Thiol Oxidase Activity of Copper,Zinc Superoxide Dismutase*

Christine C. Winterbourn‡, Alexander V. Peskin, and Helena N. Parsons-Mair

From the Department of Pathology, Christchurch School of Medicine and Health Sciences, P. O. Box 4345, Christchurch, New Zealand

The ability of copper,zinc superoxide dismutase (Cu,Zn-SOD) to catalyze autoxidation of cysteine and other thiols was investigated by measuring thiol loss and oxygen consumption. The reaction occurred equally well with the bovine and human enzymes and produced hydrogen peroxide and the corresponding disulfide. It did not occur with manganese SOD and is not, therefore, due to the dismutase activity of the enzyme. Cysteine and cysteamine were highly reactive: the $K_m$ for cysteine was 1.4 mM and $V_{max}$ (with 40 $\mu$g/ml SOD) 35 $\mu$M/min; the equivalent values for cysteamine (with 20 $\mu$g/ml SOD) were 1.4 mM and 36 $\mu$M/min. With 1 mM thiol and 40 $\mu$g/ml SOD, rates of oxidation of other thiols ($\mu$M/min) were as follows: GSH, 1.0; dithiothreitol, 2.1; dihydroli- poic acid, 1.7; homocysteine, 1.6; cysteine, 1.4; penicilla- mine, 0.6; and N-acetylcysteine, 0.1. SOD-mediated oxida- tion of cysteine, in the absence of chelating agents, proceeded only after a variable lag phase. The lag was decreased but not eliminated with Chelex-treated re- agents and is attributed to interference by submicromolar concentrations of iron and possibly other transition metal ions. SOD-catalyzed oxidation of the other thiols was variably affected by adventitious metal ions and chelating agents. Reactions were all performed in the presence of desferoxamine to obviate these effects. SOD-catalyzed oxidation of GSH and homocysteine was enhanced by cysteine through a thiol-disulfide ex- change mechanism. This study characterizes a novel pro-oxidant thiol oxidase activity of Cu,Zn-SOD. It is a potential source of reactive oxidants and may contribute to the cytotoxicity of reactive thiols such as cysteine and cysteamine.

Superoxide dismutase (SOD) is an essential component of the antioxidant defense of aerobic organisms. Three forms of the enzyme, namely Cu,Zn-SOD, Mn-SOD, and extracellular Cu,Zn-SOD, are found in mammals (1). Their prime function is to catalyze dismutation of superoxide (Reaction 1).

$$\text{O}_2^- + \text{SOD} \rightarrow \text{Cu}^{2+} \rightarrow \text{O}_2 + \text{SOD} + \text{Cu}^+$$

$$\text{O}_2^- + 2\text{H}^+ + \text{SOD} \rightarrow \text{Cu}^2+ \rightarrow \text{H}_2\text{O}_2 + \text{SOD} + \text{Cu}^2+$$

**REACTION 1**

A key feature of the enzyme is the maintenance of high dis- mutase activity while shielding the active site copper from other redox transformations. However, side reactions have been documented (2–5), and superoxide dismutase and reductase activities, involving in each case only one-half of the copper redox cycle, have recently been reported (6, 7).

If SOD does have superoxide reductase activity, then thiols are potential biological substrates. Thiols are generally viewed as antioxidants. GSH is present intracellularly in millimolar concentrations and acts with glutathione peroxidase and glutathione reductase to remove peroxides. GSH and other thiols may also scavenge radicals and in combination with SOD constitute an efficient radical removal system (8–12). However, thiols can act as pro-oxidants by undergoing autoxidation to produce hydrogen peroxide (13–17). The reaction is metal-cat- alyzed, with copper being a particularly effective catalyst. Au- toxidation is considered to be important in the well established cytotoxicity of low molecular weight thiols (18, 19). Cu,Zn-SOD, if it were a catalyst of this reaction, could be a promoter of thiol toxicity.

We have investigated the ability of Cu,Zn-SOD to catalyze autoxidation of cysteine, GSH, and a range of physiological relevant thiols. In previous studies of superoxide-mediated thiol oxidation, we noted that addition of SOD to some thiols alone appeared to promote oxidation (10). Others have made similar observations with cysteine (20, 21). The explanation given, that superoxide somehow protects cysteine against oxida- tion (15, 21), is not consistent with current knowledge of how superoxide interacts with thiols (11, 12). After further investiga- tion, we report here that the enzyme does not act in these systems simply by dismutating superoxide. Instead, we demon- strate a novel activity of Cu,Zn-SOD, namely the catalysis of thiol oxidation by oxygen. This thiol oxidase activity is high for cysteine and cysteamine but much lower with GSH and a range of other biological thiols.

**EXPERIMENTAL PROCEDURES**

Materials—Unless stated otherwise, all biochemicals, including thiol compounds, were purchased from Sigma. Catalase, bovine erythrocyte, and liver Cu,Zn-SOD, human Cu,Zn-SOD, and Mn-SOD were used without further purification. Human erythrocyte Cu,Zn-SOD was iso- lated in our laboratory from red cell lysate by successive chromatogra- phy on DEAE-Sepharose and phenyl-Sepharose and ran as single systems simply by dismutating superoxide. Instead, we demon- strate a novel activity of Cu,Zn-SOD, namely the catalysis of thiol oxidation by oxygen. This thiol oxidase activity is high for cysteine and cysteamine but much lower with GSH and a range of other biological thiols.

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Christine C. Winterbourn‡, Alexander V. Peskin, and Helena N. Parsons-Mair

From the Department of Pathology, Christchurch School of Medicine and Health Sciences, P. O. Box 4345, Christchurch, New Zealand

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were carried out at 37 °C with a rapid decrease in absorbance shown. SOD has a characteristic Cu(II) absorbance peak with a dismutation. It is not simply a consequence of its ability to catalyze superoxide dismutation. Inhibitory experiments with Mn-SOD caused no loss of cysteine. This cysteine oxidation at a comparable rate to those with the bovine erythrocyte Cu,Zn-SOD and bovine liver Cu,Zn-SOD also caused Cu,Zn-SOD there was rapid thiol loss (Fig. 1). Human erythrocyte Cu,Zn-SOD (c141, EŒ), bovine liver Cu,Zn-SOD (b11032), and with catalase (10 M SOD in 50 mM phosphate, pH 7.4, at 37 °C. Cysteine (Cys) or GSH was added at the arrow. Absorbances have been corrected for initial dilution effects.

As Cu2+ is an excellent catalyst of thiol oxidation, it was important to exclude the possibility that cysteine oxidation was simply due to adventitious or loosely bound copper in the enzyme preparation. With free Cu2+, we observed oxidation of cysteine (1 mM) at a rate of 50 μmol/minute/μmol Cu2+, i.e. about 10 times faster on a molar basis than with SOD. Others have analyzed Sigma bovine Cu,Zn-SOD preparations and found less than 3% of the copper to be loosely bound (28). Analyses that we performed, by the method of Crow et al. (5), gave results consistent with this figure. To test directly if cysteine oxidation could have been caused by adventitious copper, we pretreated the SOD with a 5-fold molar excess of DTPA, then removed the DTPA on a spin column. Dismutase activities and protein concentrations were measured before and after passage through the column to account for losses. When related to either measure, rates of thiol oxidation by the treated and untreated enzyme were identical, even with three successive DTPA treatments and passages through the column. Extensive dialysis of the SOD against Chelexed phosphate buffer, either with or without pretreatment with DTPA, also did not diminish its ability to promote cysteine oxidation. Thus, it is unlikely that this activity was caused by bound copper.

Cysteine loss in the presence of Cu,Zn-SOD was accompanied by oxygen consumption. The mole ratio of thiol loss to oxygen consumed, measured over 20 min in the same solution, was 3.8 ± 0.3 (n = 4). The oxygen consumption rate was decreased, to about 73%, by the addition of catalase, suggesting that hydrogen peroxide was produced in the reaction. Any hydrogen peroxide formed would be expected to cause additional cysteine oxidation (10, 29). We routinely added catalase when measuring thiol oxidation to avoid complications due to this reaction. In the absence of catalase (with 1 mM cysteine and 40 μg/ml SOD), cysteine oxidation was 1.38 ± 0.12 times faster (n = 5) than with catalase present. The effect of catalase was the same at 4–20 μg/ml and independent of whether the enzyme was Chelex-treated. These results are consistent with hydrogen peroxide being produced in Reaction 2 and oxidizing further cysteine as in Reaction 3.

\[
\text{SOD} \quad \text{O}_2 + 2\text{RSH} \rightarrow \text{H}_2\text{O}_2 + \text{RSSR} \quad \text{REACTION 2}
\]

\[
\text{H}_2\text{O}_2 + 2\text{RSH} \rightarrow 2\text{H}_2\text{O} + \text{RSSR} \quad \text{REACTION 3}
\]

As described below, SOD also promoted GSH autoxidation but at a very much lower rate. GSSG formation was measured in

![Fig. 1. Time course of cysteine oxidation in the presence of bovine erythrocyte Cu,Zn-SOD (○), bovine liver Cu,Zn-SOD (●), human Cu,Zn-SOD (△), Mn-SOD (▼), or no SOD (□). Reactions were carried out at 37 °C with 40 μg/ml (1.2 μM) SOD in Chelex-treated phosphate buffer, pH 7.4, with 1 μg desferrioxamine and 10 μg/ml catalase added. Results are means ± S.D. for three determinations at each time point. For human SOD, data are shown for the enzyme isolated in our laboratory, but similar results were obtained with the Sigma preparation. Inset shows early time points for bovine erythrocyte Cu,Zn-SOD.](image1.png)

![Fig. 2. Changes in Cu(II) absorbance at 680 nm on incubation of Cu,Zn-SOD with cysteine or GSH. Reagent concentrations were 1 mM thiol, 200 μM SOD in 50 mM phosphate ± 20 μM DTPA, pH 7.4, at 37 °C. Cysteine (Cys) or GSH was added at the arrow. Absorbances have been corrected for initial dilution effects.](image2.png)
TABLE I
Thiol Oxidase activity of bovine Cu,Zn-SOD

| Thiol            | Thiol oxidation | μM/min |
|------------------|-----------------|--------|
| Cysteamine       | 19.2 ± 3.1 (8)^a |        |
| Cysteine         | 11.7 ± 1.2 (8)^a |        |
| Dithiothreitol   | 2.1 ± 0.2 (4)^a  |        |
| Dihydrolipoic acid| 1.7 ± 0.2 (4)^a |        |
| Homocysteine     | 1.6 ± 0.4 (4)^a  |        |
| Cysteine-glycine | 1.4 ± 0.2 (4)^a  |        |
| Reduced glutathione| 1.0 ± 0.3 (4)^a |        |
| Penicillamine    | 0.6 ± 0.3 (4)^a  |        |
| N-Acetylcysteine | 0.1 ± 0.0 (4)^a  |        |

^a p < 0.001.
^b p = 0.02.

this reaction and accounted for at least 80% of the GSH loss. This is also consistent with Reaction 2. Although the hydrogen peroxide formed could potentially inactivate the SOD (2), pre-exposure of the SOD to cysteine (in the absence of catalase), then dialysis, caused no loss of dismutase activity or ability to promote further cysteine oxidation (data not shown).

Thiol Reactivity Profile—The reactivities of various physiologically relevant thiol compounds with bovine Cu,Zn-SOD were determined (Table I). Cysteine reacted almost twice as rapidly as cysteine. With all the other thiols, addition of SOD caused a significant increase in oxidation rate, but they were at least 5-fold less reactive that cysteine. It is noteworthy that the major intracellular thiol, GSH, was oxidized very slowly. High activity was not simply a property of aminothiols or associated with low thiol group pK, as penicillamine was poorly oxidized. DTT, dihydrolipoic acid, homocysteine, and Cys-Gly showed 1.5–2 times the reactivity of GSH, but N-acetylcysteine was almost inactive.

Although SOD showed very little oxidase activity with GSH and homocysteine, addition of cysteine promoted the oxidation of both thiols, as demonstrated by thiol loss in excess of the cysteine added (Fig. 3).

DTT is frequently added at high concentration to cell extracts containing SOD. Therefore, we measured cysteine oxidation rates at higher DTT concentrations. Rates of oxidation were linear over 2 h and increased linearly with DTT concentration to approximately 0.4 μM/h with 4 μM DTT (8 μM thiol; not shown).

Kinetics of Oxidation of Cysteine and Cysteamine by Cu,Zn-SOD—The rates of loss of cysteine and cysteamine increased with increasing thiol concentration (Fig. 4A). In each case there was evidence of saturation. With 40 μg/ml (1.2 μM) SOD, cysteine showed a K_m of 1.4 μM and V_max of 35 μM/min. Cysteamine (with 20 μg/ml enzyme) also showed a K_m of 1.4 μM and V_max of 3.2 μM/min. The rate of cysteine oxidation was linearly dependent on SOD concentration, but with cysteamine, the curve flattened above 20 μg/ml enzyme (Fig. 4B). Thus at maximum efficiency, the turnover numbers for cysteine and cysteamine were ~30 and 60/min, respectively. The rate of cysteine oxidation increased more than 10-fold when the SOD was inactivated by heating at 105°C for 40 min. This is not surprising as boiling the enzyme would release the active site copper. Adding a 2- or 4-fold excess of Mn-SOD over Cu,Zn-SOD (per catalytic site) did not affect the rate of cysteine oxidation (rate with Mn-SOD = 99 ± 10% of that without Mn-SOD, n = 4). For cysteamine, under the same conditions as for Fig. 1, initial rates of oxidation at pH 7.0, 7.4, and 8.0 were 9.3 ± 0.4, 8.9 ± 0.1, and 9.2 ± 0.4 μM/min, respectively, indicating no pH dependence in this range.

The Effect of Chelating Agents and Iron—With desferrioxamine present, initial rates of SOD-mediated thiol oxidation were linear (as shown for cysteine in Fig. 1). In the absence of chelating agents, the kinetics were very different. For cysteine (Fig. 5) there was a lag phase, and the overall oxidation rate was decreased. Oxidation rates varied unpredictably depending on the buffer preparation and were substantially increased.
FIG. 5. Time course for oxidation of cysteine in Chelex-treated buffer containing 40 μg/ml (1.2 μM) SOD and 10 μM (□) or 1 μM (■) desferrioxamine (data for both conditions fall on the same line); Chelex-treated buffer containing 10 μM DTPA (○), Chelex-treated buffer with no chelator (●), untreated buffer with no chelator (□). Other conditions are as described in the legend to Fig. 1.

FIG. 6. Time course for cysteine oxidation in Chelex-treated buffer with 40 μg/ml (1.2 μM) SOD and no addition (○), with 0.5 μM FeSO₄ (■), with 1 μM desferrioxamine (□), and with 1 μM desferrioxamine and 0.5 μM FeSO₄ (△). Other conditions are as described in the legend to Fig. 1.

with less lag, if the reagents were treated with Chelex resin. Adding the product of the reaction, cystine, at the start did not overcome the lag (not shown). DTPA almost eliminated the lag but gave a slower overall rate than with desferrioxamine (Fig. 5). The rate of cysteine oxidation was the same in the presence of 1 or 10 μM desferrioxamine, either with 1.2 μM SOD (Fig. 5) or with 6 μM SOD (not shown). This indicates that it was not necessary to have as much chelator as active site copper. Rates in the presence of desferrioxamine were the same in Chelexed and unChelexed buffer (not shown).

One possible explanation for the results shown in Fig. 5 is that the chelators acted by removing and activating loosely bound copper from the enzyme and that in the absence of chelator, cysteine alone slowly displaced the metal, accounting for the lag. This can be discounted on the basis that DTPA pretreatment did not decrease the activity of the enzyme (see above). Furthermore, the lag phase was still evident with SOD that had been pretreated with DTPA, and it disappeared when further DTPA was added. This suggests that the chelator was interacting with a constituent of the reaction mixture and not with the enzyme.

This complex set of observations could be explained by the reagents containing trace amounts of adventitious transition metal ion(s) that affect the rate of cysteine oxidation by SOD and give rise to a lag phase. The results with desferrioxamine point to iron being involved. Ehrenberg et al. (29) have noted that iron can inhibit the autoxidation of cysteine catalyzed by free copper ions. We have found this inhibition to be extremely potent, such that with 0.4 μM Cu²⁺, ferrous sulfate has an IC₅₀ of about 50 nM. Adding submicromolar concentrations of Fe²⁺ ions to SOD and cysteine caused a progressive decrease in the rate of cysteine oxidation. As shown for 0.5 μM iron in Fig. 6, the effect of the metal was entirely abrogated by desferrioxamine. These results provide strong support for the conclusion that traces of iron (and perhaps other transition metals) in the reagents interfere with SOD-mediated cysteine oxidation and that to study the system without this complication it is necessary to add desferrioxamine.

EDTA and DTPA were not as effective as desferrioxamine at eliminating the effects of adventitious metals, and in the presence or absence of either of these chelators, rates of cysteine oxidation varied in the pH 7–8 range (data not shown). SOD-mediated oxidation of the other thiols was also influenced by adventitious metals, but not necessarily in the same way as with cysteine. This complex interaction with metal ions shows similar features to their effect on thiol oxidation by free copper ions. A mechanistic study of the effects of trace metals and chelators on the thiol reactivity of copper and SOD will be published elsewhere.

DISCUSSION

We have shown that Cu,Zn-SOD promotes the autoxidation of low molecular weight thiols. The reaction involves reduction of the active site copper, consumes oxygen, and produces hydrogen peroxide and disulfide. It is not a consequence of the dismutase activity of the enzyme, as no thiol oxidation was seen with Mn-SOD. Rather, it is a characteristic of the Cu,Zn enzyme, and as demonstrated with cysteine, the human and bovine enzymes exhibit comparable reactivity. The oxidase activity was particularly high with the aminothiols, cysteine, and cysteamine. Other thiols, including GSH, were much less reactive.

It is well known that copper ions are efficient catalysts of thiol autoxidation and that aminothiols are more susceptible than DTT or GSH to copper-catalyzed oxidation (16, 17, 20, 29). On a molar equivalent basis, the cysteine oxidase activity we observed with SOD was about a tenth that of free Cu²⁺. This activity was not due to adventitious or loosely bound copper that could be removed by pretreatment of the enzyme with chelator. Bovine SOD from erythrocytes and liver, and human erythrocyte SOD prepared by two different methods, all had comparable reactivity. It is unlikely, therefore, that it was due to a contaminant or partially denatured enzyme, as these would vary between preparations. We conclude that the active site copper was involved and that Cu,Zn-SOD has thiol oxidase activity. Although less active than free copper, from a physiological perspective this activity of Cu,Zn-SOD is potentially significant. At a typical cell concentration (~100 μg/ml), SOD acting at its maximum efficiency could consume about 0.1 mM cysteine/min, with concomitant hydrogen peroxide production. Even with GSH, which reacted more than 10 times more slowly, this reaction could be a significant source of hydrogen peroxide in the cell.

Regarding the mechanism of thiol oxidation, our observation of saturating effects with cysteine and cysteamine suggests that an intermediate complex with the enzyme is involved. The effect of inhibition by excess Mn-SOD indicates that superoxide is not required to oxidize the reduced SOD. It also suggests a two-electron transfer directly from oxygen to the complex to regenerate the Cu(I) form of the enzyme and a sulfenic acid equivalent, rather than release of thiol radicals and subse-

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2 R. Munday and C. Winterbourn, unpublished data.
quent superoxide generation. One possibility is the scheme proposed in Reactions 4–6, in which the sulfenic acid would react with more thiol to give the disulfide and the SOD would be available to complex more cysteine. This is similar to the inner sphere mechanism proposed for free Cu$^{2+}$, although there is equivocal evidence for at least some superoxide production (13, 15–17, 30). Cu$^{2+}$ forms a 1:2 complex with cysteine (31). With SOD, where there is a narrow (4 Å) hydrophobic channel (32), it is more likely that only one cysteine could access the active site copper or that there is long range electron transfer.

$$\text{SOD} - \text{Cu}^{2+} + \text{RSH} \rightarrow [\text{SOD} - \text{Cu}^{2+} - \text{RS}] \leftrightarrow \text{SOD} - \text{Cu}^{2+} - \text{RS} + H^+$$

**REACTION 4**

$$[\text{SOD} - \text{Cu}^{2+} - \text{RS}] + O_2 + H^+ + H_2O \rightarrow \text{SOD} - \text{Cu}^{2+} + \text{RSOH} + H_2O_2$$

**REACTION 5**

$$\text{RSH} + \text{RSOH} \rightarrow \text{RSSR} + H_2O$$

**REACTION 6**

By generating hydrogen peroxide, the reaction between CuZn-SOD and thiols is a potential source of reactive oxidants in biological systems. The toxicity of thiols to cells is well documented, with the aminothiols, cysteine and cysteamine, being most potent (18, 19, 33–38). In a number of these studies, hydrogen peroxide formed by autoxidation has been implicated in the toxicity and a copper-mediated mechanism proposed. Our results suggest that physiologically, CuZn-SOD is a likely catalyst of this reaction. There are other instances where CuZn-SOD has been seen to enhance the toxicity of cysteine (39, 40). The effect was attributed to copper released from the enzyme damaged by oxidative modification, but our findings suggest that SOD itself may have been responsible.

GSH was much less reactive than cysteine and was minimally oxidized in our experimental system. This low reactivity should allow millimolar concentrations of GSH to coexist with CuZn-SOD in the cell without generating large amounts of peroxide. This would not be true for cysteine. Maintenance of low cyst(e)ine concentrations and evolution of GSH as the major cytosolic thiol have largely alleviated the pro-oxidant threat of SOD and readily oxidized thiols. Radical scavenging by GSH generates superoxide, so that for effective radical removal, the concerted action of GSH and SOD is required (8, 9). The low reactivity between the two should not compromise this antioxidant function.

Intracellular GSH could, however, contribute to the toxicity of reactive thiols such as cysteine. We observed that small amounts of cysteine promoted autoxidation of GSH, presumably via SOD-catalyzed oxidation of cysteine and a disulfide exchange mechanism to recycle the cystine formed. An elevation in intracellular cyst(e)ine, therefore, has the potential to expose all the GSH to oxidation. With enzymatic recycling of the GSSG, this could generate large amounts of hydrogen peroxide.

Promotion of homocysteine oxidation by cysteine and SOD was also observed. Elevated homocysteine is a recognized risk factor in cardiovascular disease (41), and production of superoxide and hydrogen peroxide from homocysteine autoxidation could be a contributing factor to the disease (42). Autoxidation of homocysteine is slow, even in the presence of copper ions, but it can be accelerated by small amounts of cyst(e)ine (43). Our finding that the same occurs with CuZn-SOD suggests that it may be more relevant than copper as a physiologically catalyst.

Of the other thiols we examined, Cys-Gly and dihydrolipoic acid showed comparable reactivity to GSH with CuZn-SOD, whereas penicillamine and N-acetylcysteine were less reactive. Especially at the concentrations likely to be encountered physiologically, we would predict they would cause little oxidative stress. DTT is frequently added to cell extracts or biological samples in high millimolar concentrations. Therefore, its reaction with CuZn-SOD has practical implications. Even though relatively slow, this reaction would be expected to give a steady rate of hydrogen peroxide production and may result in oxidative inactivation of extract constituents.

We observed that CuZn-SOD-mediated thiol oxidation was influenced by adventitious metal ions. Whether this is relevant in a physiological context is unclear, but it has practical implications. Initially we observed highly variable and unpredictable kinetics of cysteine oxidation, which we now attribute to interference by traces of iron and possibly other metal ions that we were unable to remove from the reagents. Oxidation of other thiols was also affected, in ways that we shall describe in more detail elsewhere. Interference was eliminated by adding micromolar desferrioxamine. DTPA also affected the oxidation rate, but its effect was not identical to that of desferrioxamine, and it is likely that iron complexed to DTPA still influences the reaction. Thus, experimenters should be aware that CuZn-SOD-mediated thiol oxidation is open to interference by adventitious metal ions and that this will vary depending on the purity of reagents, pH, and whether a chelator is present. Desferrioxamine can be added to overcome this complication. We have no simple explanation for how metal ions inhibit cysteine oxidation by CuZn-SOD. Substoichiometric amounts relative to the active site copper were effective, suggesting that the mechanism involves interference with electron transfer between the cysteine-copper complex and oxygen rather than forming a more oxygen-resistant complex. We and others (29) have shown that low iron concentrations inhibit cysteine oxidation by free copper, and it is likely that a similar mechanism is involved with SOD.

Although SOD has generally been considered strictly as an enzyme that breaks down superoxide, there is interest in other possible activities, particularly in the context of its association with familial amyotrophic lateral sclerosis (1). Peroxidase activity that proceeds via the oxidation of bicarbonate to a radical cation has been documented for CuZn-SOD (2–4) as has catalysis of tyrosine nitration via peroxynitrite (5). CuZn-SOD has been reported to cause breakdown of nitrosothiols to generate NO (44), although others propose that this involves hydrogen peroxide-mediated release of copper (45). This activity appears to be 2–3-fold higher with mutants associated with amyotrophic lateral sclerosis (46). Liochev and Fridovich have addressed the question of whether the superoxide oxidase and reductase activities of SOD can be dissociated (6) and proposed a superoxide reductase activity of CuZn-SOD in the catalysis of 3-hydroxyanthranilic acid oxidation (7). With thiols, we suggest that the enzyme does not classify as a superoxide reductase but rather acts as a thiol oxidase. This novel pro-oxidant function of CuZn-SOD has the potential to generate substantial amounts of hydrogen peroxide and may contribute to the toxicity of thiols such as cysteine and cysteamine and the disruption of cellular thiol equilibrium.

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