JETIR.ORG ISSN: 2349-5162 | ESTD Year : 2014 | Monthly Issue

JOURNAL OF EMERGING TECHNOLOGIES AND INNOVATIVE RESEARCH (JETIR)

An International Scholarly Open Access, Peer-reviewed, Refereed Journal

INVESTIGATION OF THE COMPLEXES FOR SUPEROXIDE DISMUTASE

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ABSTRACT

Superoxide dismutase is an enzyme that catalysis the reversible reduction of the damaging superoxide (O2) radical into the inert molecular oxygen (O2) and hydrogen peroxide (H2O2) (H 2O 2). Aerobic organisms produce superoxide, a kind of reactive oxygen species (ROS), as a byproduct of their respiratory metabolism. Through the Fenton reaction, hydrogen peroxide (H2O2) and ferric iron (Fe2+) may generate the reactive oxygen species (ROS) hydroxide ion (•HO). H2O2 is an essential sensor in redox metabolism. According to Hirs' performic acid oxidation technique, we measured total cysteine and half-cystine in triplicate. spectrophotometric approach was used to determine the concentration of tryptophan. Dimethyl sulfoxide was used in the Spencer and World technique for determining total cysteine. Differential centrifugation in isotonic sucrose is a well-established technique for isolating mitochondria from chicken liver. In order to prevent interference from cytochrome c peroxidases and oxidases, superoxide dismutase was measured in the same way as reported before (4), with the addition of 1 x 1O3 M cyanide.

KEYWORDS antioxidant; superoxide dismutase; supplementation; detoxification

INTRODUCTION

An enzyme called superoxide dismutase catalysis the reversible dismutation (or partitioning) of the superoxide (O2) radical into regular molecular oxygen (O2) and hydrogen peroxide (H2O2) (H 2O 2). In the absence of control mechanisms, the metabolic byproduct superoxide may harm many different kinds of cells. Hydrogen peroxide, albeit reduced by enzymes like catalase, is also toxic. As a result, SOD is a crucial antioxidant defense mechanism present in almost all oxygen-exposed cells in the living world. However, lactobacilli linked to Lactobacillus plantarum employ a different strategy to protect themselves against reactive O2.

Metalloenzymes called superoxide dismutase (SODs) catalyze the dismutation of superoxide anions at a pace almost as fast as the diffusion limit. Superoxide is a kind of reactive oxygen species (ROS) that is generated in aerobic living systems as a byproduct of the mitochondria's respiratory activity. Maintaining low amounts of superoxide via the work of SODs helps avoid the oxidative stress that has been linked to a wide range of illnesses. All of the SODs have a metal cation (Cu (Zn), Cu, Fe, Mn, or Ni) in their active site that alternates between two redox states to reduce superoxide to H2O2 and oxidize it to O2. There are three known types of SODs in humans. SOD1, a copper/zinc SOD, is located in the cytosol and mitochondrial intermembrane space, while SOD3, a zinc/copper SOD, is found in the extracellular environment (inside the extracellular matrix and at the cell surface). Overexpression of the inactive form of intestinal Mn SOD and under expression of the cytoplasmic Cu/Zn SOD are both associated with inflammatory bowel illnesses

(IBDs) such Crohn's disease and ulcerative colitis. As oxidative stress is known to be intimately associated to chronic inflammation, it is possible that these deficits in the SOD antioxidant system either cause IBDs or make them worse. Therefore, it would seem that an antioxidant treatment based on SOD would be an effective treatment for IBDs. In a study using a mouse model of DSS- and TNBS-induced colitis, MnSODs were shown to effectively decrease lipid peroxidation and neutrophil recruitments while also dampening inflammation. However, the half-life, induced immunogenicity, and poor cell penetration of pure enzymes continue to be barriers to their widespread application as treatments.

Metalloenzymes called superoxide dismutase (SODs) are present in both eukaryotes and some prokaryotes, and as shown in, they are found in the cytosol and the mitochondrial intermembrane (Cu, Zn-SOD or SOD1), the mitochondrial matrix and inner membrane (Mn-SOD or SOD2), and the extracellular compartment (Cu, Zn-SOD or SOD3). The importance of these compounds as a first line of antioxidant defense has been widely acknowledged since since their discovery by Joe McCord and Irwin Fridovich. Critical to understanding the function of oxidant/antioxidant mechanisms in ischemia/reperfusion-associated diseases in both people and animal models is the work of I. Fridovich and colleagues. By acting as a catalyst, SOD changes the free radical superoxide anion (•O2) into the less reactive hydrogen peroxide (H2O2) and molecular oxygen (O2). Catalase (CAT), glutathione peroxidase (GPx), and/or thioredoxin (Trx)-dependent peroxiredoxin (Prx) enzymes subsequently function to convert H2O2 to water. H2O2 is a crucial sensor in redox metabolism, and it may also produce another reactive oxygen species (ROS), the hydroxide ion (•HO), through the Fenton reaction in the presence of Fe2+. Under normal conditions, when H2O2 intracellular concentrations are 1-10 nM, it mediates the stress response involved in physiological and adaptive processes (termed oxidative eustress), whereas at higher concentrations, it is responsible for so-called oxidative distress, in which the evoked inflammatory response causes cell damage. H2O2 synthesis and elimination are regulated by an endogenous antioxidant system, the enzymes of which have both a physiological and pathological function.

REVIEW OF LITRATURE

Alvaro de Obeso Fernandez del Valle et.al (2022) Several cellular components regulate the concentrations of reactive oxygen species at physiological levels (ROS). Many enzymes, not only superoxide dismutase, have the potential to convert or eliminate reactive oxygen species (SODs). Neglected ROS damage may lead to illness, accelerated ageing, and even death. To prevent the harmful effects of superoxide anions, dismutase (SODs) converts them to water and oxygen. The purpose of this article is to provide a concise summary of the research on the role of SODs in the development and pathogenicity of eukaryotic bacteria that are important to humans. Podospora anserina, a fungal model of aging; Aspergillus species that can cause aspergillosis; Trypanosoma cruzi and Trypanosoma brucei, the causative agents of Chagas disease and sleeping disease, respectively; and Acanthamoeba spp., pathogenic amoebae. SODs have crucial roles in the growth, infection of hosts, proliferation, and control of gene expression of these organisms. Our research focuses on SODs and related factors in these bacteria, which have been demonstrated to have an effect on health and disease.

Abhay K. Patela et.al (2021) The [Cu(L)(ClO4) (H2O)] 1 and [(Cu)(L)(N3)] 2 complexes belong to a novel family of mono- and binuclear complexes. Two and Two [(Cu)(L)(OCN)] The numbers 2, 3, and [(Cu)(L)(CNS)] (where HL = (E)-N′-(phenyl(pyridin-2-yl) methylene) thiophene-2-carbohydrazide) have been produced and characterized. We have used spectroscopy and elemental analysis to fully characterized all complexes. Powder X-ray diffraction methods were used to examine the crystalline structure of complexes. Complexes 2-4 showed evidence of intra exchanged-coupled systems in their polycrystalline Epr spectra due to a clearly defined half-field signal. Electrochemical properties of all compounds were investigated using cyclic voltammetry (CV) and differential pulse voltammetry (DPV). Weak antiferromagnetic spin-exchange interactions between copper (II) ions are indicated by the magnetic moment (eff = 1.47-1.63 BM) for binuclear complexes measured at room temperature. According to the geometrical structural index (-5), the geometries surrounding the copper (II) ion always maintain their deformed square pyramidal shape. Molecular docking was used to model the interactions between all of the produced complexes and DNA. Each compound had its superoxide dismutase (SOD) activity tested.

Alexander N. Vaneev et.al (2021) There is still no more frequent clinical issue in ophthalmology than inflammatory eye disorders. Tissue degeneration, hazy vision, and even blindness may result from inflammation's secondary processes, such as the overproduction of reactive oxygen species (ROS) and the

depletion of the endogenous antioxidant system. Copper-zinc superoxide dismutase (SOD1) and other antioxidant enzymes may be effective ROS scavengers. However, owing to poor ocular penetration, getting them into the eye compartments is difficult. This study presents a new therapeutic approach for the eye based on multilayer polygon complex nanoparticles of SOD1 (Nano-SOD1), which has a strong therapeutic effect without the usual side effects (such as eye irritation, acute, chronic, and reproductive toxicity, allergenicity, immunogenicity, and mutagenicity, even at high doses) and is stable during storage. Nano-anti-inflammatory SOD1's effects on a rabbit model of immunogenic uveitis (inflammation of the eye's interior vascular tract) were studied in vivo. Preclinical research shown that Nano-SOD1 administered by topical instillations was much more effective than the free enzyme in reducing uveitis symptoms

O.M. Ighodaro et.al (2018) Endogenous enzymatic and non-enzymatic antioxidants provide the backbone of a sophisticated antioxidant defense grid inside the body. Collectively, these chemicals fight free radicals, protecting essential biomolecules and, by extension, bodily tissues from harm. Antioxidants may be divided into a "first line of defense," "second line of defense," "third line of defense," and "fourth line of defense" categories based on how they deal with free radicals in general. Superoxide anion radical (*O2) is constantly generated in normal body metabolism, in particular through the mitochondrial energy production pathway, making the role and efficacy of the first line defense antioxidants, which primarily consist of superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPX), crucial and indispensable in the overall defense strategy of antioxidants (MEPP). While there has been a lot written about antioxidants and their function in warding off oxidative stress and the harm it can do to cells, few people realize just how crucial SOD, CAT, and GPX are. This study focuses on the antioxidant enzymes catalase (CAT), glutathione peroxidase (GPX), and superoxide dismutase (SOD) as a first line

Gabrielle Schanne et.al (2022) Patients with inflammatory bowel disorders (IBDs) have been reported to have decreased levels of the antioxidant enzyme superoxide dismutase (SODs), suggesting that oxidative stress plays a significant role in the development of these conditions. In the setting of inflamed bowel diseases (IBDs), SOD mimics, also known as SOD mimetics, are potential antioxidant catalytic metallodrugs as low-molecular-weight compounds mimicking the action of SOD. Metal ion exchanges between the manganese center and metal ions present in the biological environment (such as Zn (II)) were demonstrated for a Mn (II) complex SOD mimic (Mn1) based on an open-chain diaminoethane ligand that exerts antioxidant and anti-inflammatory effects on an intestinal epithelial cellular model. Improving the kinetic inertness of Mn (II) complexes based on open-chain ligands is crucial to enhance their bioactivity in a cellular environment, since the resultant complexes (mostly Zn (II)) were demonstrated to be inactive. Here, we present the results of our research into three novel Mn (II) complexes that were formed when Mn1 was functionalized with a cyclohexyl and/or propyl group to restrict, in turn, (a) metal exchanges and (b) deprotonation of an amine from the 1,2-diaminoethane central scaffold. Increased intrinsic SOD activity and enhanced kinetic inertness in metal ion exchange processes (with Zn (II), Cu (II), Ni (II), and Co (II)) characterize the novel manganese-based SOD mimics.

Methods

Type III cytochrome c was bought from Sigma. Chicken livers, both frozen and fresh from the slaughterhouse, were sourced from area vendors. Microgranular carbosymethylcellulose (CM-52) and diethylaminoethylcellulose (DE-32) were purchased from the Reeve Company. In the past, Pharmacia produced a drug called Sephadex G-75. Copper, zinc, and manganese standard solutions were purchased from Fisher. Baker brand ammonium sulphate was used as a reagent. this division kindly donated xanthine oxidase, and it was manufactured using a method that kept it safe from proteolytic agents. The activity of superoxide dismutase was measured in the same way as reported previously, except 1 x 1O3 M cyanide was used to block the activity of cytochrome c peroxidases and oxidases. In these tests, adding ferricytochrome c just before starting the reaction with santhine oxidase proved to be optimal. The goal was to prevent cyanide from altering the cytochrome c. These tests relied on xanthine oxidase, which was inhibited by cyanide but not by xanthine. The mitochondrial superoxide dismutase was not inhibited by this concentration of cyanide. Manganese superoxide dismutase is not inhibited by cyanide although the analogous cupro-zinc enzymes are. The sedimentation equilibrium technique was used in a Beckman model E analytical ultracentrifuge with 0.1 M KCl, 0.005 M potassium phosphate, 1 X low5 M EDTA, pH 7.8 and 25", and 1 yc fi-mercaptothion. To determine the enzyme's purity, 7.5% polyacrylamide gels were electrophoresed in a disc-shaped electrophoresis apparatus, with one set of gels stained for protein and the other set stained for superoxide dismutase activity. Using the photochemical approach previously reported, superoxide dismutase

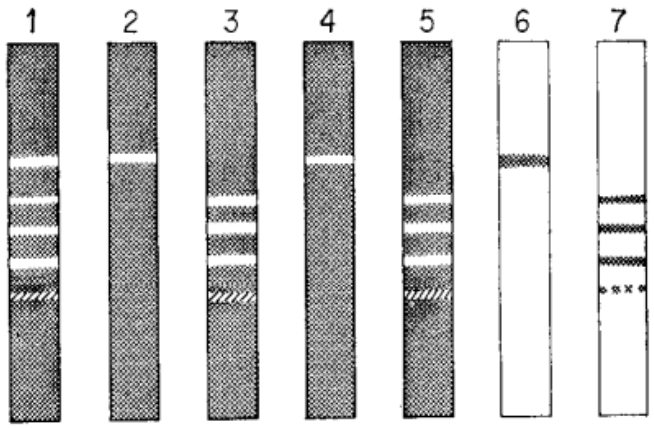
activity was localized on polyacrylamide gels by suspending the gel cylinders in 0.05 % potassium phosphate, 1 x lo-* M EDTA at pl-I 7.8 and 25" before and during illumination.

By introducing 1 x 103 M cyanide in the developing reagents to decrease the activity of the sensitive enzyme, cyanide-sensitive superoxide dismutase could be distinguished from cyanide-insensitive superoxide dismutase on polyacrylamide electrophoretograms. The method of electrophoresis in acid urea gels was carried out in accordance with the guidelines laid forth by Panyjima and Chalkley. Disc gel electrophoresis using 10% polyacrylamide gels containing sodium dodecyl sulphate was used to calculate the molecular weight of the enzyme's constituent subunits. Transferrin (77,000 Da), human albumin (68,500 Da), ovalbumin (43000 Da), pepsin (35,000 Da), carbonic anhydrase (29,000 Da), trypsin (23,000 Da), and bovine superoxide dismutase (16,300 Da) were used as molecular weight standards to calibrate the gels. Atomic absorption spectrophotometry using a Perkier-Elmer model 107 calibrated with standard solutions of salts of manganese, copper, and zinc allowed for the quantitative analysis of these elements. Protein concentrations were calculated using either ultraviolet (UV) or short-wavelength ultraviolet (UV) absorbance or the biuret technique. The biuret technique was standardized with the use of crystalline bovine serum albumin. Absorbance measurements of pure superoxide dismutase in the short ultraviolet were adjusted by a factor of 1.57, yielding more accurate results. Then, they matched up with findings from the UV biuret technique (30). The explanation for this tweak is provided in the section on the absorbance characteristics of mitochondrial superoxide dismutase. A Cary model 15 was used to capture the spectra. At 1200 volts, a LKB 440.ml electro focusing apparatus was used to concentrate a 170 ampholyte solution by isoelectric focusing. Amino acid studies included hydrolyzing duplicate samples for 24, 36, and 48 hours at 110" in 6 M HCl + 0.1% phenol under vacuum, and then chromatographing the hydrolyzed amino acids after vacuum drying and resuspending them. Hirs' performic acid oxidation method was used to measure total cysteine and half-cystine in duplicate.

RESULTS

Different Xu peroxide Dismutase Isoforms Found in Chicken Liver Following homogenization, ultrasonication, and centrifugation, soluble extracts of a single fresh chicken liver yielded at least four electrophoretic ally distinct superoxide dismutase. These, which are seen in Gel 1 of Fig. 1, may be labelled A, B, C, and D according to their relative mobility. The darkened band in Gels I, 3, 5, and 7 indicates that there were fainter bands of activity beyond D. Band A was not impacted by 1 mrl of cyanide, but Bands B, C, and D were completely wiped off. Specifically, Gel 2 shows this to be the case. Band A was the only one impacted by the Tsuchihashi combination of chloroform and ethanol; Bands B, C, and D were untouched. Gel S demonstrates this outcome. Band A was the sole protein found in a soluble extract of separated mitochondria from chicken liver, whereas Bands 13, C, and D were present in the cytosol fraction of an isotonic sucrose homogenate of chicken liver. As shown by Gels 4,

FIG 1



has a pH of 8.9, which is neutral. The dark gels were stained for superoxide dismutase activity while the light gels were stained for protein as described in the text. Extract from isolated mitochondria; Cytosol fraction; Pure mitochondrial superoxide dismutase; Pure cytoplasmic superoxide dismutase; Gel 1, crude homogenate; Gel d, crude homogenate plus cyanide; Gel 3, crude homogenate after treatment with

chloroform and ethanol; Gel 4, crude homogenate; Gel 5, cytosol fraction; Gel 6, pure mitochondrial superoxide dismutase; Gel 7, crude homogenate.

Band A, which was due to the cyanide-insensitive superoxide dismutase and which was eliminated by the chloroform ethanol procedure, proved to be due to a Mangan enzyme. Protein staining, rather than activity staining, was used to identify the pure Mangan superoxide dismutase in gel 6. Bands B, C, and D proved to be due to a cupro-zinc superoxide dismutase present in the cytosol. There seems to be a family of bands that originate from this superoxide dismutase, each of which has a unique electrophoretic profile. Protein staining was used to identify the presence of chicken liver cupro-zinc superoxide dismutase in Gel 7, rather than enzymatic activity. The addition of mercaptothion had no effect on the distribution of these bands. Unless otherwise specified, all procedures for purifying chicken liver Cupro-zinc superoxide dismutase were done at 4 degrees oxygen. Homogenization of five pounds of frozen chicken liver was place for two minutes in a gallon-sized stainless-steel liver. Waring Blendor, which was run at high speed. After centrifuging the sludge at 13,000 x g for 30 minutes, the muddy water was become crystal clear. The water the supernatant solution was mixed with 0.25 volume of ethanol and 0.15 volume of was initially at room temperature, but was chilled by the frozen chloroform and was stirred for 15 min, during which time its color changed to a light tan. In order to utilize entire frozen liver as a convenient supply of the cytosol enzymes without interference from the mitochondrial enzyme, it must first be treated with organic solvents, which kills off the mitochondrial superoxide dismutase. After centrifuging the suspension at 13,000 x g for 15 minutes, the supernatant solution was cooled to room temperature. After 15 minutes of stirring at room temperature, the salted-out stages were allowed to separate, and solid KH2PO1 (300 g per litre) was added. We gathered the ethanol-rich, less dense phase, then refrigerated and cleared it using centrifugation. After adding two volumes of cold acetone while stirring, the resulting precipitate was centrifuged to collect the solids and then suspended in a small volume of a 0.015 M sodium acetate buffer with a pH of 5.0 and a conductivity of 0.7 mmho. Dialyzing this enzyme solution against three 50-volume changes of the acetate buffer took place over the course of 24 hours. After centrifuging out the precipitate that formed during dialysis, 100 grammes of CM-52 that had been pre-washed and equilibrated in the same acetate buffer were stirred into the clear, brownish solution for thirty minutes. ChI-52 was collected on a 10-cm Buchner funnel and washed in 0.015 M &c acetate buffer until the absorbance of the effluent was less than 0.010 at 280 nm. Next, the CM-52 was eluted using a 0.065 M sodium acetate buffer at pH 5.0 until the fractions collected in tubes (13 x 150 mm) no longer showed any detectable green color. Ultrafiltration over a 150 mm imexon PM-10 membrane was used to concentrate the green fractions into a small amount of dark green liquid, which was subsequently diluted to 30 ml with 0.1 M KCl, 0.005 M potassium phosphate, and 1 X lo-5 M EDTA at pH 7.8. Gel exclusion chromatography was performed on a Spades G-75 column (2.5 x 100 cm) equilibrated in the same Cyclophosphane buffer. There was an enzyme elution from this column, and it was separated into two bands. Each member of the main band had the same level of superoxide dismutase activity. Figure 2 depicts this elution pattern. The blue-green, active substance collected from this column was pooled as shown in Fig. 2 and then concentrated using ultrafiltration. Specific activity was 3295 units per mg, with a yield of 235 mg of enzyme.

FIG 2

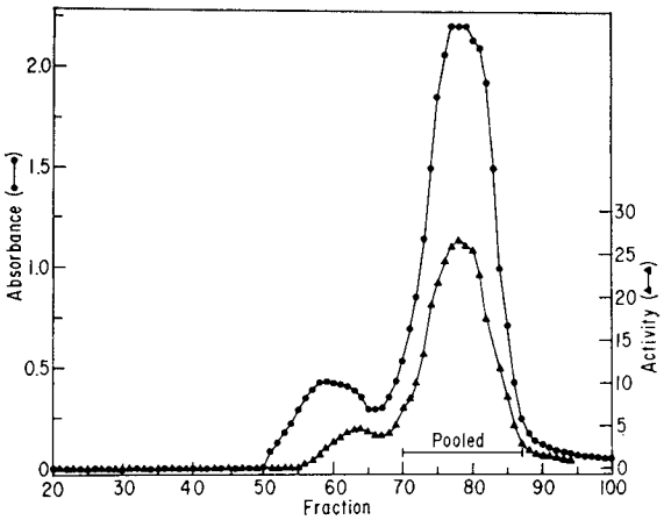


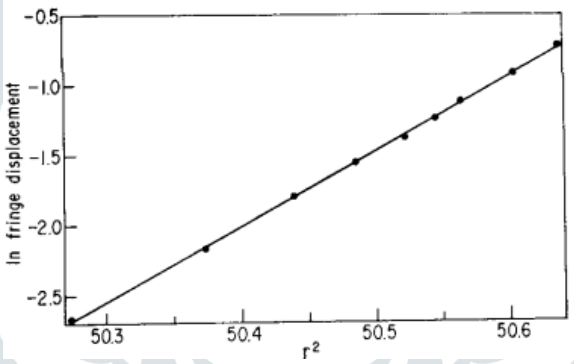
Fig. 2. Chromatography of G-75 shown in Gel exclusion chromatography was performed on a column (2.5 X 100 cm) of Sephadex G-75 that had been equilibrated with the dilution buffer, using fractions pooled from the CM-52 column that had been concentrated to a tiny amount across a PM10 ultrafiltration membrane. The final volume was 30 ml. High-specific-activity fractions 70–87 were combined to create a concentrated fraction. A polymer of superoxide dismutase is likely responsible for the tiny activity peak at Fraction.

table the outcomes of each phase of this process are summarized below. Purity Standards- Electrophoresis on a polyacrylamide gel clearly showed that this substance could be separated into many bands. Nonetheless, all of these frequencies were in use. There were thus no protein fractions that lacked superoxide dismutase activity.' The data from the thiosulfate chain length polymorphism assay revealed that chicken liver cupro-zinc superoxide dismutase polymerizes through the production of trisulfide bridges.

TABLE 1 Purijkation of chicken liver cytoplasmic superoxide dismutase

Preparation stage	Volume	Protein concentration	Total protein	Total units	Specific activity	Yield	Purification factor
	ml	*mg/ml*	*g*	$\times 10^{-5}$	*cm/mg*	%	
Supernatant from homogenate	3575	65.5	234.0	6.00	26.0	100	1.0
Supernatant from Tsuchihashi fractionation	3740	21.5	80.4	3.78	47.0	62	1.8
Ethanolic phase	1340	8.96	12.0	2.65	222	44	8.5
Acetone precipitate resuspended and dialyzed	700	5.57	3.90	1.95	500	32	19.7
After chromatography on CM-52	40	20.0	0.800	1.20	1500	20	57.7
After chromatography on G-75	75	3.13	0.235	0.773	3295	12.7	126.7

FIG 3



Superoxide dismutase in the cytoplasm settles to an equilibrium position (see Fig. 3). The enzyme was equilibrated at 34,000 rpm in a solution containing 0.1 M KCl, 0.005 M potassium phosphate, 1 X 10m6 M EDTA, pH 7.8, and 1% p-mercaptoethanol at a concentration of 1.0 mg/ml. The In fringe displacement was mapped out as a function of the square of the distance from the centre of rotation, using an average of five fringe readings from an interference optics photograph. According to Yphantis's (24), technique, we get a molecular weight of 30,600.

TABLE 2 Amino acid composition of cytoplasmic chicken liver superoxide dismutasea

Amino acid	Amino acid content[b]	Residues per mole of enzyme (nearest integer)
	moles/mole enzyme	
Lysine	19.5	20
Histidine	14.2	14
Arginine	8.4	8
Aspartic acid	31.6	32
Threonine	17.6[c]	18
Serine	14.1[c]	14
Glutamic acid	23.0	23
Proline	11.5	12
Glycine	50.1	50
Alanine	21.5	22
Valine	28.2[d]	28
Methionine	4.0	4
Isoleucine	14.1[d]	14
Leucine	15.8[d]	16
Tyrosine	2.0	2
Phenylalanine	7.8	8
Cysteic acid	14.3[e]	14
Tryptophan	0[f]	0
Total residues		299

Hydrolysis times were recorded at 24, 48, and 72 hours for triplicate samples. The molecular weight of 30,400 was used in all of the computations. The c values were extrapolated to a time of zero for hydrolysis. To account for an indefinite amount of time, the values were extrapolated. Based on UVA absorption spectrum interpretation (number 37), 8 triplicate samples were hydrolyzed with dimethylsulfoxide using the technique of Spencer and Wold. At 258 nm, the molar extinction coefficient was 8,750 l/molcm. Amino Acid Composition of Cytosol Enzyme-Amino acid analyses were done following acid hydrolysis as indicated under “Materials and Methods.” Table 2 provides a summary of these kind of studies' findings. There were no direct analyses for tryptophan. According to the UV absorption spectra, its lack of presence was predicted. Those parts of a concentrated (250 mg per ml) solution of superoxide dismutase in 0.05 _II potassium phosphate-l x 10e4 M EDTA, pH 7.8, that were more than 0.75 cm from the air interface became yellow over a period of weeks when left at 4." This yellow enzyme absorbed light in exactly the same way that decreased superoxide dismutase does when mercaptoethanol is used. The auto-reduced yellow materials and the blue-green ones were equally active. Using the 2,2'-biquinoline technique (38), we observed that although the yellow material had 88% of its copper in the cuprous state, the blue-green material only contained 6%. All the copper in the blue-green superoxide dismutase was reduced to the cuprous form when mercaptoethanol was added. In a 0.05 M potassium phosphate-l x 10m4 M EDTA, pH 7.8 solution, newly isolated superoxide dismutase was found to have 1.2 f 0.2 sulfhydryl groups per subunit upon titration with pchloromercuriphenyl sulfonate. In contrast, solutions that had been frozen or kept at 4°C for many weeks were found to have just 0.2–0.3 sulfhydryls group per subunit. Dialyzing the pink, aged enzyme against 0.1 M KCI, 0.05 nM potassium phosphate, 1 x 104 M EDTA, pH 7.8 and then running gel exclusion chromatography on a column (2.5 X 120 cm) of Sephadex G-200 equilibrated with this buffer restored its sulfhydryl titer to 1 per sub- lut,ion. The outcome of this method is shown in Fig. 9. A single peak of constant specific activity eluted from the enzyme. Superoxide dismutase activity was measured at 3400 units per mg of protein, and the ultimate production was 52.2 mg with a recovery of 407. In Table 3 , we can see the overall outcomes of the purification process broken down into its constituent steps. Purity electrophoresis performed at a pH of 8.9 on a polyacrylamide gel showed a single protein band that correlated with the activity band. One time 103 M cyanide had no discernible effect on this activity. A single protein band was obtained from polyacrylamide gel electrophoresis in acid urea after incubation with 1-year-old P-mercaptoethanol. A new minor band was seen by polyacrylamide electrophoresis in acid urea when 6. mercaptoethanol treatment was skipped.

TABLE 3 Purification of chicken liver mitochondrial superoxide dismutase

Purification Stage	Volume (ml)	Protein* Conc. (mg/ml)	Specific Activity (u/mg)	Total Units	Total Protein (mg)	Yield (%)	Purification Factor
Supernatant from sonicate	4400	57.1	1.75	$4.4x10^5$	251,240	100	1.0
Heat Step	3800	35.3	2.75	$3.7x10^5$	134,140	84.1	1.6
Streptomycin Sulfate	3800	19.3	5.08	$3.7x10^5$	73,340	84.1	2.9
60% Ammonium Sulfate	4560	11.3	7.58	$4.1x10^5$	51,528	93.2	4.5
90% Ammonium Sulfate. Precipitate Redissolved	435	9.1	115	$4.6x10^5$	3,959	104.5	66
Dialysis and Centrifugation	600	3.7	164	$3.6x10^5$	2220	81.8	94
After Passing through DE-32	940	1.0	376	$3.5x10^5$	940	79.7	214
After Column Chromotography on CM-52	8.70	10.47	2420	$2.21x10^5$	91.1	50.1	1383
After Column Chromotography on G-200	5.64	12.93	3400	$1.77x10^5$	52.2	40.1	1941

Assuming E:tm = 1.0 at 280 nm. After column steps, the ultraviolet biuret method was used

Amino Acid Andysis-- Table 4 compares the amino acid composition of mitochondrial superoxide dismutase to that of several other superoxide dismutases from different organisms, both prokaryotic and eukaryotic. There are notable discrepancies between bacterial and eukaryotic levels for six residues (glutamate, glycine, alanine, leucine, tyrosine, and tryptophan). Although tryptophan has not been quantified, it has been shown that two enzymes (E. coli superoside dismutase (1) and S. mutans super- oxide dismutasc (2)) exhibit 280-am absorption peaks characteristic of tryptophan. The mitochondrial enzyme is most similar to the eukaryotic superoside tlismutases with regard to 5 of the 6 residues.

TABLE 4 Amino ucid composition of chicken liver mitochondrial superoxide dismutase

Amino Acid	Amino Acid Content[b]	Residues per mole of enzyme (nearest integer)	Chicken Liver Mitochondria (per subunit)	E.Coli (1) (per subunit)	S.Mutans (2) (per subunit)	Chicken Liver cytoplasm (per subunit)	Bovine erythrocyte (5) (per subunit)
	moles/mole enzyme						
Lysine	48.8	49	12	15	10	10	11
Histidine	28.8	29	7	6	6	7	8
Arginine	19.8	20	5	5	3	4	5
Aspartic Acid	77.2	77	19	21	21	16	18
Threonine	39.4[c]	39	10	9	10	9	13
Serine	41.4[c]	41	10	11	4	7	10
Glutamic Acid	74.4	74	19	18	22	12	12
Proline	30.2	30	8	8	7	6	7
Glycine	65.0	65	16	13	12	25	25
Alanine	50.6	51	13	24	29	11	11
Valine	42.2[d]	42	11	10	12	14	14
Methionine	10.4	10	3	2	2	2	0
Isoleucine	33.6[d]	34	8	7	12	7	8
Leucine	68.8[d]	69	17	19	19	8	10
Tyrosine	32.0	32	8	6	8	1	1
Phenylalanine	21.6	22	5	9	7	4	5
Cysteic Acid	9.0[e]	9	2	-	-	7	-
Tryptophan	18.4[f]	18	5	-	-	0	0
Total Residues		711	178	183[g]	184[g]	150	158[h]

The molecular weight of 79, GOO was used for all computations. c the values were expanded to the point when hydrolysis takes place instantly. d There was no variation among time periods. 6 According to Hirs's performic acid procedure (31). In accordance with Edelhoch's spectral technique. Does not include cysteic acid or tryptophan. It excludes cysteic acid.

Table 5 summarises the findings of these various purification methods. After the Sephadex G-75 gel column, we observed a peak specific SOD activity of 4818.2 IUmg/mg.

The enzyme was filtered via a Sephadex G-75 column and its molecular weight was found to be 32 000 1.000. SDS-PAGE electrophoresis Fig. 4 calculated the subunit size of isolated SOD to be 16 000500. These findings point to a dimer structure, with two identical subunits, for the native enzyme. Purified SOD was found to have 1.00.02 atoms of Cu and 0.850.01 atoms of Zn per subunit, as measured by atomic absorption spectroscopy.

Table 5 The purification steps of chicken liver SOD

Purification step	IU/ml	Total activity	Total protein (mg)	Specific activity (U/mg)	Yield (%)	Purification fold-factor
Crude extract	725	14500	860	16.86	100	—
Hb-free crude extract	712	12816	171	74.95	88	4.5
PEG-3350 precipitation	702	12636	74.7	169.15	87	10.0
DEAE-cellulose	286.6	4300	6.1	704.9	29.7	41.8
Sephadex G-75	84.8	1060	0.22	4818.2	7.3	285.8

Substrate Specificity–Milk xanthine oxidase can slowly oxidize reduced diphosphopyridine nucleotide and xanthopterin, in addition to a wide range of aldehydes (22-24) and ox purines. The xanthine dehydrogenase in chicken liver oxidized both xanthine and hypoxanthine at a similar rate. Table 6 details the enzyme's activity in the presence of aldehydes at concentrations of 0.05 M. Similar to the previously published manometric method, but the buffer had a pII of

TABLE 6 Relative Rates of Oxidation of Various Substrates by Purified Chicken Liver Xanthine Dehydrogenase in Comparison with Activity toward Hypoxanthine – Xanthine

Substrate	Xanthine dehydrogenase		Milk xanthine oxidase*
	Manometric	Dye reduction	
Hypoxanthine	100	100	100
Xanthine	100	96	100
p-Hydroxybenzaldehyde	33	40	130
Acetaldehyde		76	72
Furfural		88	75
Benzaldehyde		57	80
Butyraldehyde		54	0.6

Values reported by Booth (24).

CONCLUSION

Lactobacillus plantarum and similar lactobacilli stand out because they use a unique method to shield themselves from the oxidizing effects of reactive O2. These SOD antioxidant system deficits may be responsible for or at least contribute to the oxidative stress associated with IBDs and chronic inflammation. The superoxide anion free radical (•O2) is neutralized by SOD, which then catalysis the formation of hydrogen peroxide (H2O2) and molecular oxygen (O2). Then, enzymes like catalase (CAT), glutathione peroxidase (GPx), and/or peroxiredoxin (Prx) that rely on thioredoxin (Trx) convert the H2O2 back into water. Variations of Superoxide Dismutase in Chicken Liver The soluble extracts of one fresh chicken liver that were homogenized, ultraconfident, and centrifuged contained at least. four different superoxide dismutase as shown by electrophoresis to conduct electrophoresis in acid urea gels, we followed the methods of Panyim and Chalkley. Disc gel electrophoresis on 10% polyacrylamide gels in the presence of sodium dodecyl sulphate was used to calculate the molecular weight of the enzyme's constituent subunits.

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