The Manganese Superoxide Dismutase of *Escherichia coli* K-12 Associates with DNA*

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Superoxide dismutases (SODs) are vital components in the resistance of aerobic organisms to the toxicity of oxygen. *Escherichia coli* contains two highly homologous cytoplasmic SODs, a manganese- and an iron-containing enzyme (MnSOD, FeSOD). We previously demonstrated that MnSOD and FeSOD have different physiological functions and that MnSOD is more effective in preventing oxidative damage to DNA. In this report, purified *E. coli* MnSOD was shown to bind nonspecifically to DNA by electrophoretic mobility shift assay and nitrocellulose filter binding methodologies. From electrophoretic mobility shift assay, the equilibrium dissociation constants for interaction with a variety of double-stranded and single-stranded oligonucleotides ranged from 1.5 ± 0.2 to 8.4 ± 1.3 μM at 20 °C. This range of concentrations corresponds to MnSOD concentrations in aerobically grown *E. coli*. *In vivo* binding of MnSOD to DNA was supported by co-localization of MnSOD and the *E. coli* nucleoid in immunoelectron microscopy. Both MnSOD and DNA were inhomogeneously distributed in the cytosol, the concentration of each being higher in the center of the cell and relatively low near the inner membrane. In contrast, there was no evidence for physiologically relevant interaction of FeSOD with DNA. Binding to DNA *in vitro* was weak, *K*  40–220 μM, concentrations 7–40 times higher than found *in vivo*. In addition, the cytoplasmic distribution of FeSOD did not correlate with DNA. FeSOD concentration was higher near the inner membrane and lower in the center of the cytosol. These results demonstrate that *E. coli* MnSOD associates with DNA *in vitro* and *in vivo*. Combined with prior data demonstrating that MnSOD preferentially protects DNA *in vivo* while an equal enzymatic activity of FeSOD does not (Hopkin, K. A., Papazian, M. A., and Steinman, H. M. (1992) *J. Biol. Chem.* 267, 24253–24258), our data suggest that *E. coli* MnSOD acts as a “tethered antioxidant”; association of MnSOD with DNA localizes dismutase activity near a target of oxidative stress and increases protection of DNA from oxidative damage. This model has implications for the therapeutic use of SODs as antioxidants.

The superoxide dismutases (SODs)\* are a family of metalloenzymes that catalyze the decomposition of superoxide free radical. The importance of SODs in the defense against reactive oxygen intermediates (ROI) was first documented in *Escherichia coli* K-12. *E. coli* contains a manganese- and an iron-containing SOD (MnSOD, FeSOD) that reside in the cytosol. A mutant of *E. coli* lacking both Mn- and FeSOD is extremely sensitive to the oxidative stresses of hyperoxia hydrogen peroxide, and to paraquat and other drugs that generate superoxide radical. The phenotypes of this mutant have shown that DNA, certain 4Fe4S enzymes, and the cell envelope are targets of ROI damage that are protected by *E. coli* SODs (1–8).

The Mn- and FeSOD of *E. coli* are 44% identical in amino acid sequence and have identical rate constants for superoxide dismutation. Why does *E. coli* need 2 cytoplasmic enzymes so similar in structure and activity? Regulation has been proposed as one answer. MnSOD is inducible in response to oxidative stress, while FeSOD is constitutive. We recently identified a second answer from physiological comparisons of isogenic *E. coli* strains containing equal specific activity of MnSOD or FeSOD. The 2 SODs serve different physiological roles *in vivo* and preferentially protect different targets. MnSOD is more effective in preventing damage to DNA. Assessed by rate of mutation to rifampicin-resistance and resistance to killing by paraquat in rich medium, DNA damage is reduced by 50%. FeSOD is more effective in preventing inactivation of a superoxide-sensitive 4Fe4S enzyme. With FeSOD, the specific activity of 4Fe4S 6-phosphogluconate dehydratase is close to 50% that in a SOD* strain. With MnSOD, the dehydratase activity is no greater than in a SOD* control. These results were the first demonstration of functional differences between any SODs (9).

It is unlikely that differences in *in vivo* kinetic parameters of MnSOD and FeSOD are a major reason for their functional differences. If that were the case, one SOD would be a better *in vivo* catalyst of superoxide dismutation, consistently more effective in preventing oxidative damage irrespective of the target of oxidative stress. Our data clearly showed target specificity. To explain the functional differences, we proposed a model based on analysis of SOD structure. MnSOD is more basic (pI = 6.9 compared with 4.9 for FeSOD). The extra basic regions lie at the carboxyl terminus and in a loop. These sites are adjacent in the three-dimensional structure and could comprise a locus for association of MnSOD with an anionic ligand such as the polyphosphate in DNA. Since this site is far from the subunit interface and the active site, it could mediate MnSOD binding to DNA without impairing SOD activity or stability. We hypothesized that *E. coli* MnSOD is more effective in protecting DNA because it binds DNA *in vivo* and *E. coli* FeSOD does not (9–10).

We now report that purified *E. coli* MnSOD binds DNA in electrophoretic mobility shift assay (EMSA) and nitrocellulose filter binding assays. Binding occurs at physiologically relevant SOD concentrations and is nonspecific with respect to DNA sequence and structure. In addition, we report that MnSOD localizes with the *E. coli* nucleoid in immunoelectron microscopy. By contrast, interaction of *E. coli* FeSOD with DNA requires SOD concentrations far in excess of those found *in vivo*, and the cytosolic distribution of FeSOD is quite different from

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\* The abbreviations used are: SOD, superoxide dismutase; ROI, reactive oxygen intermediates; EMSA, electrophoretic mobility shift assay.
that of MnSOD and DNA. In sum, our data indicate that E. coli MnSOD preferentially associates with DNA in vitro and in vivo. Our prior results (9) can now be viewed with additional significance. When the physiological function of two homologous superoxide dismutases were compared at equal specific activities, enhanced protection of DNA was shown only by the SOD that associates with DNA. We therefore propose that by association with DNA, MnSOD becomes a tethered antioxidant that can preferentially protect DNA from ROI damage.

**EXPERIMENTAL PROCEDURES**

**Materials—**E. coli Mn- and FeSOD (Sigma) were brought to 0.1 mM potassium phosphate, pH 7.2, by repeated concentration and dilution in a Centricon-10 (Amicon). Protein concentration was determined by UV absorbance (1). By SDS-polyacrylamide gel electrophoresis, MnSOD was greater than 95% pure, and FeSOD was slightly less. There was no contamination of one SOD with the other based on activity stains following nondenaturing gel electrophoresis (11). Monospecific rabbit polyclonal antibodies to each SOD, raised to SOD preparations different from those used here, have been described before (12).

A 635-bp pair restriction fragment containing the promoter region of the E. coli gal operon and part of the galE gene was obtained as a 34-mer was end-labeled with 32P and purified as described above (13). This restriction fragment was end-labeled with 32P and purified as described before (14).

Oligonucleotides, synthesized at the Oligonucleotide Synthesis Facility of Albert Einstein College of Medicine, were purified by electrophoresis in 15% acrylamide followed by phenol and chloroform treatments and ethanol precipitation. For annealing single stranded oligonucleotides, 100 ml of 1 x T4 kinase buffer containing 2 uM each of oligonucleotide was heated for 2 min in 300 ml of boiling water that was then cooled overnight to room temperature. The 34-mer was 5'-CTCTCTAGCTGAATAACCGGAAGTAACTCATCG-3'. This 34-mer was then cooled overnight to room temperature. The 34-mer was 5'-CTCTCTAGCTGAATAACCGGAAGTAACTCATCG-3'. This 34-mer was then cooled overnight to room temperature. The 34-mer was then cooled overnight to room temperature.

**RESULTS AND DISCUSSION**

**DNA Binding by E. coli MnSOD—**We previously proposed that preferential protection of DNA by MnSOD could be attributed to binding of DNA by MnSOD and not by FeSOD (9). We report here the results of EMSA directly demonstrating DNA binding by purified MnSOD. Initial experiments conducted with a 640-base pair restriction fragment are shown in Fig. 1. A band-shift is clearly observed between 2 and 15 pmol MnSOD dimer. No DNA bands of intermediate mobility are seen for MnSOD; the radioactivity in top and bottom bands, respectively, of the transition curve (14). The values of K may not represent the intrinsic binding constants since the number of SOD molecules capable of binding to the short oligonucleotides was not determined.

**Nitrocellulose Filter Binding—**Binding reactions were conducted as described above and then filtered at a constant flow rate of 1 ml/min through Millipore HAW 0.45-µm filters (17). Filters were immediately washed with 0.5 ml of binding buffer and placed while moist into scintillation fluid in counting vials. The titration curves were analyzed as described above.

**Electronic Microscopy—**E. coli strain MG1655 (wild type, SOD') was grown to mid log phase in LB and M9-glucose minimal media at 37 °C. Chilled cells were fixed in 0.1 M cacodylate buffer containing 3% paraformaldehyde and 0.1% Tween 20. Grids were blocked with TST containing 0.1% nonfat dry milk and then incubated overnight at room temperature with primary antibody in TST and milk. After washing, grids were incubated for 1 h at room temperature with secondary antibody. After washes with TST and milk and TST and water, grids were poststained with uranyl acetate and lead citrate and viewed with a JEOL 1200 EY electron microscope. For E. coli SODs, the primary antibodies were rabbit polyclonal antibodies described above and secondary antibody goat anti-rabbit IgG conjugated to 10-nm colloidal gold (EY Laboratories). For DNA, the primary antibody was a mouse monoclonal anti-DNA antibody, class IgG, purified on a protein G column, and the secondary antibody was protein A conjugated to 15-nm colloidal gold (EY Laboratories). The anti-DNA antibody was tested for double stranded DNA binding activity with ELISA and antinuclear antigen test (ANA test kit, Immuno Concepts). Digestion of the band containing the unbound oligonucleotide. Separate rectangles were obtained for LB and M9 glucose media. The concentration of MnSOD dimer in the assay ranged from 0.62-0.05 pmol. The DNA had no effect on the rate of spontaneous autooxidation.

**DNA Binding by E. coli MnSOD—**We previously proposed that preferential protection of DNA by MnSOD could be attributed to binding of DNA by MnSOD and not by FeSOD (9). We report here the results of EMSA directly demonstrating DNA binding by purified MnSOD. Initial experiments conducted with a 640-base pair restriction fragment are shown in Fig. 1. A band-shift is clearly observed between 2 and 15 pmol MnSOD dimer. No shift is observed at concentrations of FeSOD up to 56 µM FeSOD dimer. No DNA bands of intermediate mobility are seen for MnSOD; the radioactivity in lanes 7 and 8 is located in the sample well at the top of the gel. This result suggests that multiple molecules of the MnSOD bind to the long fragment, implying nonspecific association with DNA.

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The concentration of SOD dimer increased in was binding buffer with 75 mM KCl. D designates the lane with free DNA. Anode is at the right.

Rich medium and 8 and 4 MnSOD occurred over a range of MnSOD concentrations that are estimated to be about 12 and 1.6 FeSOD is at least 2:1. Using this value, MnSOD and FeSOD, respectively. It is noteworthy that the DNA-binding transition for growth is about 18 (21). From our experience and data in the literature (22, 23), any significant DNA binding by MnSOD is the predominant activity, and the ratio of MnSOD:FeSOD is from 0.26 to 33. From rich and 12 MnSOD was even lower than estimated above. These considerations and the experimental data clearly established that DNA binding was uniquely attributable to MnSOD, antibody inhibition of DNA binding was demonstrated.

Antibody-mediated supershifting, the decreased mobility of a protein-DNA complex in the presence of antibody, has been used to implicate an antigen as the DNA-binding protein. Supershifting was not feasible here, because complexes of MnSOD with oligonucleotides did not enter the gel. Thus, an antibody-mediated reduction in mobility would not be evident. However, binding sites for antibody and DNA may overlap or an antibody may interfere in some other way with DNA binding. In that case, net binding to DNA should decrease in the presence of antibody specific for the DNA binding protein. With antibody present, the binding curve should shift toward higher concentrations of the DNA-binding protein. This is the approach we used.

The effect of monospecific polyclonal antibody on binding of E. coli MnSOD to the 34-mer duplex oligonucleotide is shown in Fig. 2. The presence of antibody unambiguously shifted the binding curve to higher MnSOD concentrations compared with the control. This shift corresponded to a factor of 3 difference in $K_d$. We consider these changes significant based on the following arguments. First, in an experiment with polyclonal antibody to FeSOD at the same concentration as MnSOD antibody, the binding curve for MnSOD did not shift. That is, MnSOD associated with DNA equally well in the presence of anti-FeSOD and in the absence of any antibody (data not shown). Second, the shift was significant because the MnSOD antibody was not in excess. The molar ratio of antibody/MnSOD ranged from 0.4 to 1.0 in the region where the curve was shifted to the right. For this calculation, we assumed that 10% of the antibody was antigen-specific, a reasonable figure for a polyclonal antibody. Finally, some of the anti-MnSOD antibody was expected to bind MnSOD without reducing DNA binding. Thus, the actual ratio of inhibitory antibody/MnSOD was even lower than estimated above. These considerations and the experimental data clearly established that MnSOD was the DNA-binding protein.

Fig. 2 also shows that DNA binding of this 34-mer to FeSOD...
(●) is considerably weaker, consistent with earlier data. A complete titration curve could not be obtained for FeSOD (Fig. 2). In order to establish a minimal value for the $K_d$ of FeSOD, a value of 1.0 was assumed for the upper limit of the titration curve. This assumption is reasonable since at binding saturation, the optical density of the free DNA band being quantitated approaches the background. It is clear that binding could be considerably weaker and the true $K_d$ could be considerably larger. A similar situation was found with FeSOD for other binding conditions and other DNA ligands (see below).

**Nitrocellulose Filter Binding**—Binding to the flash end 34-mer duplex in the 75 mM potassium chloride buffer was independently assessed by the nitrocellulose filter binding assay, a methodology based on very different principles from EMSA. No detectable binding was observed for FeSOD. For MnSOD, binding was comparable with that assessed by EMSA (Fig. 3). The midpoints for the binding transition for both assays were from -0.6 to -0.3 for MnSOD. The $K_d$ values, equilibrium dissociation constants calculated from curve fitting, were 1.8 ± 0.3 and 3.0 ± 0.5 M for EMSA and filter binding, respectively.

**Binding to Other Oligonucleotides: Effects of DNA structure and salt concentration**—Quantitative band shift analysis was used to study binding of SODs to duplex oligonucleotides with 5' and 3' overhangs, an internal base mismatch and to single-stranded DNA. Duplexes with 4-base overhangs were prepared by annealing the single-stranded 34-mer (see “Experimental Procedures”) to 38-mers. The internal mismatch was a GA base pair, analogous to the misincorporation of A that occurs opposite of the oxidatively damage G, 8-hydroxyguanine (24). In most studies, ionic strength was altered by changing the concentration of potassium glutamate. Glutamate is the major anion of *E. coli* proteins with a concentration that far exceeds that of chloride (25). The equilibrium dissociation constants, $K_d$, are listed in Table I. The following conclusions can be drawn.

Consistent with the initial observations, the affinity of FeSOD for DNA was substantially less than MnSOD for all DNA ligands and at all salt concentrations. Under certain conditions (asterisks in Table I) DNA binding was too weak to accurately estimate the equilibrium dissociation constant of FeSOD from the binding data using the procedure described above. On the average, the estimated $K_d$ for FeSOD was greater than that for MnSOD by at least a factor of 30–40, for the same DNA ligand and salt conditions. Secondly, $K_d$ for MnSOD varied only slightly with the DNA ligand. The presence of 3'- or 5'-overhangs or an internal one-base mismatch did not dramatically alter the interaction of MnSOD with DNA.

Finally, the effect of salt on MnSOD binding was modest. Increasing KCl concentration from 75 to 250 mM increased the equilibrium dissociation constant by a factor of 2 and 4, for duplex and single-stranded DNA, respectively. This was considerably less than the effect of ionic strength on DNA binding of 2 well characterized DNA binding proteins, bacteriophage T4-coded gene 32 protein, and *E. coli* single-stranded binding protein. For these proteins, the salt dependence of binding, $\delta \log K/\delta \log (\text{salt})$, was $-0.5$ to $-7$ (26, 27). For MnSOD, the corresponding values were $-0.6$ and $-1$ for dsDNA and single-stranded DNA, i.e. a factor of 6–10 smaller. This result suggested that a small number of phosphates on DNA were involved in association with MnSOD and a small number of ions were displaced on binding. This is consistent with our analysis of SOD three-dimensional structure that identified a basic patch in MnSOD containing 4 additional Lys and Arg residues not present in FeSOD (9).

**Intracytoplasmic Location of MnSOD and FeSOD by Immunelectron Microscopy**—The preceding data established that MnSOD binds DNA in vitro. We used immunogold electron microscopy to obtain evidence for an in vivo association between MnSOD and DNA. The *E. coli* nucleoid is condensed toward the center of the cytoplasm (28, 29). Therefore, the concentration of DNA is expected to be lower near the inner

![Image](https://example.com/image.png)

**Figure 3.** Filter retardation assay of DNA binding. The DNA ligand was the blunt, 34-mer oligonucleotide duplex and binding conditions the same as for Fig. 1. Binding was quantitated by the nitrocellulose filter retardation method for FeSOD (●) and MnSOD (○). Binding quantified by EMSA for MnSOD (■).

**Table I**

| Oligonucleotide | $K_d$ |
|-----------------|-------|
| **A. Duplex DNA** |       |
| Salt | $\mu$M | 75° | 150° | 250° |
| SOD | -7 | 1.8 ± 0.3 | 2.5 ± 0.4 | 3.6 ± 0.6 |
| Fe | >79 | >130 | >40 |
| KCl | Mn | 6.0 ± 1 | 4.2 ± 0.5 | 5.0 ± 0.8 |
| Fe | >110 | >66 | >130 |
| 5' Overhang | Mn | 8.4 ± 0.8 | 4.2 ± 1.2 | 2.1 ± 0.3 |
| Fe | >93 | >220 |
| 3' Overhang | Mn | 5.0 ± 0.8 | 3.6 ± 0.7 | 2.1 ± 0.3 |
| Fe | >190 | >56 | >130 |
| Mismatch | Mn | 3.6 ± 0.6 | 6.1 ± 1 | 4.2 ± 1.2 |
| Fe | >56 | * | * |
| **B. Single-stranded DNA** |       |
| Salt | $\mu$M | 75° | 150° | 250° |
| SOD | Mn | 1.5 ± 0.2 | 2.1 ± 0.3 | 6.0 ± 1 |
| Fe | >190 | >130 | >66 |
| KCl | Mn | 6.0 ± 1.4 | ND | 8.4 ± 2.2 |
| Fe | >93 | ND | * |

* indicates salt concentration (mM).
$\beta$ Binding too weak to estimate $K_d$ by curve fitting.
ND, not determined.
The cytosol was divided into four concentric regions, region 1 being grown in LB medium, as described under "Experimental Procedures." For MnSOD and DNA, the relative density of gold particles in each region was determined as described under "Experimental Procedures." The relative density in region 1 and that at right depicts relative density in region 4. The data show that the distribution of MnSOD was identical to that of DNA. Both antigens were present at lower concentrations near the inner membrane and higher concentrations at inner regions of the cytosol. For MnSOD and DNA, the relative density for region 1 is statistically different from that for regions 2–4 (p < 0.001 by one-way analysis of variance). The distribution of FeSOD was different and reciprocal to that of MnSOD and DNA. FeSOD is more concentrated near the inner membrane and higher in the central region of the cytosol. If MnSOD binds DNA to any significant degree, the distribution of MnSOD should be the same as that of the nucleoid. Since in vitro binding of FeSOD to DNA is very weak, occurring at higher than physiological concentrations, no significant in vivo association with DNA is expected. The distribution of FeSOD should then be different from that of MnSOD.

To determine the distribution of MnSOD, FeSOD, and DNA, the cytoplasmic region of E. coli in the electron micrographs was divided into four concentric regions, region 1 being closest to the inner membrane and region 4 encompassing the cell center. The relative density of gold particles in each region was determined as described under "Experimental Procedures" for MnSOD, DNA, and FeSOD. The results are shown in Fig. 4. Within each set of bars, the bar at the left depicts relative density in region 1 and that at right depicts relative density in region 4. The data show that the distribution of MnSOD was identical to that of DNA. Both antigens were present at lower concentrations near the inner membrane and higher concentrations at inner regions of the cytosol. For MnSOD and DNA, the relative density for region 1 is statistically different from that for regions 2–4 (p < 0.001 by one-way analysis of variance). The distribution of FeSOD was different and reciprocal to that of MnSOD and DNA. FeSOD is more concentrated near the inner membrane and at lower concentrations in the cell interior. Values of relative density for FeSOD in regions 1 and 2 are statistically different from values for inner cytosol regions 3 and 4 (p < 0.001 by one-way analysis of variance).

Colocalization of MnSOD and the E. coli nucleoid was clearly demonstrated by the immunoelectron microscopy data. The results agreed exactly with the above predictions and provided evidence that MnSOD associates with DNA in vivo. In addition, the data showed that even though both E. coli Mn- and FeSOD are cytoplasmic, they are distributed differently and neither is found homogeneously throughout the cytosol. The increased concentration of FeSOD on the cytoplasmic side of the inner membrane (Fig. 4) is consistent with the osmotic shock data (23). FeSOD is easily released by osmotic shock while MnSOD is not. Other cytoplasmic proteins in E. coli have been shown to be readily released by osmotic shock but retained in spheroplasts (30). These proteins were presumed to be localized on the cytoplasmic side of the inner membrane and thus more readily released by osmotic shock. However, this hypothesized localization was not corroborated as was done here with immunoelectron microscopy for FeSOD. An asymmetric distribution of cytoplasmic glycolytic enzymes in Zymomonas mobilis has been shown by immunoelectron microscopy (20).

Conclusions and Significance—The short lifetime of reactive oxygen intermediates such as superoxide radical has been a major consideration in understanding their biological effects. How can ROI be as damaging as they are or as vital to regulation of gene expression if they exist only transiently in vivo? Site-specific generation of ROI has been proposed as an answer. The biological consequences of ROI may be enhanced if they are generated at the site of their biological effects. For example, metal chelation and site-specific generation have been demonstrated in cleavage of DNA by ROI (31, 32). Oxidative inactivation of E. coli glutamine synthetase and modification of albumin have been associated with a metal binding site and local generation of the inactivating ROI species (33, 34).

A similar question can be raised for antioxidant defenses against short-lived ROI. How can an antioxidant enzyme be effective if its substrate has such a short lifetime in vivo? Our data suggest an answer. Just as site-specific generation may be crucial in the chemistry of short-lived ROI, optimal protection may involve "tethered antioxidants," i.e. the site-specific presence of antioxidants near sensitive targets of ROI damage. From a combination of five independent approaches, four experimental studies, and an analysis of SOD protein structure, we propose that the physiological function of E. coli MnSOD is consistent with the tethered antioxidant model.

1) We have shown that E. coli Mn- and FeSOD have different functions in vivo (9). We made an osmotic deletion mutant and introduced Mn- and FeSOD plasmids permitting comparison of strains containing equal amounts of SOD activity attributable to either Mn- or FeSOD. At equal mutarotate activity, the MnSOD and FeSOD strains showed differences in sensitivity to oxidative stress. The strain with MnSOD showed a 2-fold greater protection of DNA assessed by mutation rate and sensitivity to the redox active drug paraquat. The strain with FeSOD showed dramatically increased protection of a superoxide-sensitive FeS enzyme. These results clearly demonstrated that SOD metalloisoenzymes exhibit differential protection of in vivo targets of ROI and that the MnSOD was more effective in the protection of DNA.

2) Binding of purified MnSOD was demonstrated in vitro by EMSA and filter binding techniques. Transitions occurred in the range of physiologically meaningful concentrations of MnSOD. Binding of purified FeSOD to DNA was substantially weaker and at concentrations not physiologically relevant.

3) Colocalization of MnSOD with the E. coli nucleoid was demonstrated. FeSOD was distributed in the cytosol in a manner that was contrary to that of the nucleoid.

4) Consistent with the tethered antioxidant hypothesis, we demonstrated that the specific activity of MnSOD is not decreased by excess DNA. For the experiments with purified MnSOD described under "Experimental Procedures," values of 0.049 ± 0.004 units µl−1 and 0.050 ± 0.004 units µl−1 were determined in the presence and absence of salmon sperm DNA.

5) Analysis of the primary and three-dimensional structures of E. coli Mn- and FeSOD suggests that the preferred protection of DNA by the manganese isoenzyme was attributable to its increased basicity. A basic patch and potential site for DNA interaction was identified in Mn- but not in FeSOD (9). This patch was not adjacent to the metal cofactor or the dimer interface, suggesting that interaction with DNA could occur without compromising the catalytic activity or stability of MnSOD DNA in E. coli.
MnSOD. That is, structural analysis suggested that DNA binding and superoxide dismutase function could function independently. The combination of these five approaches provides a strong argument that MnSOD acts at least in part as a tethered antioxidant, localized near DNA and thus reducing ROI-mediated mutational damage. Knowing now that MnSOD of \textit{E. coli} binds DNA and FeSOD does not, our prior physiological studies become a critical aspect of this argument. They demonstrated that when homologous superoxide dismutases are present in the cell at identical SOD activity, enhanced protection of DNA is seen only with the SOD that binds DNA \textit{in vitro} and \textit{in vivo}. This is rather convincing evidence in favor of MnSOD being a tethered antioxidant.

The DNA binding activity of \textit{E. coli} MnSOD is a new activity for this enzyme. DNA binding is not indicative of a new function but is related to the function of SOD in the defense against oxidative stress. The nonspecific binding of MnSOD to DNA is covalent with a protein that widely binds to and protects DNA from ROI damage. The electron microscopy data are consistent with a significant fraction of \textit{E. coli} MnSOD being bound to DNA. If only a small fraction of MnSOD were bound to DNA \textit{in vitro}, it is unlikely that the cytosolic distributions of DNA and MnSOD would be different, as we found. Assuming 10 μM MnSOD (21) in aerobically grown \textit{E. coli} and 50% bound to DNA, there would be one SOD dimer every 1000 base pairs. This is a by association with an SOD. We observed that mutational rates at sites in the 5'-end of SOD hydratase or inaccessible from DNA folding. The role of MnSOD in protection of DNA can be tested further by fusing MnSOD with a prototethered antioxidant.

In the variety of clinical studies in which oxidative stress is etiologically implicated (36, 37). The above considerations may be pertinent to increasing the efficacy of SODs as therapeutic agents.

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