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Extracellular Superoxide Dismutase

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Abbreviations:
CuZnSOD, copper, zinc-superoxide dimutase (SOD1); EC-SOD, extracellular superoxide dismutase (SOD3); LDL, low density lipoprotein; MnSOD, manganese-superoxide dismutase (SOD2).

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Abstract

EC-SOD is a tetrametric, slightly hydrophobic glycoprotein that has a molecular weight about 135,000 Da. This enzyme has high reactivity with the superoxide radical. It is mainly located in extracellular fluids, including plasma and the extracelluar matrix of tissues. The highest expression of human EC-SOD is in the hearts, followed by the pancreas, and the lungs. Because SOD3 gene shares 40% - 60% similarity with the SOD1 gene at exon level, EC-SOD displays many similarities with CuZnSOD. Due to the antioxidant activity of EC-SOD, it suppresses LDL oxidation and protects lungs from inflammation. Owning a peroxidase activity, active EC-SOD can remove H_2O_2 in extracellular space.

Introduction

The evolution of aerobic organisms that can survive in oxygen-rich environments requires an effective defense system against reactive oxygen species (ROS), which are produced following single electron reductions of molecular oxygen [1]. An unbalanced, elevated concentration of ROS may contribute to the development of various diseases, such as cancer, hypertension, diabetes, atherosclerosis, inflammation, and premature aging [2]. The major defenses against the superoxide anion radical are three superoxide dismutases (SODs). CuZn-SOD (SOD1) exists in the cytosol, while MnSOD (SOD2) exists in the mitochondrial matrix, and extracelluar SOD (EC-SOD; SOD3) [3] is located in extracellular fluids, including plasma and the extracelluar matrix of tissues. EC-SOD is a predominant extracellular antioxidant enzyme [4]. It plays a critical role to scavenge superoxide anion ($O_2^{\bullet -}$) generated in extracellular space. The structure and properties of EC-SOD is very important for us to understand the mechanisms of some diseases and cancers.

Primary structure and properties

EC-SOD is a tetrametric, slightly hydrophobic glycoprotein that has a molecular weight around 135,000 Da. Upon chromatography on heparin-sepharose [5], it is divided into three fractions, A, B and C, with different affinities for heparin. [6]. The enzyme is cyanide-sensitive, composed of four equal non-covalently bound subunits and has a high reactivity with the superoxide radical. It appears to possess four copper atoms and possibly also four zinc atoms/molecules [7].

Tissue-specific expression of human EC-SOD

One study reported an experiment about the expression of human EC-SOD [4]. mRNA from eight different human tissues was isolated and analyzed by RNA gel blot hybridization to a radiolabeled anti-sense cDNA probe derived from their full-length human EC-SOD cDNA. Figure 1 shows us expression of EC-SOD mRNA corresponding the bands (data not shown) in RNA gel blot quantitated by laser densitometric scanning in different tissues. As can be seen, the heart showed the most expression, followed by the pancreas, and the lung. Expression in skeletal muscle was slightly higher than kidney and liver. The brain showed the lowest expression of EC-SOD mRNA.

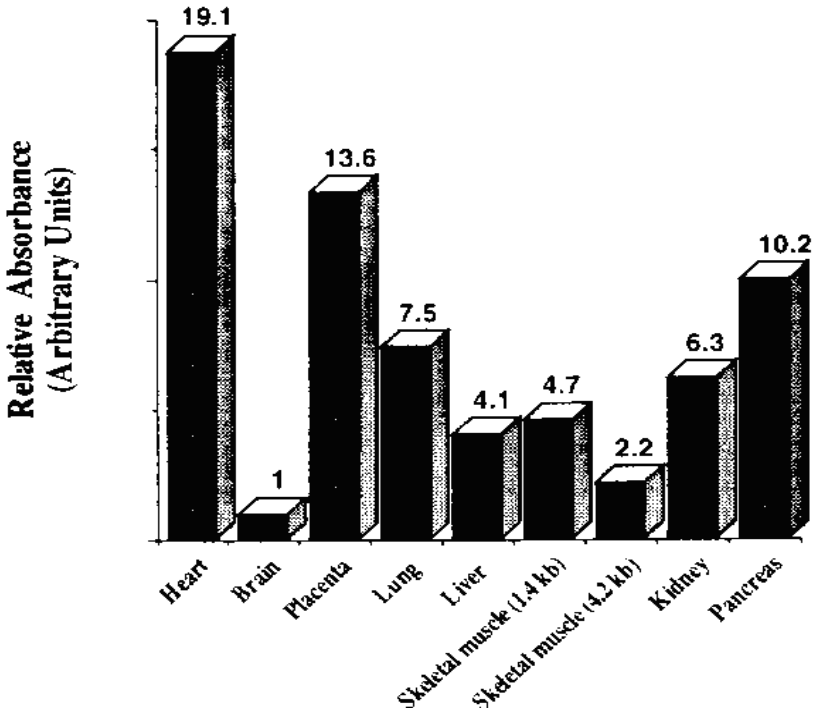
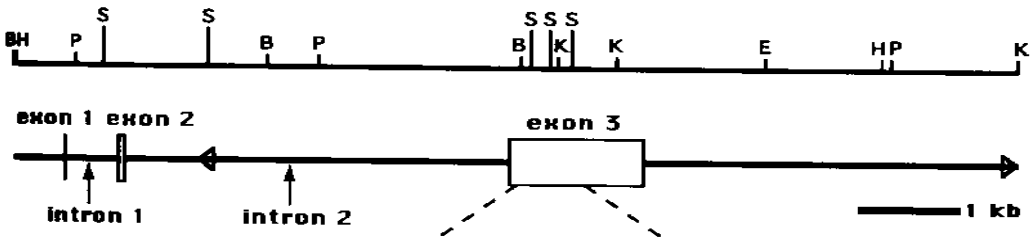


Figure 1. Expression of EC-SOD mRNA in different tissues [4]

Genomic characterization

Figure 2 (A) shows us a partial restriction map of human EC-SOD genomic clone in the 5' to 3' orientation. The exon/intron structure of the human EC-SOD is shown. Figure 2 (B) shows the four structural domains of human EC-SOD protein. The signal peptide is indicated by an arrow. This is followed by the mature, lycosylated (CHO), amino-terminal peptide domain. A third region has very high amino acid sequence homology to human CuZnSOD. The carboxy-terminal domain has multiple charged basic residues (+), which are critical for binding heparin glycosaminoglycans.

A. EC-SOD restriction map and gene structure



B. EC-SOD protein structure

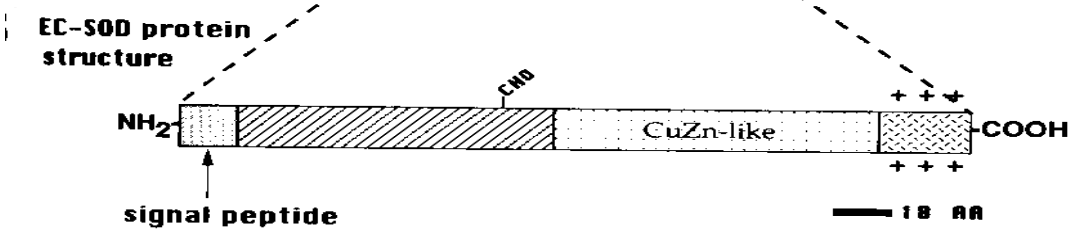


Figure 2. Partial restriction map, genome structure, and protein structure of human EC-SOD [4].

Comparison with CuZnSOD

The Cu- and Zn-containing superoxide dismutase CuZnSOD (SOD1) was the first SOD isoenzyme to be described. It is composed of two 16-kDa subunits, each containing one Cu and one Zn atom. CuZnSOD is located in the cytosol or peroxisomes of eukaryotic cells [8]. The structure and location of EC-SOD (SOD3) described above are different. Despite these physical differences, EC-SOD displays many similarities with CuZnSOD. Both isoenzymes catalyze first-order disproportionations of superoxide anions. Their specific activities are similar, and both are inhibited by cyanide, azide, diethyldithiocarbamate, and hydrogen peroxide [9]. The SOD3 gene shares 40%-60% similarity with the SOD1 gene at exon level (Figure 3) [10].

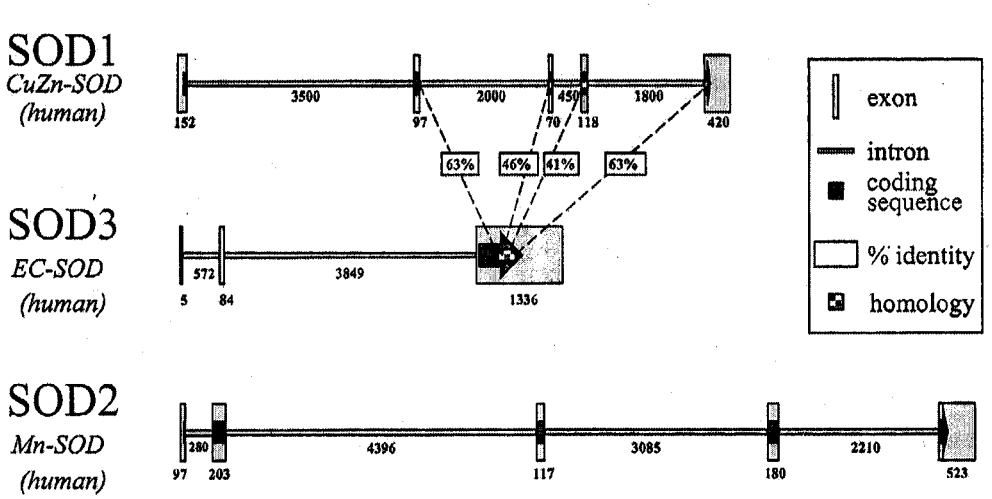


Figure 3. Genomic organization of the three known members of the human SOD enzyme family [10].

Function of EC- SOD

The catalytic reaction

SOD catalyzes the dismutation of $O_2^{\bullet-}$ to H_2O_2 and dioxygen, as seen in the reaction (1) below [8].

$$O_2^{\bullet-} + O_2^{\bullet-} + 2H^+ \xrightarrow{\text{SOD}} H_2O_2 + O_2 \quad (1)$$
$$k = 2 \times 10^9\ M^{-1}s^{-1}$$

EC-SOD suppresses LDL oxidation

EC-SOD is located in the extracellular space and binds to sulfated glycosaminoglycans. The extracellular matrix is the place where LDL may be mainly oxidized by endothelial cells, smooth muscle cells or macrophages. One study constructed the recombinant adenovirus AxCAEC-SOD expressing human EC-SOD by CAG promoter. They transfected endothelial cells with increasing MOI of AxCAEC-SOD (3, 30, 300 MOI) and vector control AxCALacZ 300 MOI. Table 1 shows that the percentage LDL modification with AxCAEC-SOD was decreased by 50% at an MOI of 300 compared with AxCALacZ. The results indicated that EC-SOD could inhibit endothelial-cell mediated LDL oxidation [11].

TABLE 1
Effect of AxCAEC-SOD Infection on Endothelial-Cell-Mediated LDL Oxidation on Electrophoretic Mobility

	Electrophoretic mobility (mm)	LDL modification (%)
Native LDL	4.00 ± 0.00	0.0
Vehicle	13.19 ± 0.25	100.0
EC-SOD (MOI = 3)	11.36 ± 0.52*	88.0*
EC-SOD (MOI = 30)	10.38 ± 0.26*	69.4*
EC-SOD (MOI = 300)	8.74 ± 0.42*	50.3*
LacZ (MOI = 300)	13.56 ± 0.44	104.0
Cu^{2+}-induced oxidized LDL	24.75 ± 0.48*	225.8*

EC-SOD protects lung from inflammation

It has been implicated in pathologies of the organ that the lung is exposed to high oxygen tension and oxygen free radicals [4]. Extracellular superoxide dismutase occurs in high concentration in the lung and protects against hyperoxia-induces inflammation. One study used

the lungs from old EC-SOD-null and wild type mice. They observed alveolar, bronchial inflammation and degenerative changes in the lung (Table 2) [12]. More pathological signs occurred in the EC-SOD-null mice, especially chronic bronchial inflammation and fibrosis.

Table 2. Morphology of Lungs from Old Mice

Pathology		+/+ (n = 11)	−/− (n = 11)
Bronchial inflammation	Acute	0	2
	Chronic	1	4
	Edema	4	3
Alveolar inflammation	Acute	0	1
	Chronic	4	7
	Edema	2	3
Fibrosis		1	5
Emphysema		7	7

The figures indicate number of animals in each group of 11 mice (6 males and 5 females), with pathological findings.
+/+ = wild-type mice; −/− = EC-SOD–null mice.

Peroxidase activity of EC-SOD

Cytoplasmic Cu/Zn superoxide dismutase not only catalyzes dismutation of $O_2^{\bullet-}$ to H_2O_2, but also has peroxidase activity, with H_2O_2 used as a substrate, forming a copper-bund hydroxyl ($HO^{\bullet}$) radical [13]. EC-SOD (SOD3) is also a Cu/Zn-containing SOD that shares about 50% sequence homology with SOD1 within its catalytic region. One study demonstrated the peroxidase activity of SOD3. As can be seen from figure 4, the active SOD3 reacts with H_2O_2 to form an intermediate (SOD3-OH radical) that can interact with the bicarbonate to ultimately lead to the formation of a DEPMPO-OH adduct. In the absence of a cosubstrate, SOD3 (SOD1) is inactivated by H_2O_2. In the presence of physiological levels of uric acid, however, SOD3 (SOD1) inactivation is prevented. The urate radical formed has low oxidative potential and may react with ascorbic acid to regenerate uric acid.

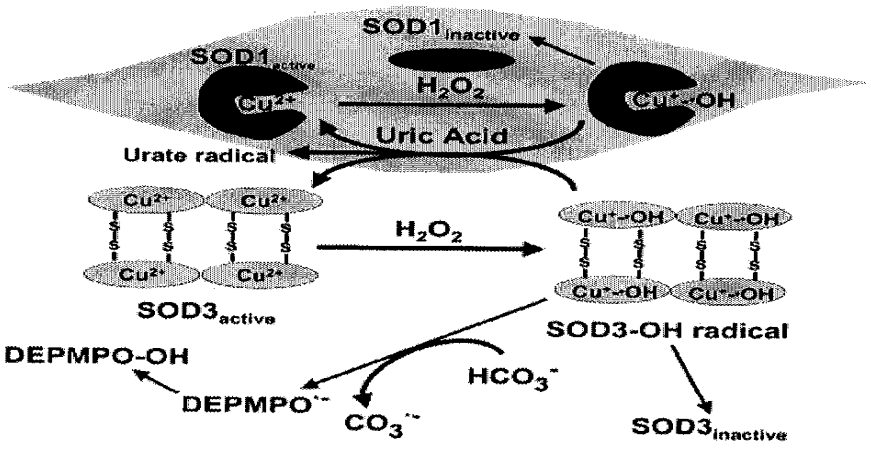


Figure 4. Peroxidase activity of SOD1 and SOD3 [13].

Summary

Extracellular superoxide dimutase (EC-SOD) is a predominant antioxidant enzyme that plays a critical role to scavenge superoxide in extracellular space. Because EC-SOD gene shares 40%-60% similarity with CuZnSOD gene at exon level, it displays many similarities with CuZnSOD. The levels of expression of human EC-SOD are various in different tissues. EC-SOD not only has functions to suppress LDL oxidation and protects lungs from inflammation, but also owns peroxidase activity to remove H_2O_2 in extracellular space.

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