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Nitroxides—Metal-Independent SOD Mimics

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Abbreviations:

DMPO: 5,5-dimethyl-1-pyrroline-N-oxide
DTBN: di-tert-butylnitroxide
ESR: electron spin resonance
NMR: nuclear magnetic resonance
OXANO: 2-ethyl-2,5,5-trimethyl-3-oxazolidinoxyl
OXANOH: 2-ethyl-1-hydroxy-2,5,5-trimethyl-3-oxazolidine
SOD: Superoxide dismutase
Tempo: 2,2,6,6-tetramethyl-piperidinoxyl
Tempol : 4,OH-2,2,6,6- tetramethyl-piperidinoxyl
TPO: 2,2,6,6,-tetramethyl-piperidinoxyl

Outline

Abstract

Nitroxides are the most important metal-independent superoxide dismutase mimics. They act as antioxidants mainly by reacting with superoxide. Nitroxides are low molecular weight, cell membrane permeable, and relatively stable radicals. The SOD-mimic activities of nitroxides are catalytic processes in which nitroxides and their oxidized form exchange among themselves without depletion of nitroxides. Many experiments have confirmed that such SOD-mimic activities of nitroxides play a prime role in preventing biological free radical damage.

Introduction

The ability of superoxide dismutase (SOD) to catalyze superoxide ($O_2^{\bullet -}$) dismutation was first discovered by McCord and Fridovich in 1969. It is believed that it protects cells from $O_2^{\bullet -}$ toxicity [1]. The catalytic dismutation of $O_2^{\bullet -}$ by SOD involves alternate reduction and oxidation of the transition metal ion in a " ping-pong"-like mechanism [2]. Increasing intracellular levels of SOD or administering exogenous SOD can lessen inflammation, decrease ischemia-induced reperfusion injury, decrease damage from tumor necrosis factor, and mitigate

the damage caused by rheumatoid arthritis [3]. However, native SOD has a few drawbacks in its use. The main problems are due to its short half life, which is about 10 minutes, and its inability to penetrate into the cells because of its larger molecular weight and also inability to reach lipophilic regions. Hence, compounds with SOD-like activity with low molecular weight, biological stability, and membrane permeability are being sought.

SOD mimics

In order that a compound with a high SOD-like activity would be able to mimic SOD *in vivo*, it is required that:

1. It should be non-toxic.
2. It should have a long metabolic half life.
3. It should have a high cell permeability.
4. It should be relatively stable to cell metabolism, which means that SOD mimics should have a high stability constant [3].
5. Its reduced form should not be reoxidized by oxygen or H_2O_2.
6. It should have a reduction potential of $-0.16\ V < E^0 < 0.89\ V$, be nonimmunogenic and active within the physiological pH range.
7. It should not form ternary complexes with the cell components, or alternatively, the ternary complexes should retain most of above mentioned properties.
8. It should be able to be directed preferentially either to a lipophilic or hydrophobic regions according to the demand [4].

These requirements suggest that finding a SOD mimic to operate *in vivo* will not be an easy task.

Types of SOD Mimics

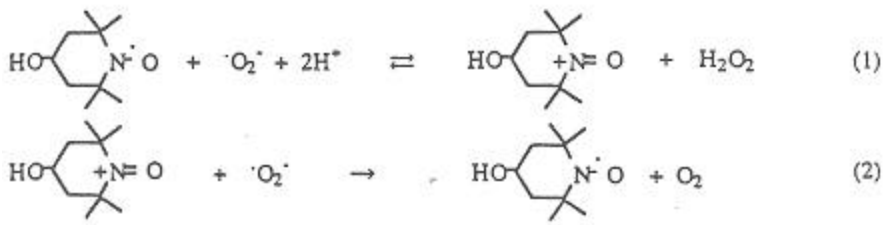
There are two kinds of SOD mimics: metal-dependent, and metal-independent. A number of chelates of iron, manganese, and copper are efficient catalysts for $O_2^{\bullet -}$ dismutation. Unfortunately, the metal-dependent SOD mimics often easily dissociate in the cell, exhibit high affinity toward proteins and amino acids and may lose their activity upon binding to cellular components [2]. Thus, metal-independent SOD mimics would be a desirably added criterion for a potential biological use.

Metal-Independent SOD mimics

Many SOD mimics without metals have been examined for their anti-oxidant and SOD activity. The most important metal-independent SOD mimics are nitroxides [5]. Stable nitroxide radicals have been used as biological tools for ESR spectroscopic studies, particularly of membranes and proteins, and more recently as contrast agents for *in vivo* NMR imaging and spin label oximetry. The nitroxide compounds, 2,2,6,6-tetramethyl-piperidinoxyl (Tempo) and 4,OH-2,2,6,6- tetramethyl-piperidinoxyl (Tempol) have been used most often in biological studies. Tempo and Tempol have been shown to act as SOD mimics at rates of (1.8± 0.4) X 10^6 $M^{-1}S^{-1}$ and $(6.9 \pm 2.9) \times 10^5$ $M^{-1}S^{-1}$ at pH 7, respectively [5].

Mechanism of Nitroxides as SOD mimics

Nitroxides act as antioxidants through several mechanisms, including detoxifying ferryl-heme species, facilitating heme-mediated catalytic removal of H_2O_2, trapping carbon-centered radicals and terminating radical chain reactions [6]. However, the most important mechanism is that nitroxides inhibit oxidative damage by removing both extra- and intracellular $O_2^{\bullet-}$, not stoichiometrically, but rather catalytically as SOD-mimics [6]. Nitroxides were found to be reduced to the corresponding hydroxylamine by $O_2^{\bullet-}$. This finding originally stemmed from the observation of $O_2^{\bullet-}$ induced spin-loss of OH spin-adduct of 5,5-dimethyl-1-pyrroline-N-oxide (DMPO). The ESR signals of persistent nitroxide spin-adducts such as DMPO-OH or DMPO-CH_3 and of several stable nitroxide radicals, such as OXANO (2-ethyl-2,5,5-trimethyl-3-oxazolidinoxyl) rapidly decayed to a lower steady state when exposed to a continuous $O_2^{\bullet-}$ flux. The major pathway by which nitroxide spin labels decay within cells is through a one-electron reduction to diamagnetic ("ESR-silent") hydroxylamines that can be reversibly oxidized. One particular hydroxylamine, 2-ethyl-1-hydroxy-2,5,5-trimethyl-3-oxazolidine (OXANOH), is readily oxidized at pH 7.8 by $O_2^{\bullet-}$ to a stable radical, 2-ethyl-2,5,5-trimethyl-3-oxazolidinoxyl (OXANOH), with a rate constant of 6.7×10^3 M^{-1} S^{-1}. Superoxide induced depletion and regeneration of the nitroxide are coupled through following two reactions [7].



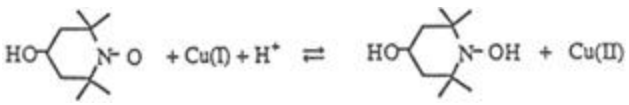
Adding these two reactions together yield the following dismutation reaction.

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

Thus, nitroxides are identified as SOD mimics.

Krishna *et al* reported that stable nitroxides (*e.g.* piperidinyl and pyrrolidine derivatives) catalyze the dismutation of $O_2^{\bullet-}$ by utilizing the oxoammonium cation/nitroxide redox couple [8]. The $O_2^{\bullet-}$ / H_2O_2 couple has a redox potential of + 0.89 V, and the catalytic dismutation rate is found to be directly related to the midpoint redox potential of the corresponding nitroxide. Tempo, Tempol, and Tempamine have catalytic rates of $O_2^{\bullet-}$ dismutation of 1.2×10^5 $M^{-1}S^{-1}$, 6.5×10^4 $M^{-1}S^{-1}$, and 6.5×10^4 $M^{-1}S^{-1}$, respectively [8]. The reactions of nitroxides with $O_2^{\bullet-}$ reveal that the piperidinyl and pyrrolidine derivatives are reduced in an $O_2^{\bullet-}$-dependent manner only in the presence of two-electron donors such as NADH and NADPH [8].

The nitroxides as anti-oxidants have other biological activities. In addition to dismutating $O_2^{\bullet-}$, nitroxides can oxidize semiquinones, thus decreasing their cytotoxicity. Nitroxides react with alkoxyl, peroxyl, carbon-centered and lipid radicals. Nitroxides can act as mild oxidants to oxidize Fe(II) and Cu(I) preventing the Fenton-type production of damaging hydroxyl radicals [9].



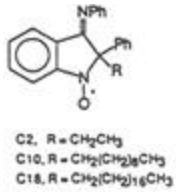
Nitroxides have been shown to induce a catalase activity of some heme proteins [5]. Nitroxides can be reversibly reduced to hydroxylamines, then amines or oxidized to oxoammonium ions [5]. The reduced form of nitroxides, hydroxylamines, act as very good

general anti-oxidants and have been claimed to prevent or retard cataract formation. It is believed that the piperidinoxyl compounds such as Tempo and Tempol can cycle between nitroxides and oxoammonium moiety to dismutate $O_2^{\bullet -}$ [5] .

The Chemistry and Metabolism of Nitroxides

Antosiewicz *et al* reported that one of the determinants of the antioxidant power of nitroxides is the structure and mobility inside cell membranes [10]. They designed an experiment to determine how to what extent the structure of indolinic nitroxides can influence their antioxidant activity in biological systems. Three indolinic nitroxides bearing hydrocarbon chains of different lengths were synthesized: C2, 1,2-dihydro-2-ethyl-2-phenyl-3H-indole-3-phenylimino-1-oxyl; C10, 1,2-dihydro-2-decyl-2-phenyl-3H-indole-3-phenylimino-1-oxyl; C18, 1,2-dihydro-2-octadecyl-2-phenyl-3H-indole-3-phenylimino-1-oxyl.

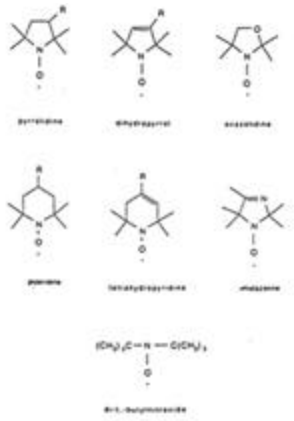
Figure 1. One of the determinants of anti-oxidant power of nitroxides is dependent on their chemical structures [10].



They found that all the nitroxides were effective in preventing oxidation of bovine serium albumin, but to different extents, with the longer chain derivatives being more efficient. However, the C2 compound was the most efficient in preventing lipid peroxidation in microsomal membranes [10].

Fuchs *et al* reported that the nitroxide ring structure and proximal substituents can affect their biostability [11].

Figure 2. Structurally different formula of nitroxides have different biostability [11].

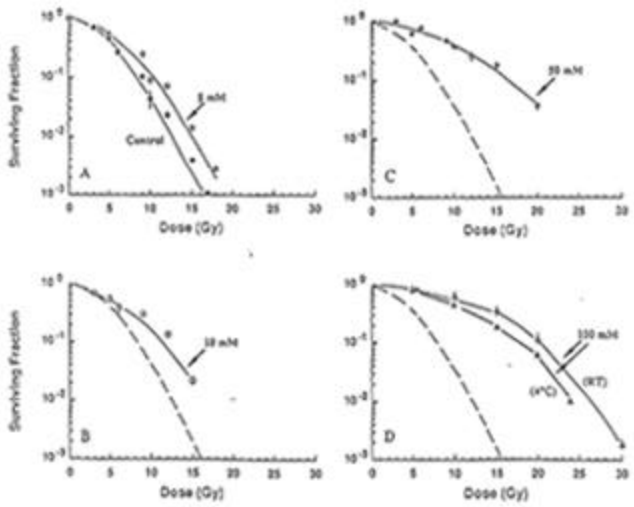


They found that the metabolism of nitroxides in keratinocytes in skin is generally similar to their metabolism in other biological systems, such as fibroblasts, hepatocytes, and liver, kidney, and muscle homogenates [11]. Imidazoline and pyrrolidine nitroxides are the most biostable nitroxides in mouse and human skin. The order of nitroxide biostability is : imidazodine > pyrrolidine > di-*t*-butylnitroxide (DTBN) > piperidine > oxazolidine. Cationic nitroxides are more unstable than neutral and anionic nitroxides [11].

Biological Significance of Nitroxide

It was reported that nitroxides (*e.g.* Tempol) provide partial protection against X-ray and NCS-induced mutagenicity and DNA damage, as well as cytotoxicity, mainly due to their SOD-like activity [12]. Goffman *et al* reported that treatment of Chinese hamster V79 cells with stable nitroxide radical Tempol afforded significant protection against $O_2^{\bullet-}$, H_2O_2, and X-ray mediated cytotocixity [13]. Mitchell *et al* reported that Tempol, a SOD mimic, can inhibit oxygen-dependent radiation-induced damage. Tempol can afford significant enhancement in cell survival [14].

Figure 3. Tempol can protect Chinese hamster V79 cells from X-ray induced damage [14].



Also, nitroxides can protect Cu,Zn-SOD from hydrogen peroxide-induced inactivation by reducing SOD-Cu^{2+}-$HO^{\bullet}$ to the active SOD-Cu^{2+}[15]. Nitroxide radicals inhibit lipid peroxidation in rat liver microsomes [16]. Samuni *et al* reported that nitroxide radicals can prevent leakage of lactate dehydrogenase and preserve normal cardiomyocyte contractility, presumably by intercepting intracellular $O_2^{\bullet-}$, or reacting with reduced transition metal ions or by detoxifying secondary organic radicals [17].

Summary

Nitroxides are the most important metal-independent SOD mimics. The research on nitroxides has been particularly interesting because of their anti-oxidant activities. We can predict that nitroxides as SOD mimics will be applied in the clinical areas.

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