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Only one of a wide assortment of manganese-containing SOD mimicking compounds rescues the slow aerobic growth phenotypes of both *Escherichia coli* and *Saccharomyces cerevisiae* strains lacking superoxide dismutase enzymes

William Munroe [a], Carolyn Kingsley [a], Armando Durazo [a], Edith Butler Gralla [a], James A. Imlay [b], Chandra Srinivasan [c,*], Joan Selverstone Valentine [a,*]

[a] *Department of Chemistry and Biochemistry, UCLA, Los Angeles, CA 90095-1569, United States*
[b] *Department of Microbiology, University of Illinois, Urbana, IL 61801, United States*
[c] *Department of Chemistry and Biochemistry, California State University, Fullerton, CA 92834-6866, United States*

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We dedicate this paper to our colleague, Ed Stiefel, an inspirational researcher, teacher, and friend.

Abstract

A variety of manganese-containing coordination compounds, frequently termed superoxide dismutase (SOD) mimics, have been reported to have SOD activity *in vitro* and to be effective at improving conditions related to increased oxidative stress in multicellular organisms. We tested the effectiveness of several of these compounds in substituting for authentic SOD enzymes in two simple systems – the prokaryote *Escherichia coli* and the single-celled eukaryote, *Saccharomyces cerevisiae* – where strains are available that completely lack cytoplasmic SOD activity and are thus significantly impaired in their ability to grow aerobically. Most of the compounds tested, including Euk-8 and Euk-134, manganese salen derivatives developed by Eukarion; M40403, a manganese complex of a bis(cyclohexylpyridine)-substituted macrocyclic ligand developed by Metaphore; and several manganese porphyrin derivatives, were ineffective in both systems. Only the manganese tetrapyridyl porphyrin complex MnTM-2-PyP and two close relatives were effective in rescuing aerobic growth of *E. coli* lacking SOD, and, in the case of *sod1*Δ yeast, only MnTM-2-PyP itself was fully effective. Surprisingly, several compounds reported to be beneficial in other *in vivo* model systems (Euk-8, Euk-134, M40403) were actually toxic to these organisms lacking SOD, although they had no effect on the wild-type parent strains. Our results suggest the possibility that the beneficial effects of some of the so-called "SOD mimic drugs" may be due to some property other than *in vivo* superoxide dismutase activity.

Keywords: Superoxide dismutase; SOD mimic; Eukarion; Manganese; Porphyrin; Metaphore

1. Introduction

Oxidative stress has been implicated in a variety of detrimental health conditions including ischemia-reperfusion injury, chronic inflammation, neurodegenerative disease, and aging [1]. The potential medical utility of a compound (drug or supplement) that would reduce oxidative stress has led to the development and testing of a variety of manganese-containing coordination compounds that have been termed superoxide dismutase (SOD) mimics. In some cases, substantial beneficial effects have been reported for *in vivo* tests of these compounds (see below for references).

The exact molecular mechanisms by which the manganese-containing SOD mimics function *in vivo* are unknown,

* Corresponding authors. Tel.: +1 310 825 9835; fax: +1 310 206 9880.
E-mail addresses: chandra@fullerton.edu (C. Srinivasan), jsv@chem.ucla.edu (J. Selverstone Valentine).

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and we therefore decided to address this question using two very simple model systems – bacteria (*Escherichia coli*) and yeast (*Saccharomyces cerevisiae*) that completely lack their cytoplasmic SOD enzymes – to screen compounds for their ability to substitute for bona fide SOD enzymes. We chose a prokaryotic system because of its simplicity and lack of intracellular compartmentalization and a eukaryotic single-celled organism because of its more complex subcellular organization and closer resemblance to higher organisms.

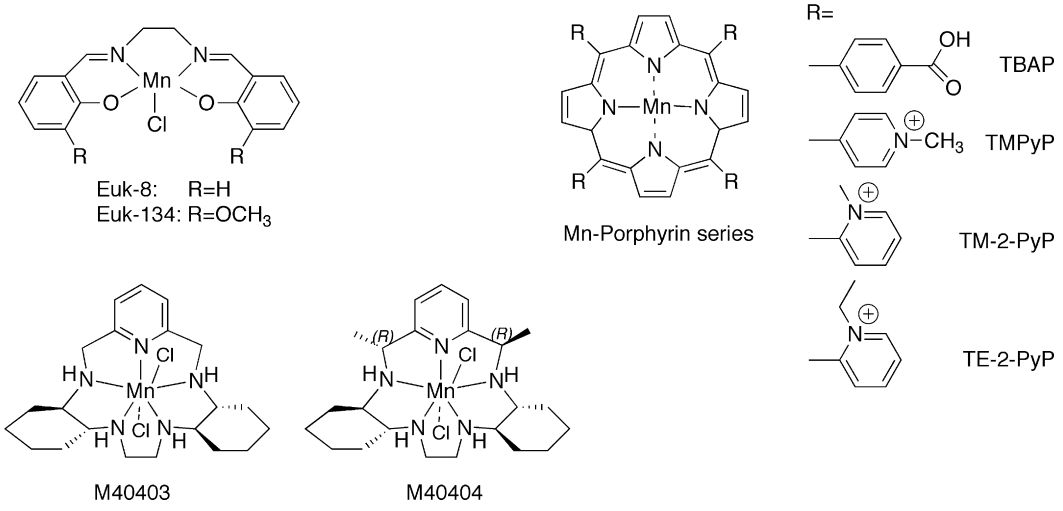
Wild-type *E. coli* have iron-containing and manganese-containing cytoplasmic SODs. *E. coli* lacking both of these cytosolic SODs, termed *sodA*$^-$*sodB*$^-$, exhibit several phenotypes that can be directly attributed to the loss of SOD activity, e.g., poor aerobic growth [2] and auxotrophies for branched-chain [3], aromatic [4] and sulfur-containing amino acids [5]. Most eukaryotes, including yeast, have a single abundant, copper- and zinc-containing SOD (CuZnSOD or SOD1) in the cytosol (which is also present in the nucleus and some other cellular locations) and a much less abundant MnSOD in the mitochondrial matrix. Yeast lacking CuZnSOD (*sod1*Δ) exhibit a variety of aerobic phenotypes including poor growth, lysine and methionine auxotrophies, and decreased stationary phase survival [6–10]. In both of these systems, i.e., *sodA*$^-$*sodB*$^-$ *E. coli* and *sod1*Δ *S. cerevisiae*, growth under anaerobic conditions reverses these phenotypes.

We recently demonstrated that all known phenotypes of *sod1*Δ yeast were completely reversed when the growth medium was supplemented with high levels (5 mM) of ionic manganese [11]. Simple manganese salts have also been reported partially to reverse the phenotypes of *sodA*$^-$*sodB*$^-$ *E. coli*, including slow aerobic growth rates, lowered aconitase activity, and sensitivity to hydrogen peroxide [2]. Much lower levels of manganese salts (10–100 μM) are effective in the latter case. The exact molecular mechanisms by which ionic manganese functionally substitutes for the SOD enzymes in yeast and *E. coli* lacking SOD enzymes are not yet known, and we wondered whether the mechanisms of action of the manganese-containing SOD mimics could be related.

The structures of the SOD mimics that we examined are shown in Scheme 1. The Euk class are manganese salen derivatives that are reported to possess both SOD and catalase activities *in vitro*, properties which could in theory allow these molecules to protect against a wider range of reactive oxygen species (ROS) and thereby lower oxidative stress [12]. Euk-8 is the prototypical manganese salen SOD mimic; alterations made to the ligand doubled the catalase activity (Euk-134) or improved the solubility of the compound in organic solvents (Euk-189) without significantly affecting the SOD activity. It should be noted, however, that Euk-8 and Euk-134 have been reported to lose activity upon treatment with EDTA [13].

The EUK compounds have been shown to be effective treatments for several different types of oxidative stress-related conditions, such as ischemia-reperfusion injury in a rat stroke model [14], ROS-induced apoptosis [15], and paraquat-induced dopaminergic cell death in neurons [16]. They have also been reported to improve survival of *sod2* null mice [17], and dose-dependent increases in life span occurred in *Caenorhabditis elegans* under some growth conditions [18,19] indicating these compounds entered the cell. Another study of Eukarion compounds in *C. elegans* found adverse rather than beneficial effects with dose-dependent decreases in life span and fertility [13,20]. The Eukarion compounds also did not extend life span in the housefly [21].

The SOD mimics from Metaphore are highly stable manganese complexes of a bis(cyclohexylpyridine)-substituted macrocyclic ligand derivatives that retain SOD activity in the presence of EDTA [22]. They are reported to react specifically with superoxide and not with other ROS or reactive nitrogen species [23,24]. The SOD-active compound, M40403 (see Scheme 1) was shown to be effective in a variety of model systems, such as ischemia-reperfusion injury in rat heart [25] and inflammation in rat [26,27].



Scheme 1.