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SOD mimics

by

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Abbreviation List:

H_2O_2	hydrogen peroxide
ROS	reactive oxygen species
SOD	Superoxide dismutase

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Abstract

Superoxide dismutases (SODs) are enzymes that catalyze the removal of superoxide radicals ($O_2^{\bullet -}$), and convert $O_2^{\bullet -}$ to hydrogen peroxide (H_2O_2) and dioxygen (O_2). SODs have metal in their active sites. Low molecular weight complexes that can remove $O_2^{\bullet -}$ are desirable as SOD mimics because native SOD enzymes, if treated outside the cells, cannot penetrate cell membrane. There are two classes of SOD mimics: metal-dependant and metal-independent. The metal-dependant mimics have three forms: Mn, Cu and Fe containing forms. Mn containing forms are commonly used. Two of such compounds are discussed in this paper: M40403 and $Mn^{II}OBTMPyP^{4+}$.

Introduction

Reactive oxygen species (ROS), including superoxide ($O_2^{\bullet -}$), hydrogen peroxide (H_2O_2), and hydroxyl radicals ($^{\bullet}OH$), are generated by a number of pathways [1]. Accumulation of these active oxygen species in aerobic organisms may cause DNA strand breakage, peroxidation of membrane lipids, and inactivation of enzymes [2]. Defense systems derived from enzymatic and nonenzymatic antioxidants in living organisms can minimize the deleterious effects of active oxygen free radicals. Superoxide dismutases, catalase, and peroxidase are among these important antioxidant enzymes [3]. SODs are a group of metal-containing enzymes that catalyze the dismutation of superoxide radical to oxygen and hydrogen [4]. These enzymes may be classified into three types according to their metal cofactor requirements: manganese (MnSOD), copper/zinc (CuZnSOD), and iron (FeSOD) forms.

Properties of SOD

Superoxide dismutases (SODs) are a family of metalloenzymes that provide a defense against the toxicity of superoxide. Superoxide is a free radical arising from oxygen metabolism. SODs catalyze the removal of superoxide radicals providing a major protective effect in our bodies. They catalyze the conversion of $O_2^{\bullet -}$ to hydrogen peroxide (H_2O_2) and dioxygen (O_2), seen in the reaction below [6].

$$2O_2^{\bullet -} + 2H^+ \longrightarrow H_2O_2 + O_2$$

Studies showed MnSOD is widespread in bacteria, plants and animals. In most animals, MnSOD is largely located in the mitochondria [1]. The reaction mechanism of MnSOD catalysis can be written as

$$Mn^{3+} + O_2^{\bullet -} \Leftrightarrow [Mn^{3+}\text{-}O_2^{\bullet -}] \longrightarrow Mn^{2+} + O_2$$

$$Mn^{2+} + O_2^{\bullet -} \Leftrightarrow [Mn^{2+}\text{-}O_2^{\bullet -}] + 2H^{+} \longrightarrow Mn^{3+} + H_2O_2$$

At pH 7.0, the rates of $O_2^{\bullet -}$ dismutation for MnSOD enzyme in *E. coli* is around 1.8×10^9 $M^{-1}s^{-1}$. But the rates of this reaction for MnSOD decrease at alkaline pH, such as at pH 10.2, the rate constant is 0.33×10^9 $M^{-1}s^{-1}$ [5].

In higher organisms, MnSOD usually contains four protein subunits and usually have 0.5 or 1.0 ions of Mn per subunit. The catalytic activity will loss if manganese is removed from the active site and the manganese can not usually be replaced by other transition-metal ion to yield a functional enzyme [1].

Decrease in the levels of SOD within a cell causes injuries to the cell [7]. Exogenous treatment of these cells with native SOD does not solve this problem because native SOD cannot penetrate cell membrane. Because iron, copper and manganese are cofactors in the active site of native SOD, low molecular weight chelates of these ions can be used as mimics of SOD. Certain SOD mimics based on copper can react rapidly with $O_2^{\bullet -}$ but is found to be biologically ineffective because they can react with molecular oxygen and dissociate with chelates at high rate [8]. In contrast, manganese was found not to dissociate with its chelates and, at the same time, can remove $O_2^{\bullet -}$ efficiently [9].

Properties of SOD mimics

A transition metal complex must meet the following criterions to be an effective mimic of SOD: (1) has a low molecular weight and high cell permeability. (2) soluble in water, (3) stable, (4) specific for its substrate (superoxide, $O_2^{\bullet-}$), and (5) reduction potential between –0.33 V(the standard reduction potential of the couple $O_2/O_2^{\bullet-}$) and +0.65 V (the standard reduction potential of the couple $^1O_2/O_2^{\bullet-}$) so that catalysis can take place whereas singlet oxygen cannot be formed[10, 11]. In order to work *in vivo*, the complex should be non-immunogenic, nontoxic. They should also have a catalytic rate constant between 10^8 and 10^9 $M^{-1}s^{-1}$ [11].

There are two major classes of SOD mimics, those that contain metals and those that are metal-independent. The three metals contained in complexes normally studied are copper, iron, and manganese. Metal-independent SOD mimics are various nitroxides complexes.

SOD mimics catalyze the dismutation of $O_2^{\bullet-}$ to hydrogen peroxide (H_2O_2) and dioxygen (O_2) as illustrated in reactions (1) and (2). M represents a metalloenzyme, that is, a metal containing complex that acts to catalyze reactions. M^{n+} is this complex in the oxidized state and $M^{(n-1)+}$ is in the reduced state.

$$M^{n+} + O_2^{\bullet-} \longrightarrow M^{(n-1)+} + O_2 \quad (1)$$

$$M^{(n-1)+} + O_2^{\bullet-} + 2H^+ \longrightarrow M^{n+} + H_2O_2 \quad (2)$$

But metals have such problems as that they can react with H_2O_2 to make the more reactive oxygen species, the hydroxyl radical ($^{\bullet}OH$). This product is very reactive and reacts very quickly. Thus, it enhances cell toxicity rather than protecting against $O_2^{\bullet-}$ induced damage. The general redox process of molecules containing metals is illustrated in reactions (3) and (4) [11].

$$M^{n+} + O_2^{\bullet-} \longrightarrow M^{(n-1)+} + O_2 \quad (3)$$

$$M^{(n-1)+} + H_2O_2 \longrightarrow M^{(n-1)+} + OH^- + {}^{\bullet}OH \quad (4)$$

For a metal containing mimic to be deemed protective the rate of reaction (2) must be greater than the rate of reaction (4) [12].

Manganese complexes

Besides copper- and iron-containing complexes, another group of metal-dependant SOD mimics are those that contain manganese and are mainly manganese-porphyrins. The primary structure of these molecules is shown in Figure 1 [13].

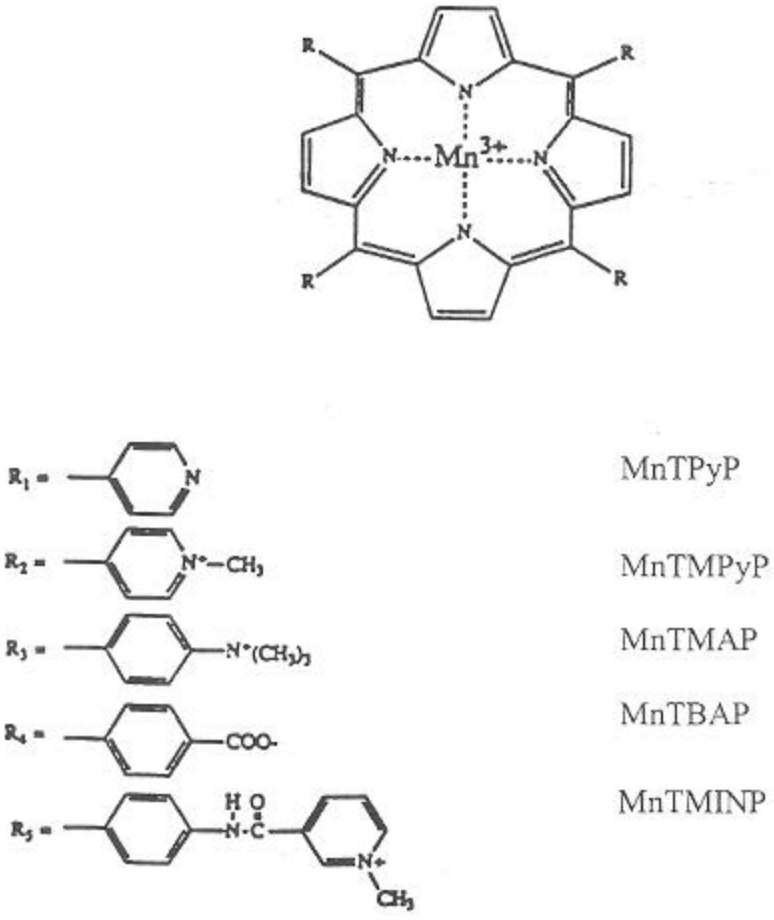

Figure 1. Manganese-porphyrins, the R-group structures and their names [13].

The names used in Figure 1 are: MnTPyP, which is Mn(III) *tetra*(4-pyridyl) prophyrin; MnTMPyP, Mn(III) *tetrakis*(1-methyl-4-pyridyl) prophyrin; MnTMAP, Mn(III) *tetrakis*(trimethylammonio) phenyl prophyrin; MnTBAP, Mn(III) *tetrakis*(4-benzoic acid) prophyrin; and MnTMINP: Mn(III) *a a a b-tetrakis*[(1-methylisoniconcinamido) phenyl] prophyrin;

The most common strategy to use manganese as an SOD mimic is to use manganese as a redox active center with Mn^{3+} being reduced to Mn^{2+} by superoxide radical followed by oxidation of Mn^{2+} to Mn^{3+} by a second superoxide radical, as shown in reaction (1) and (2). The metal is liganded within a macrocyclic porphyrin ring system [7].

A manganese(II) complex with a *bis*(cyclohexylpyridine)-substituted macrocyclic ligand known as M40403, is not the first nonprotein SOD mimic researchers have identified, but it is more specific in its action than the others [14]. This compound transforms superoxide at rates similar to those of native SOD and does not break down in the body. When injected into rats, M40403 greatly decreased several key indicators of inflammation such as swelling, tissue damage, and white blood cell accumulation at an injury site. In another series of animal tests, M40403 reduced the "reperfusion" injury that occurs when clot-busting drugs are used to treat a heart attack or stroke. The structure of M40403 is shown in Figure 2a [14].

Another compound, manganese β-octabromo-*meso*-tetrakis-(N-methylpyridinium-4-yl) porphyrin, known as $Mn^{II}OBTMPyP^{4+}$, was also synthesized and characterized. The structure is shown in Figure 2b [15]. The rate constant of this compound for the dismutation of $O_2^{\bullet-}$ is $k_{cat} = 2.2\times10^8$ $M^{-1}s^{-1}$. And slow dissociation of manganese from $Mn^{II}OBTMPyP^{4+}$ enables the

compound to be stable and exhibit catalytic activity even at the nanomolar concentration level and at biological pH.

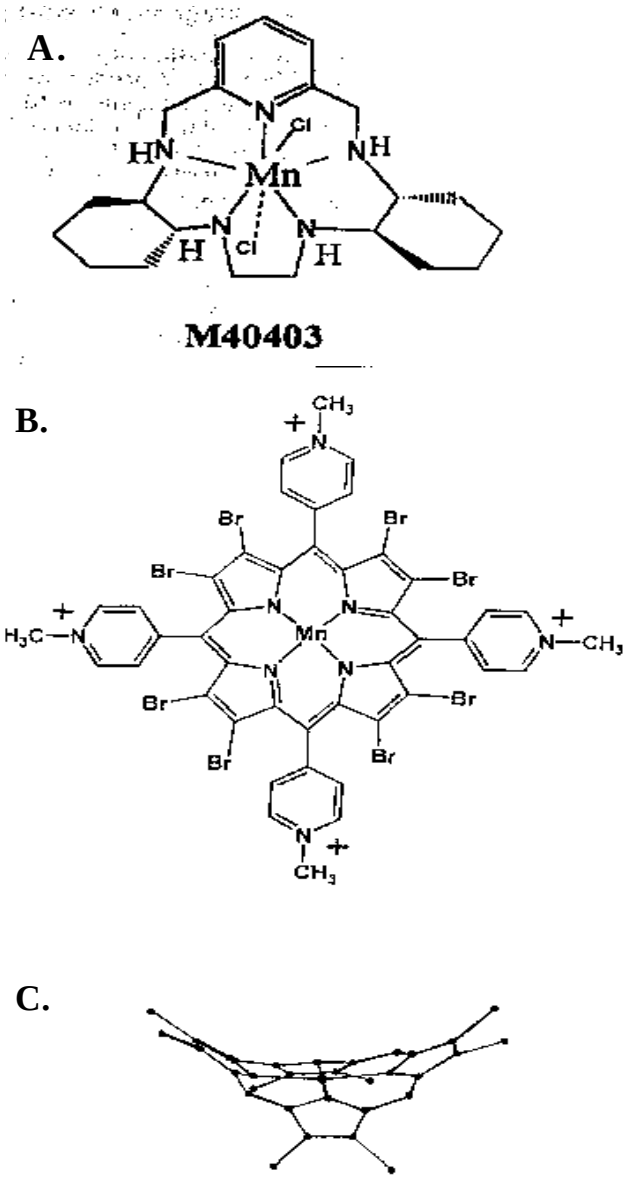

Figure 2. Structures of two manganese containing complexes. A. Structure of M40403. **B.** Structure of $Mn^{II}OBTMPyP^{4+}$, with β and *meso* substituents indicated. **C.** Edge-on view of the skeleton of $Mn^{II}OBTMPyP^{4+}$ [14, 15].

Summary

Superoxide dismutases (SODs) are a family of metalloenzymes that catalyze the removal of superoxide radicals ($O_2^{\bullet -}$), and convert $O_2^{\bullet -}$ to hydrogen peroxide (H_2O_2) and dioxygen (O_2). Because native SOD enzymes cannot penetrate cell membrane, low molecular weight complexes that can remove $O_2^{\bullet -}$ are more desirable as SOD mimics. There are two classes of SOD mimics: metal-dependant and metal-independent. The metal-dependant mimics have three forms: Mn, Cu and Fe containing forms. Mn containing forms are commonly used, among these are two compounds: M40403 and $Mn^{II}OBTMPyP^{4+}$.

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