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DNA and RNA strand scission by copper, zinc and manganese superoxide dismutases

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Copper/zinc (Cu/ZnSOD) and manganese (MnSOD) superoxide dismutases which catalyze the dismutation of toxic superoxide anion, O_2^-, to O_2 and H_2O_2, play a major role in protecting cells from toxicity of oxidative stress. However, cells overexpressing either form of the enzyme show signs of toxicity, suggesting that too much SOD may be injurious to the cell. To elucidate the possible mechanism of this cytotoxicity, the effect of SOD on DNA and RNA strand scission was studied. High purity preparations of Cu/ZnSOD and MnSOD were tested in an *in vitro* assay in which DNA cleavage was measured by conversion of phage ϕX174 supercoiled double-stranded DNA to open circular and linear forms. Both types of SOD were able to induce DNA strand scission generating single- and double-strand breaks in a process that required oxygen and the presence of fully active enzyme. The DNA strand scission could be prevented by specific anti-SOD antibodies added directly or used for immunodepletion of SOD. Requirement for oxygen and the effect of Fe(II) and Fe(III) ions suggest that cleavage of DNA may be in part mediated by hydroxyl radicals formed in Fenton-type reactions where enzyme-bound transition metals serve as a catalyst by first being reduced by superoxide and then oxidized by H_2O_2. Another mechanism was probably operative in this system, since in the presence of magnesium DNA cleavage by SOD was oxygen independent and not affected by sodium cyanide. It is postulated that SOD, by having a similar structure to the active center of zinc-containing nucleases, is capable of exhibiting non-specific nuclease activity causing hydrolysis of the phosphodiester bonds of DNA and RNA. Both types of SOD were shown to effectively cleave RNA. These findings may help explain the origin of pathology of certain hereditary diseases genetically linked to Cu/ZnSOD gene.

Keywords: *in vitro* DNA/RNA cleavage, oxygen radicals, superoxide dismutase, transition metals

Introduction

Superoxide dismutase (SOD) is a family of metalloenzymes ubiquitously present in all oxygen-consuming organisms (McCord *et al.* 1971, Gregory *et al.* 1974). SODs catalyze the dismutation of the toxic superoxide anion, O_2^-, yielding O_2 and H_2O_2 (McCord & Fridovich 1969), and together with catalases and peroxidases constitute the first line of the defense against the damaging effect of reactive oxygen species. In mammalian tissues there are three types of SOD: cytosolic Cu/ZnSOD, mitochondrial MnSOD and extracellular (EC)SOD (Bannister *et al.* 1987). These enzymes are encoded by separate genes (Sinet 1977) and are structurally unrelated (Harris & Steinman 1977).

Although the primary role of SOD is to provide defense against toxicity of oxygen in which the superoxide radical plays a major role (Fridovich 1978), the overproduction of SOD does not result in increased protection but rather creates a variety of unfavorable effects. It has been reported that elevated intracellular SOD activity sensitizes bacteria to ionizing radiation (Scott *et al.* 1987), induces cell killing (Norris & Horrisby 1990), enhances lipid peroxidation (Elroy-Stein *et al.* 1986),

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interferes with the transport of biogenic amines (Elroy-Stein & Groner 1988) and causes hemolysis (Mavelli *et al.* 1984). In Down's syndrome, where there is an extra copy of the Cu/ZnSOD gene (Summit 1981), overproduction of the enzyme has been found to be a primary cause of many symptoms of the disease (Epstein *et al.* 1987, Avraham *et al.* 1988). Some of those unfavorable effects may be linked to the presence of transition metals in the active center of the enzyme and their ability to catalyze the formation of free radicals in Fenton-type reactions, considered to be the primary mechanism of oxidative damage to many biomolecules (Halliwell & Gutteridge 1990). Indeed, there are reports in which Cu/ZnSOD (Yim *et al.* 1990, Sato *et al.* 1992) and hemoproteins (Davies 1988) have been shown to generate the formation of hydroxyperoxide-derived radicals, supporting the view that protein-bound transition metals can catalyze this re-action. All the above evidence leads to the assumption that SOD itself may have the potential to induce damage to nucleic acids.

Using supercoiled phage DNA and well-defined RNA run-off transcript as substrates in an *in vitro* assay, we tested the ability of high purity commercial preparations of Cu/ZnSOD and MnSOD to induce DNA strand breaks or cleavage of RNA. We have found that both types of SOD can mediate DNA/RNA strand scission through two different mechanisms, one involving reactive oxygen species (ROS) and the second involving non-specific nuclease-like cleavage.

Materials and methods

Chemicals and reagents

Sodium cyanide, diethyldithiocarbamic acid, EDTA, riboflavin, Nitro-blue tetrazolium, actin, magnesium chloride, and ferrous and ferric chlorides were from Sigma (St Louis, MO). Bovine serum albumin was purchased from Calbiochem (La Jolla, CA). High purity preparations of human erythrocytes Cu/ZnSOD were purchased from Sigma and Fluka Chemie (Buchs, Switzerland). Human placental recombinant Cu/ZnSOD was obtained from Calbiochem. *Escherichia coli* MnSOD was from Sigma. Purified (IgG fraction) polyclonal antibodies against human Cu/ZnSOD were obtained from The Binding Site (Birmingham, UK) or from Calbiochem and those against human retinoblastoma protein from Oncogen Science (Uniondale, NY). Anti-human catalase polyclonal antibodies were from Calbiochem. Protein A–agarose beads were supplied by Schleicher & Shuell (Kenee, NH). ϕX174 RF I and M13 RF I DNA was from New England BioLabs (Beverly, MA). Electrophoretic grade agarose was from Gibco/BRL (Gaithesburg, MD). RNA transcription kit was purchased from Stratagene (La Jolla, CA).

Assay for DNA strand breaks

DNA strand breaks were measured by conversion of supercoiled ϕX174 RF I double-stranded DNA to open circular (single-strand breaks) and linear (double-strand breaks) forms. The standard conditions of the assay were as follows: 0.2 μg of DNA were mixed with 12 units of SOD in Chelex-purified water and incubated at 37°C at a final volume of 20 μl for 3 or 18 h. An incubation time of 18 h was used for convenience even though a 3 h incubation showed no difference in the patterns of the reactions. Anaerobic incubations were performed in an argon saturated water overlaid with few drops of mineral oil. Samples were analyzed by electrophoresis through a 1% agarose slab gel in a TAE buffer. After electrophoresis gels were stained with 0.5 μg ml^{-1} solution of ethidium bromide and photographed on Polaroid Type 55 positive/negative film. The percentage of DNA in each form was determined densitometrically after scanning negatives with a Scanmaster 3+ (Howtek, Hudson, NH) scanner and processing the data by image analysis computer software from BioImage System (Millipore, Bedford, MA).

Assay for RNA cleavage

RNA substrate consisted of a run-off transcript of a 500 bp cDNA fragment of a hamster hexokinase gene subcloned into pBluescript plasmid. The transcription reaction was carried out using reagents from the RNA transcription kit (Stratagene) under conditions recommended by manufacturer. RNA (0.3 μg) was mixed with 12 units of SOD in nuclease-free water and incubated at 37°C in a final volume of 20 μl for 3 h. Samples were analyzed by electrophoresis through 2% agarose gel. RNA cleavage was estimated by changes in the electrophoretic mobility of the substrate.

Assay for SOD activity

The method employed was essentially that of Winterbourne *et al.* (1975) and is based on the ability of SOD to inhibit the superoxide caused reduction of nitro-blue tetrazolium (NBT) to the blue formazan. Superoxide radicals were generated photochemically during the spontaneous reoxidation of photo-reduced riboflavin. A series of enzyme samples ranging from 0.1 to 10 μg was prepared in 2.7 ml of 0.067 M potassium phosphate buffer, pH 7.8, to which 0.2 ml of 0.1 M EDTA/0.3 mM sodium cyanide and 0.1 ml of 1.5 mM nitro-blue tetrazolium were added. Tubes were placed in a light box and incubated for 10 min to achieve a standard temperature. At zero time and at timed intervals, 0.05 ml of 0.12 mM riboflavin were added followed by incubation in the light box for 12 min. The percent of inhibition of NBT reduction was determined spectrophotometrically by measuring absorbtion at 560 nm.

Immunodepletion of SOD

Protein A–agarose-bound antibodies (PAS-Ab), were made by mixing 100 μl of 19 mg ml^{-1} IgG fraction of anti-human Cu/ZnSOD antibodies overnight at 4°C with 2 mg of preswollen PAS beads. Antibody-coated beads were then washed five times with 1 ml of binding buffer [20 mM HEPES, pH 8, 150 mM NaCl, 0.05% (v/v) Triton X-100] and resuspended in 120 μl of the same buffer. For immunodepletion experiments 40 μl of 1 mg ml^{-1} of human Cu/ZnSOD (Sigma) were mixed with 1 mg of antibody-coated beads for 1 h at 4°C with gentle rocking. The beads were collected by centrifugation in a microcentrifuge and the supernatant was transferred to a tube containing a fresh preparation of PAS-bound antibodies. The procedure was repeated three times. The supernatant after the last round of immunoprecipitation was tested for activity in the DNA strand break assay.

SDS–PAGE electrophoresis

Aliquots of 10 μl sample buffer (0.62 mM Tris–HCl, pH 6.8, 2% SDS, 5% glycerol) containing 100 ng of each enzyme preparation were loaded on a 15% SDS polyacrylamide Laemmli 1 cm thick mini gel (BioRad, Hercules, CA) and electrophoresed (45 min, 145 V). Protein bands were visualized by staining with silver using BioRad Silver Stain.

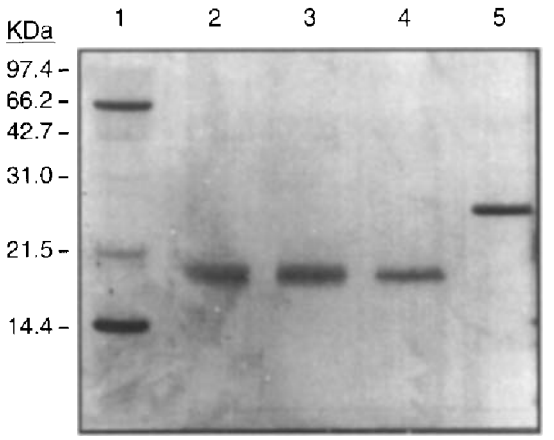

Figure 1. SDS–PAGE showing the purity of SOD preparations used in the study. Samples containing 100 ng of each preparation were loaded on a 15% (w/v) Laemmli mini gel and electrophoresed. The gel was stained with BioRad Silver Stain. Lanes: 1, BioRad low range molecular weight standards; 2–4, Cu/ZnSOD from Sigma, Fluka and Calbiochem, respectively; 5, MnSOD.

Results

High purity preparations, as determined by SDS–PAGE electrophoresis shown in Figure 1, from Sigma, Fluka and Calbiochem (recombinant) of human Cu/ZnSOD and *Escherichia coli* MnSOD, generated DNA single- and double-strand breaks when incubated with the supercoiled form of phage ϕX174 RF I DNA (Figure 2). Comparable concentrations of bovine serum albumin and actin had no effect, as well as metal-containing proteins such as cytochrome *c* and glutathione peroxidase (data not shown). Cu/ZnSODs from Fluka and MnSOD were the most active; however, they also contained more protein per unit of SOD activity. All enzyme solutions were standardized by units of SOD activity not by protein concentration, therefore their protein content varied with Fluka and MnSOD preparations being higher (4.9 and 3.6 μg per 12 units) than Sigma (2.6 μg per 12 units) and the recombinant (2.8 μg per 12 units) enzymes. Phage M13 RF I and pBR322 plasmid DNA were also cleaved with similar efficiency under standard conditions of the assay (data not shown).

Data presented in Table 1 show that inactivation of SOD activity also attenuated DNA cleaving activity. Agents such as sodium cyanide or diethyldithiocarbamic acid (DDC), which are known to inactivate Cu/ZnSOD but not MnSOD, also inhibited DNA cleaving activity of the former but did not alter either dismutase or DNA cleaving activity of the manganese-containing enzyme. Both enzymes,

Table 1. Effect of inhibitors of Cu/ZnSOD on Cu/ZnSOD- and MnSOD-mediated DNA strand breaks

	Cu/ZnSOD			MnSOD		
Treatment	Enzyme activity (%)	Open circle (%)[a]	Linear (%)[a]	Enzyme activity (%)	Open circle (%)[a]	Linear (%)[a]
Control	100	12	36	100	27	37
Boiling	8	0	0	10	0	0
NaCN (10 mM)	22	0	0	100	30	35
DDC (1 mM)	40	10	8	100	26	37

[a] The percentage of DNA in each form was subtracted with the corresponding values for DNA control

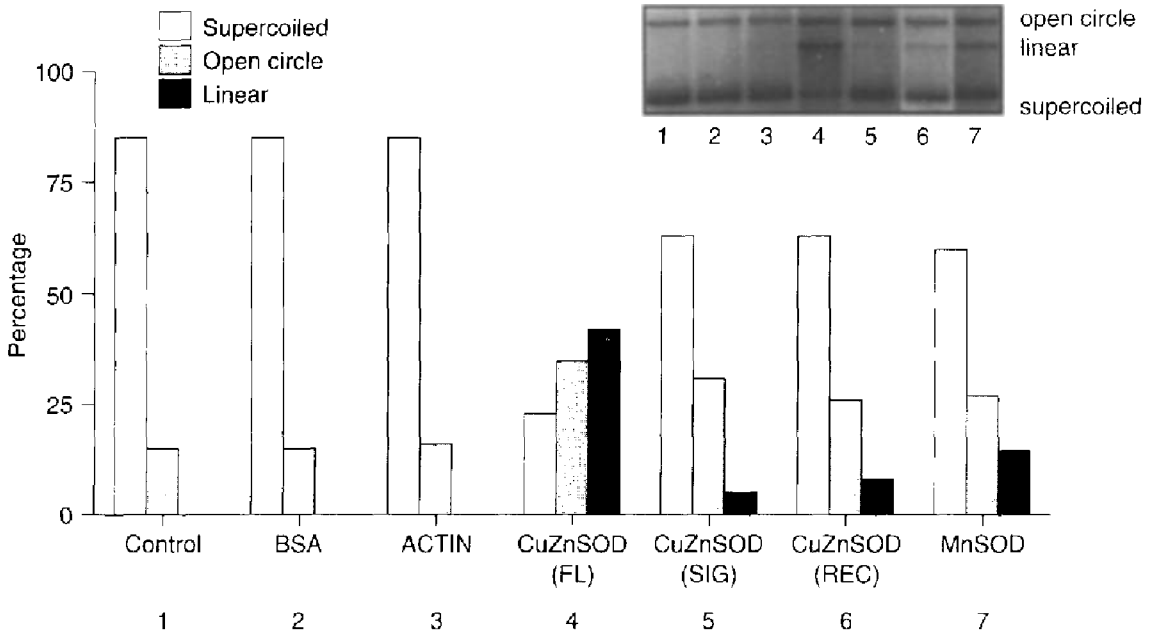

Figure 2. DNA strand breaks induced by various preparations of human Cu/ZnSOD and *E. coli* MnSOD. DNA strand breaks were measured by the conversion of supercoiled DNA to open circular and linear forms under standard conditions of the assay as described in Materials and methods. Shown in the inset is an ethidium bromide stained agarose gel of samples which were quantitated and expressed as bar graphs.

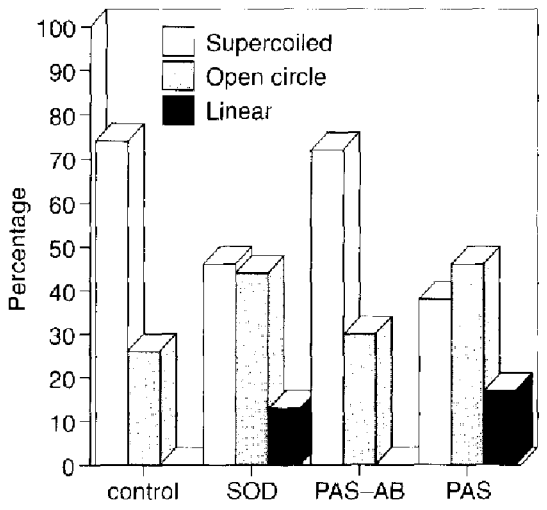

Figure 3. Effect of immunodepletion of Cu/ZnSOD on DNA strand break formations. Samples containing 1 μg μl^{-1} of Cu/ZnSOD were subjected to three rounds of immunoprecipitation, each with a fresh preparation of PAS-bound anti-Cu/ZnSOD antibodies. After the third round supernatants were assayed for DNA cleaving activity as described in Materials and methods. A parallel sample exposed to PAS alone was used as a control for dilution of activity originally present in the sample.

when inactivated by boiling, were unable to break DNA. Thus, the superoxide dismutating activity of the enzyme must be intact to induce DNA strand breaks.

Whether the nucleolytic activity is an intrinsic property of Cu/ZnSOD or due to possible contamination by nucleases, was addressed by two different approaches using highly specific anti-human Cu/ZnSOD antibodies. In the first approach, the effect of SOD immunodepletion with PAS-aB was tested. To achieve a substantial immunodepletion, samples were subjected to three rounds of immunoprecipitation. After the third round only 15% of initial SOD activity remained in the sample. As shown in Figure 3, the DNA cleaving activity of the SOD-immunodepleted sample was markedly reduced, displaying only 5% more single-strand breaks than control. The activity of samples treated with PAS beads alone was not affected.

In the second approach, anti-Cu/ZnSOD antibodies were added directly to the reactions containing Sigma or Fluka preparations (Figure 4A and B). Addition of 5 μl (7.5 μg of IgG) of anti-Cu/ZnSOD antibodies completely inhibited the formation of DNA strand breaks. The same amount of antibodies directed against human retinoblastoma protein (AbRB) or catalase (not shown) had no effect (Figure 4A). The degree of inhibition was

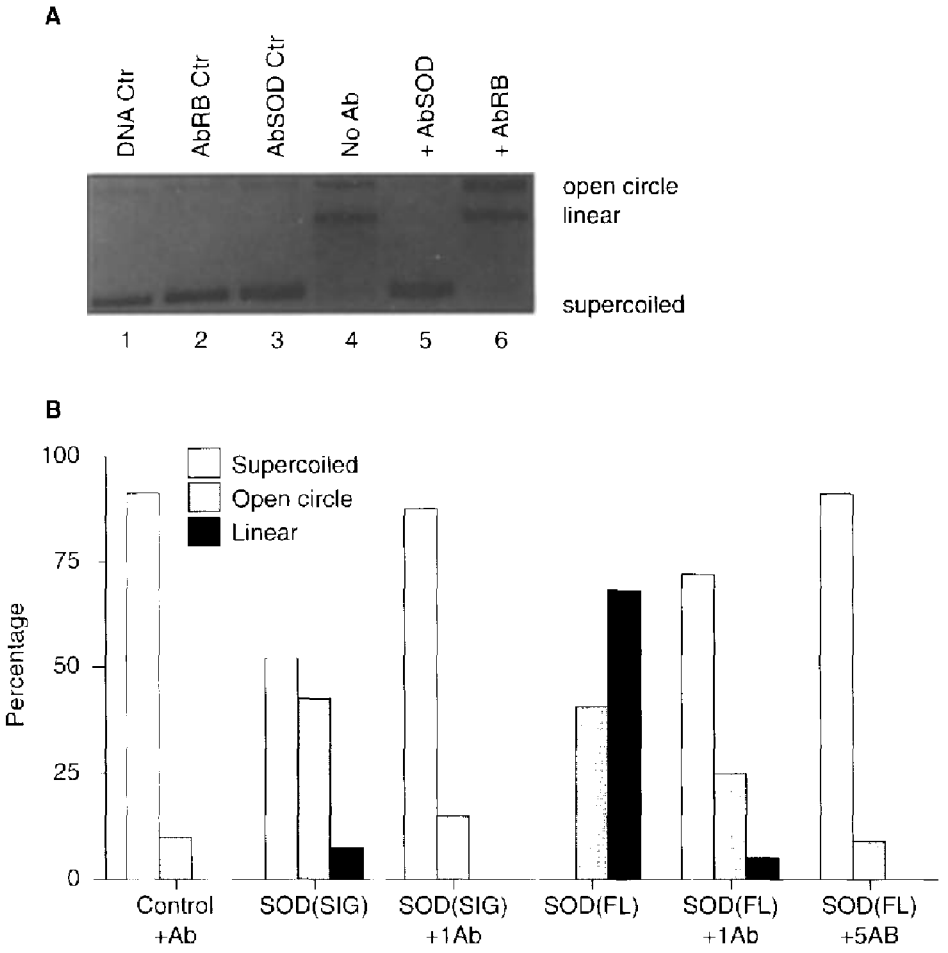

Figure 4. Effect of anti-Cu/ZnSOD antibodies on Cu/ZnSOD-mediated DNA strand breaks. (**A**) Ethidium bromide stained agarose gel of samples of the enzyme from Fluka incubated with 5μl of anti-human Cu/ZnSOD (AbSOD) or anti-human RB (AbRB) antibodies. Controls included DNA substrate alone (DNA Ctr) and each antibody with DNA (AbSOD Ctr and AbRB Ctr). (**B**) DNA strand breaks induced by two preparations (SOD Sigma and SOD Fluka) of human Cu/ZnSOD in the presence of 1 μl (1 Ab) and 5 μl (5 Ab) of anti-human Cu/ZnSOD antibodies.

correlated with the amount of protein in the samples. Results presented in Figure 4(B) show that preparations containing more protein per unit of SOD activity, represented here by enzymes from Fluka, also required more antibody to be fully inhibited. From this set of data it can be concluded that nucleolytic activity is an intrinsic property of the SOD enzyme, since it cannot be separated from SOD protein.

To approach the possible mechanism involved, the effect of metal ions such as Cu(II), Zn(II), Fe(II), Fe(III) and Mg(II), was investigated. Addition of 100 μM of either $CuCl_2$ or $ZnCl_2$ to the reaction significantly inhibited DNA strand scission (data not shown). The effect of the same concentration of $FeCl_3$, $FeCl_2$ and 1mM of $MgCl_2$ on nucleolytic activity of Cu/ZnSOD and MnSOD enzymes is presented in Figure 5. Fe(II), but not Fe(III), and Mg(II) ions greatly enhanced DNA cleaving activity. Both enzymes, when incubated in the presence of Fe(II) ions, converted all of the supercoiled form into mostly open circles (single-strand breaks). The observation that ferrous, but not ferric ions, alter the DNA cleaving activity of SOD enzymes together with the inhibitory effect of Cu(II) and Zn(II) ions suggests that the generation of DNA strand breaks may be in part mediated by hydroxyl radicals formed in metal-catalyzed Haber–Weiss reactions. Another observation supporting this mechanism is the oxygen dependency of the process. As shown in Table 2, both SOD enzymes do not cleave DNA under anaerobic conditions. Since Cu/ZnSOD and MnSOD

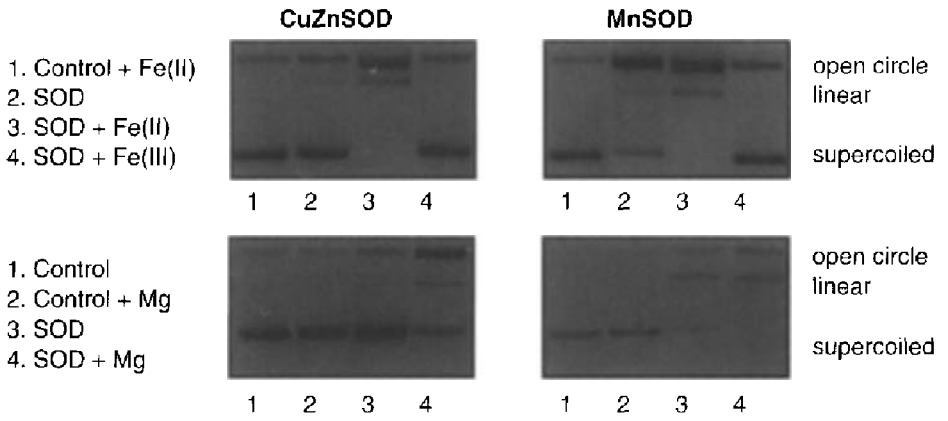

Figure 5. Effect of Fe(II), Fe(III) and Mg(II) ions on DNA strand breaks induced by human Cu/ZnSOD and *E. coli* MnSOD preparations. Ethidium bromide stained gels of ϕX176 RF I DNA incubated with Cu/ZnSOD or MnSOD in the presence of 100 μM of either $FeCl_2$ or $FeCl_3$ (upper row) or 1 mM $MgCl_2$. The conditions of the assay were the same as those described in Materials and methods.

Table 2. Effect of boiling, NaCN, anti-Cu/ZnSOD antibodies and anaerobic conditions on Fe(II)- and Mg(II)-dependent DNA cleaving activity of Cu/ZnSOD and MnSOD

		Percentage of inhibition[a]	
Treatment	Sample	Cu/ZnSOD	MnSOD
Boiling	SOD	90–100	95–100
	SOD + Fe(II)	90–100	80–100
	SOD + Mg(II)	90–100	90–100
NaCN	SOD	85–100	0
	SOD + Fe(II)	90–100	0
	SOD + Mg(II)	30– 40	0
	SOD + Mg(II) anaerobic	0–10	0
Anti-Cu/ZnSOD antibodies	SOD	100	0
	SOD + Fe(II)	95–100	0
	SOD + Mg(II)	90–100	0
Anaerobic conditions	SOD	80–100	90–100
	SOD + Fe(II)	85–100	80–100
	SOD + Mg(II)	0	0

[a] The percentages of inhibition of the amount of single- and double-strand breaks recorded in untreated controls are reported as a range of values derived from three independent experiments.

respond in a similar way to those treatments, it can be concluded that the mechanism by which they cleave DNA is also similar.

Addition of 1 mM $MgCl_2$ to the reactions containing either Cu/ZnSOD or MnSOD significantly increased DNA cleavage (Figure 5). Magnesium-dependent activity can be distinguished from activity exerted by SOD alone or SOD in the presence of Fe(II) by being independent of oxygen and resistant to NaCN (see data in Table 2). Differences in the reaction time course and quantity of enzyme required to obtain a similar effect (data not shown) further distinguish these two activities. Data presented in Table 2 also show that magnesium-dependent activity was effectively blocked by anti-human Cu/ZnSOD antibodies. The same antibodies have no effect on MnSOD induced strand breaks. Therefore, this activity must reside in the SOD molecule itself. These observations lead to the conclusion that SOD in the presence of Mg(II) cleaves DNA through a mechanism that is oxygen independent.

Both SODs also cleaved RNA when tested under similar conditions with the DNA assay. Figure 6 shows the agarose gel of RNA substrate incubated with three preparations of Cu/ZnSOD (lanes 2–4), MnSOD (lane 5) and the preparation from Sigma in the presence of anti-Cu/ZnSOD antibodies (lanes 6–9). In this assay, RNA cleavage could be estimated from the presence of shorter fragments and the extent of the shift in their electrophoretic mobility. By these criteria, Sigma, Fluka and MnSOD preparations displayed similar activity, whereas the recombinant enzyme was less active.

Attempts to block RNA cleaving activity with anti-Cu/ZnSOD antibodies were hampered by contamination of IgG preparation with RNase activity (data not shown). To eliminate this activity it was necessary to include RNasin (a potent inhibitor of eukaryotic RNases) in each reaction. RNasin alone had no effect (lane 6) and provided substantial

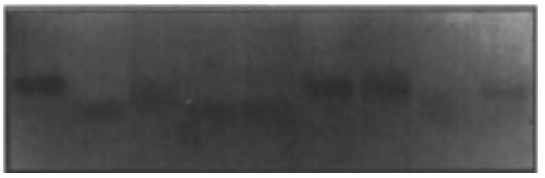

Figure 6. RNA cleaving activity of high purity preparations of human Cu/ZnSOD and *E. coli* MnSOD. Ethidium bromide stained agarose gel of RNA, represented here by a run-off transcript of a hamster hexokinase gene, incubated with 10 units of the indicated SOD preparations. For samples 6–9, 120 units of RNasin per reaction were added to protect RNA substrate from antibodies contaminated with RNase activity. For samples 7 and 9, 1 μl of anti-human Cu/ZnSOD antibodies was added.

protection of the substrate in the presence of 1 μl of antibodies (lane 7), but did not interfere with the cleavage induced by SOD. Thus, the protective effect observed in the sample containing RNasin, SOD and antibodies (lane 9) could be attributed to the neutralization of SOD by specific reaction with antibodies.

Discussion

The data presented in this paper show that Cu/ZnSOD and MnSOD enzymes exhibit DNA and RNA cleaving activity in an *in vitro* assay. The DNA strand scission induced by the SOD preparation was temperature, time and dose dependent. There was no specificity toward the DNA substrate since the phage replicative forms of DNA and bacterial plasmids were cleaved with similar efficiency.

The superoxide dismutating activity of the enzyme had to be preserved in order to cleave DNA. Agents such as NaCN and diethyldithiocarbamic acid, which inhibited SOD activity of the copper/zinc-containing enzyme, also inhibited DNA cleavage. On the contrary, the DNA cleaving activity of MnSOD, which was resistant to inactivation by both agents, was not affected at all. Depletion of Cu/ZnSOD from the sample by immunoprecipitation with highly specific antibodies resulted in loss of the nucleolytic activity. The same antibodies, when added directly to the reaction, effectively blocked the formation of DNA strand breaks as well as the cleavage of RNA. Although the possibility of contamination with nucleases is always the concern in this type of study, the above observations seem to argue against this possibility. In addition, it should be noted that all enzyme preparations used in this study were of high purity, displaying one single band of the expected size on SDS–PAGE gel stained with silver. Furthermore, it is very unlikely that any of those presumably contaminating nucleases would be inhibited by NaCN or DDC. Finally, RNasin (a potent inhibitor of eukaryotic RNases) had no effect on either DNA or RNA cleavage in the presence of SOD.

The effect of ferrous and ferric ions, requirement for oxygen as well as inhibition of DNA cleavage in the presence of excess Cu(II) and Zn(II) fit the picture of DNA damage being mediated in part by hydroxyl radicals formed in a transition metal catalyzed Haber–Weiss reaction. Both enzymes contain transition metals at the active site which can react with H_2O_2, the end product of the SOD catalyzed reaction, and generate highly reactive $OH^{\cdot}$ radicals known to be very effective in causing DNA strand breaks (Imlay & Linn 1988, Kasprzak 1991). Since the reaction required a reduced form of the metal [Fe(II) or Cu(I)] and all superoxide anions which serve as donors of electrons to the metal are being dismutated, an excess of non-reduced metal ions [Fe(III) or Cu(II)] should inhibit DNA strand scission. This was exactly the case. The inhibitory effect of a non-redox metal such as zinc can be explained by its ability to compete with copper or iron for binding sites on DNA (Thomas *et al.* 1986). Further support for the proposed mechanism can be gained from the studies by Yim *et al.* (1990) and Sato *et al.* (1992) in which Cu/ZnSOD has been shown to catalyze hydroxyl radical production from H_2O_2.

The mechanism by which SOD cleaves DNA in the presence of magnesium must be different since magnesium dependent activity did not require oxygen and it was insensitive to inhibition by NaCN. The latter observation implied that zinc rather than copper played a role in the nucleophilic reaction. In this context it is important to note that many

naturally occurring nucleases are zinc-containing proteins. There are other similarities between those nucleases and SODs. For example, alkaline phosphatase, a prototype enzyme to study the role played by zinc in the non-specific hydrolysis of the phosphodiesters of DNA and RNA (Basile & Baston 1989), has zinc ligated to three or four histidines (Otvos & Brown 1980), which closely resembles the structure of metal binding sites of Cu/ZnSOD (Tainer *et al.* 1982) and MnSOD (Stallings *et al.* 1985). Thus, the existence of a nuclease-like catalytic domain in SOD enzymes may be responsible for the observed DNA cleavage in the presence of magnesium. Magnesium may induce local conformational changes of DNA which are recognized by the nuclease-like catalytic center of SOD and then cleaved.

Evidence presented in this paper showing that SOD can carry DNA/RNA nucleolytic activity may be of significant importance in explaining the mechanism underlying the pathology of certain hereditary diseases genetically linked to the Cu/ZnSOD gene such as Down's Syndrome (DS) and familial amyothrophic lateral sclerosis (FALS). In DS, many symptoms of the syndrome have been attributed to the overexpression of Cu/ZnSOD due to the presence of one extra copy of the gene (Epstein *et al.* 1987, Avraham *et al.* 1988, Elroy-Stein & Groner 1988), while in FALS the pathogenicity of the disease was closely linked to single point mutations in the gene (Rosen *et al.* 1993). In FALS, where mutations apparently confer some new and deleterious activity on SOD rather than causing a loss of enzyme activity (Borchelt *et al.* 1994, Brown 1995, Gurney *et al.* 1994, Ripps *et al.* 1995), it is tempting to speculate that those mutations could potentiate normally marginal and harmless nucleolytic activity of SOD. This activity by cleaving RNA may contribute to the slow progressive damage of neural cells. The selective vulnerability of neuron cells to this side reaction of SOD is probably determined by their longevity and inability to divide. In such cells, even slightly modified protein products generated from SOD-damaged RNA templates, could with time accumulate to a detrimental level. The fact that neural cells are usually poor in catalase activity (Hartz *et al.* 1973) could also be of some importance in making them more prone to the nucleolytic activity of SOD. Whether mutations of Cu/ZnSOD observed in patients with FALS potentiate the ability of the enzyme to cleave DNA/RNA remains to be determined.

In many studies on DNA damage by enzymatic and non-enzymatic generators of the superoxide ion, the involvement of superoxide has been determined by the effect of externally added SOD. The ability of SOD enzymes to cleave DNA adds to the complexity of experimental interpretation and could account for many conflicting results obtained in such experimental systems.

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