **52**, 253–265
29. Halliwell, B. (1994) *Nutr. Rev.* **52**, 253–265
30. Halliwell, B. (1994) *Nutr. Rev.* **52**, 253–265
31. Halliwell, B. (1994) *Nutr. Rev.* **52**, 253–265
32. Halliwell, B. (1994) *Nutr. Rev.* **52**, 253–265
33. Halliwell, B. (1994) *Nutr. Rev.* **52**, 253–265
34. Halliwell, B. (1994) *Nutr. Rev.* **52**, 253–265
35. Halliwell, B. (1994) *Nutr. Rev.* **52**, 253–265
36. Halliwell, B. (1994) *Nutr. Rev.* **52**, 253–265
37. Halliwell, B. (1994) *Nutr. Rev.* **52**, 253–265
38. Halliwell, B. (1994) *Nutr. Rev.* **52**, 253–265
39. Halliwell, B. (1994) *Nutr. Rev.* **52**, 253–265
40. Halliwell, B. (1994) *Nutr. Rev.* **52**, 253–265
41. Halliwell, B. (1994) *Nutr. Rev.* **52**, 253–265
42. Halliwell, B. (1994) *Nutr. Rev.* **52**, 253–265
43. Halliwell, B. (1994) *Nutr. Rev.* **52**, 253–265
44. Halliwell, B. (1994) *Nutr. Rev.* **52**, 253–265
45. Halliwell, B. (1994) *Nutr. Rev.* **52**, 253–265
46. Halliwell, B. (1994) *Nutr. Rev.* **52**, 253–265
47. Halliwell, B. (1994) *Nutr. Rev.* **52**, 253–265
48. Halliwell, B. (1994) *Nutr. Rev.* **52**, 253–265
49. Halliwell, B. (1994) *Nutr. Rev.* **52**, 253–265
50. Halliwell, B. (1994) *Nutr. Rev.* **52**, 253–265