Nanomaterials for Selective Superoxide Dismutation

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ABSTRACT | Superoxide is a primary reactive oxygen species (ROS) formed from aerobic metabolism. Superoxide dismutase (SOD), by catalyzing the conversion of superoxide to form hydrogen peroxide and molecular oxygen, is a first-line defense against superoxide toxicity. Due to the protein nature of endogenous SOD, small molecule SOD mimetics are commonly used to protect against superoxide-mediated pathophysiological processes and identify the causal role of this ROS in diseases pathogenesis. Existing small molecule SOD mimetics, however, also are reactive toward other ROS and related species, including hydrogen peroxide, nitric oxide, and peroxynitrite, which makes it difficult to delineate the involvement of superoxide in the disease process. Nanomaterials, such as cerium oxide nanoparticles and fullerenes, also possess SOD activity, but again lack specificity for superoxide. A recent study by Samuel et al. reported in the Proceedings of the National Academy of Sciences of the United States of America (2015 Feb 24; 112(8):2343–8. doi: 10.1073/pnas.1417047112) demonstrates that poly(ethylene glycolated) hydrophilic carbon clusters (PEG-HCCs) efficiently catalyze the conversion of superoxide to form hydrogen peroxide and molecular oxygen, and more importantly, the carbon nanoparticles are inert to nitric oxide and peroxynitrite. This work represents a major advancement in the development of SOD biomimetics that are highly specific for superoxide. Application of such specific SOD biomimetics would have significant impact on the field of superoxide biology and medicine.

KEYWORDS | Hydrophilic carbon clusters; Nanomaterials; Reactive oxygen species; Superoxide; Superoxide dismutase

ABBREVIATIONS | EPR, electron paramagnetic resonance; MPO, myeloperoxidase; PEG-HCCs, poly(ethylene glycolated) hydrophilic carbon clusters; RNS, reactive nitrogen species; ROS, reactive oxygen species; SOD, superoxide dismutase

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1. SUPEROXIDE AS A PRIMARY REACTIVE OXYGEN SPECIES

Utilization of molecular oxygen by aerobic organisms inevitably leads to the formation of reactive oxygen species (ROS). ROS simply refers to oxygen-containing reactive species. It is a collective term to include superoxide anion radical (or superoxide for short) \(O_2^-\), hydrogen peroxide \(H_2O_2\), hydroxyl radical \((\cdot OH)\), singlet oxygen \((\cdot O_2)\), peroxyl radical \((\cdot LOO^-)\), alkoxyl radical \((\cdot LO^-)\), lipid hydroperoxide \((\cdot LOOH)\), hypochlorous acid \((\cdot HOCl)\), carbonate radical \((\cdot CO_3^-)\), nitrogen dioxide radical (commonly known as nitrogen dioxide) \((\cdot NO_2^-)\), and ozone \(O_3\), among others. As shown in Figure 1, superoxide is a primary ROS that leads to the formation of many secondary ROS or reactive nitrogen species (RNS). The term RNS refers to nitrogen-containing reactive species, such as nitric oxide \((\cdot NO)\), nitrogen dioxide, and peroxynitrite. Nitric oxide and nitrogen dioxide are free radicals, whereas peroxynitrite is a non-radical. Because biologically relevant RNS are almost exclusively also oxygen-containing species, the compound term ROS/RNS is frequently used in the literature. Also for this same reason, the oxygen containing RNS are sometime classified into the ROS category.

2. SUPEROXIDE DISMUTASE AS THE INTRINSIC CELLULAR DEFENSE AGAINST SUPEROXIDE TOXICITY

Although the harmful effects of oxygen were noticed in the 19th century, insights into the molecular mechanisms of oxygen toxicity only began to emerge during 1950’s and 1960’s. In 1954, Rebeca Gerschman et al. published an article in the Science magazine, hypothesizing that oxygen poisoning and radiation injury have at least one common basis of action, possibly through the formation of oxidizing free radicals [1]. Subsequently, in 1969 Joe M. McCord and Irwin Fridovich reported the discovery of an enzymatic function of a protein containing both copper and zinc, which then was known alternatively as erythrocuprein, hepatocuprein, or cerebrocuprein [2]. The function of this enzyme is the catalysis of dismutation of superoxide to produce hydrogen peroxide and molecular oxygen, and is known as Cu,Zn superoxide dismutase (Cu,ZnSOD). This discovery has triggered extensive investigations into the free radical mechanisms of oxygen toxicity. Substantial evidence accumulated over the past several decades suggests a critical involvement of superoxide and other ROS in the pathophysiology of a variety of human diseases. Figure 2 illustrates the molecular mechanisms underlying superoxide toxicity.

It is now known that SOD exists in three forms in mammals: (i) Cu,ZnSOD, as mentioned above, (ii) manganese superoxide dismutase (MnSOD), and (iii) extracellular superoxide dismutase (ECSOD). Cu,ZnSOD is a homodimer with a molecular mass of 32 kDa. Both MnSOD and ECSOD are homotetramers with a molecular mass of 86–88 and 135 kDa, respectively. ECSOD also contains copper and zinc. Cu,ZnSOD is primarily present in cytosol. It may also be present in other subcellular compartments, including nucleus, mitochondrial intermembrane space, as well as peroxisome [3, 4]. The presence of
Cu,ZnSOD in nucleus is in line with a recent finding that Cu,ZnSOD may act as a transcription factor to regulate oxidative stress resistance and repair genes [5]. MnSOD exists in mitochondrial matrix. ECSOD is associated with plasma membrane or present in extracellular space.

All of the three isozymes of SOD catalyze dismutation of superoxide to form hydrogen peroxide and molecular oxygen with a similar reaction rate constant of ~1.6 × 10⁹ M⁻¹s⁻¹ (Reaction 1). The reaction rate constant of SOD-catalyzed dismutation of superoxide is over three orders of magnitude greater than that of spontaneous dismutation, which is ~5 × 10⁴ M⁻¹s⁻¹ at a pH of 7.0.

2 O₂⁻ + 2 H⁺ $\rightarrow_{SOD}$ H₂O₂ + O₂ (1)

The overall mechanism by which SOD functions has been called “ping-pong” mechanism as it involves the sequential reduction and oxidation of the
3. SMALL MOLECULE SOD MIMETICS

The potential involvement of superoxide in disease pathophysiology has led to the development of small molecule SOD mimetics that easily penetrate cell membrane to detoxify intracellular superoxide [6]. This would overcome limitations associated with use of the native SODs, including the size of the protein molecule, limited membrane permeability, short half-life, antigenicity, and high costs. Commonly used SOD mimetics are compounds that contain manganese (Mn) as the redox-active center, and include the following 3 classes: (i) Mn(III) metalloporphyrins (e.g., MnTBAP, MnTMPyP); (ii) Mn(III) salen complexes (e.g., EUK-8, EUK-134); and (iii) Mn(II) pentaaazamacrocyclic ligand-based complexes (e.g., M40401, M40403). However, these SOD-mimetics lack specificity for superoxide, and may also scavenge other ROS/RNS and related species, including hydrogen peroxide, nitric oxide, and peroxynitrite, among others, which would make it impossible to delineate the causal involvement of superoxide in a pathophysiological process.

4. NANO-SOD BIOMIMETICS

Nanomaterials have received increasing attention in biology and medicine due to their unique interactions with biological systems and their potential applications as redox-active antioxidants [7, 8]. Several nanomaterials, including cerium oxide nanoparticles, platinum nanoparticles, and fullerene derivatives have been shown to possess antioxidative activities, including scavenging superoxide in biological systems. However, like small molecule SOD mimetics, these nanomaterials also show activities toward other ROS/RNS and related species [9–14] (Figure 3). In contrast to the above SOD biomimetics, the study by Samuel et al. shows that poly(ethylene glycolated) hydrophilic carbon clusters (PEG-HCCs) possess selective activity to superoxide and are inert to nitric oxide and peroxynitrite [15], thus offering a unique approach to studying superoxide.

Reactive Oxygen Species
Using 5-(diethoxyphosphoryl)-5-methylpyrrole-N-oxide (DEPMPO)-spin trapping technique in combination with electron paramagnetic resonance (EPR) spectrometry, the authors first demonstrated a scavenging activity of PEG-HCCs toward superoxide generated from potassium superoxide (KO₂) [15]. KO₂ decomposes to release superoxide in aqueous solutions and is widely used as a generator of superoxide. PEG-HCCs were also found to scavenge hydroxyl radicals generated from the Fenton reaction (Fe²⁺ + H₂O₂ → Fe³⁺ + OH⁻ + OH⁻) [15]. It is not surprising for an antioxidant compound to scavenge hydroxyl radicals, the most oxidizing ROS that can react with virtually all cellular constituents at a diffusion-limited rate.

To investigate the catalytic property of PEG-HCCs, the authors next applied a steady-state kinetic assay using low-temperature EPR and demonstrated that PEG-HCCs behaved as catalysts because the molar ratio of O₂⁻⁻ consumed to PEG-HCCs was far beyond the number of active sites on the PEG-HCCs. Using oxygen polarography and by measuring hydrogen peroxide formation, the authors showed the increased formation of oxygen and hydrogen peroxide (with a ratio of O₂ to H₂O₂ of 1) from PEG-HCC-catalyzed reaction of superoxide [15]. The authors further demonstrated that both self-dismutation and turnover of superoxide by PEG-HCCs followed the same mechanism leading to OH⁻ formation, and the stoichiometry between O₂⁻⁻ and OH⁻ was 1:1. Notably, the PEG-HCC nanoparticle was found to be a stable free radical species (PEG-HCC⁻), which might explain its high reactivity toward superoxide [15]. Collectively, the results led the authors to suggest that PEG-HCCs catalyzed O₂⁻⁻ conversion via a dismutation process involving 2 reactions (Figure 4): (i) PEG-HCC⁻ + O₂⁻⁻ → PEG-HCC⁺ + O₂ and (ii) PEG-HCC⁻ + O₂⁻⁻ + 2 H₂O → PEG-HCC⁺ + H₂O₂ + 2 OH⁻ [15]. As indicated, the PEG-HCC nanoparticle acts as a catalyst to accelerate the dismutation of two molecules of superoxide to form one molecule of hydrogen peroxide and two molecules of hydroxide ions. To investigate the efficiency of the PEG-HCC nanoparticle as an SOD biomimetic, the authors compared the activity of PEG-HCCs with that of Cu,ZnSOD and observed that on a molar basis, PEG-HCCs were as efficient at turning over O₂⁻⁻ as Cu,ZnSOD [15]. Lastly, by using a standard hemoglobin assay, the authors showed that PEG-HCCs were not reactive toward nitric oxide (NO⁻) [15]. Because nitric oxide reacts with superoxide at an almost diffusion-limited rate to form peroxynitrite (Figure 1), the reactivity toward peroxynitrite was also determined through examining peroxynitrite-induced quenching of the dye pyrogallol red. Again, PEG-HCCs were found to be inert to peroxynitrite [15].

5. IMPLICATIONS

The high selectivity of PEG-HCCs for superoxide makes it a valuable SOD biomimetic for investigation of superoxide in biological systems. Commercialization and subsequent wide use of PEG-HCCs in research would inevitably lead to a better understanding of the involvement of superoxide in both physiological and pathophysiological processes.
RESEARCH HIGHLIGHTS


