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Nitric Oxide as An Antioxidant

by

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Abbreviations:
ABAP, 2,2'-azobis(2-amidinopropane hydrochloride)
BSA, bovine serum albumin
DEA/NO, $(C_2H_5)_2N[N(O)NO]$-Na^+
LDL, low-density lipoprotein
NOS, nitric oxide synthase
$ROO^{\bullet}$, alkyl peroxide
ROS, reactive oxygen species
SLO, soybean lipoxygenas
SNAP, S-nitroso-N-acetylpenicillamine
SNP, sodium nitroprusside

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Abstract

Nitric oxide ($^{\bullet}NO$) is a free radical that has been identified as a biologically important molecule involved in a number of physiological processes. Nitric oxide can react rapidly with species containing unpaired electrons, such as molecular oxygen (O_2) and superoxide anion ($O_2^{\bullet-}$). Nitric oxide can play a protective role under oxidative stress resulting from $O_2^{\bullet-}$, hydrogen peroxide (H_2O_2), and alkyl peroxides ($ROO^{\bullet}$). It can provide protection against lipid oxidation, especially against low-density lipoprotein (LDL) peroxidation *via* termination of lipid radical chain propagation reaction. This paper will focus on the protective properties of $^{\bullet}NO$ as an antioxidant.

Introduction

Nitric oxide (•NO) is a hydrophobic, paramagnetic gas that has been shown to be synthesized in biological systems [1]. It is a short-lived free radical whose messenger function, cytotoxic and cytoprotective effects are mainly dependent on its liganding to, and chemical reactions with, metal centers (*e.g.*, heme and non-heme iron and copper) and reactive oxygen species [2, 3]. In the last decade, the antioxidant activity of •NO against free radicals peroxidation has been studied. It has been proposed to be an important antioxidant.

Chemical properties and reactions of nitric oxide

I. Chemical properties

Nitric oxide is a fairly nonpolar molecule that would be expected to freely diffuse through membranes. It contains an unpaired electron. The formal bond order in •NO is 2.5. The unpaired electron of •NO is in a 2p-p antibonding orbital. Figure 1 shows the molecular orbital diagram of •NO [4].

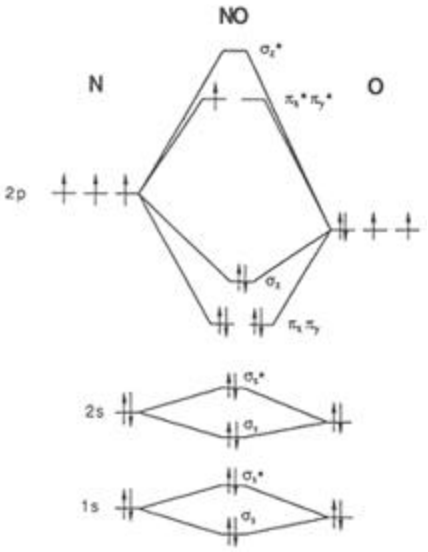

Figure 1. Molecular orbital diagram for •NO. The unpaired electron resides in an antibonding orbital (* = antibonding orbital). The total bonding is described as three net bonds gained from the filled s_z, p_x, p_y molecule orbitals minus half a bond from the partially filled p* orbital. The bond order in •NO is 2.5. Adapted from [4].

II. Reactions of nitric oxide

Due to the unpaired electron, nitric oxide can react rapidly with species containing unpaired electrons, such as molecular oxygen (O_2), superoxide anion ($O_2^{\bullet -}$).

1. Reaction with O_2

The reaction of $^{\bullet}NO$ with O_2 in the gas phase results in the formation of nitrogen dioxide (NO_2). The kinetics of this reaction is second order in $^{\bullet}NO$ (reaction 1). In the gas phase NO_2 is the terminal product. But in aqueous solution, NO_2 can react with another $^{\bullet}NO$ to give N_2O_3, N_2O_3 decomposition in H_2O then give exclusively NO_2^- (reaction 2 and 3) [4].

$$2\,{}^{\bullet}NO + O_2 \xrightarrow{k = 2.4 \times 10^6\ M^{-2}s^{-1}} 2\,{}^{\bullet}NO_2 \qquad (1)$$

$$^{\bullet}NO + {}^{\bullet}NO_2 \underset{k_r = 8.4 \times 10^4\ M^{-1}s^{-1}}{\overset{k_f = 1.1 \times 10^9\ M^{-1}s^{-1}}{\rightleftharpoons}} N_2O_3 \qquad K = 1.3 \times 10^4 \qquad (2)$$

$$N_2O_3 + H_2O \xrightarrow{k = 1.6 \times 10^3\ s^{-1}} 2\,H^+ + 2\,NO_2^- \qquad (3)$$

The net reaction is:

$$4\,{}^{\bullet}NO + O_2 + 2\,H_2O \longrightarrow 4\,H^+ + 4\,NO_2^- \qquad (4)$$

The rate of this process is governed by a rare, third-order rate constant where $k = 2.4 \times 10^6\ M^{-2}s^{-1}$ at 37 °C [5]. The activation energy is 2 kcal/mol [6].

This reaction is considered to be insignificant *in vivo*, but it is of great importance when making solutions of $^{\bullet}NO$ as well as in tissue culture experiment. Most of the $^{\bullet}NO$ produced by cells or "given" to cells in these experiments probably disappears *via* this reaction.

2. Reaction with $O_2^{\bullet -}$

Nitric oxide will react with the superoxide anion ($O_2^{\bullet -}$) to produce the potent oxidant, peroxynitrite ($ONOO^-$). It is a very rapid reaction [7]. $ONOO^-$ is a very reactive species, initiating oxidations and nitrations.

$$^{\bullet}NO + O_2^{\bullet -} \xrightarrow{k = 6.7 \times 10^9\ M^{-1}s^{-1}} O{=}NOO^- \quad (5)$$

Nitric oxide as an antioxidant

1. Nitric oxide protection against reactive oxygen species (ROS)

It is known that chemical species derived from the reduction of oxygen, *i.e.*, H_2O_2 and $O_2^{\bullet -}$, can cause cell death in both eukaryote and prokaryote organisms. Exposure of Chinese hamster V79 cells to varying concentrations of H_2O_2 resulted in increasing cell death as assayed by clonogenic methods [8]. However, in the presence of NO released from $(C_2H_5)_2N[N(O)NO]$-Na^+ (DEA/NO), a compound known as a NONOate, the cytotoxicity resulting from H_2O_2 or $O_2^{\bullet -}$ was markedly reduced. These results show that H_2O_2 mediated cytotoxicity can be prevented by the presence of a NO-generating compound [9].

2. Nitric oxide protection against alkyl hydroperoxides

Protection research has been further extended to include alkyl hydroperoxides. The cumene and t-butyl alkyl hydroperoxides were exposed to Chinese hamster V79 cells for 2 h. A dose-dependent increase in cell killing was observed. Addition of DEA/NO resulted in no protection contrary to that observed with H_2O_2 [10]. Since DEA/NO liberates NO within the first few minutes of exposure, the NONOate, PAPA/NO ($NH_2(C_3H_6)$-(N[N(O)NO] C_3H_7)) was chosen which can release NO for over an hour. When either cumene or t-butyl

hydroperoxide was exposed with 0.1 M PAPA/NO, complete protection was observed [10]. This suggests that NO can abate the toxicity of alkyl hydroperoxides.

3. Nitric oxide protection against LDL oxidation

The oxidation of LDL has been implicated in the early stages of atherosclerosis. Recent studies showed that $^{\bullet}NO$ could inhibit LDL oxidation, causing chain termination reactions during LDL peroxidation.

Studies using sodium nitroprusside (SNP)/light, S-nitroso-N-acetylpenicillamine (SNAP) and authentic $^{\bullet}NO$ solution during the oxidation of LDL by Cu^{2+}, and 2,2'-azobis(2-amidinopropane hydrochloride (ABAP) showed that under these conditions, $^{\bullet}NO$ acts only as an antioxidant [11]. There are at least three mechanisms by which $^{\bullet}NO$ can suppress Cu^{2+}-dependent oxidation of LDL. These are: (i) chelation of Cu^{2+} by $^{\bullet}NO$ to form an inactive complex, (ii) removal of LOOH within the LDL particle, and (iii) scavenging of $L^{\bullet}$ and $LOO^{\bullet}$ by $^{\bullet}NO$ [11].

Other studies examined the effect of $^{\bullet}NO$ on iron-induced lipid peroxidation in human leukemia cells (HL-60). HL-60 cells were exposed to an oxidative stress (20 μM Fe^{2+}) and the consumption of O_2 was as a measure of lipid peroxidation. The O_2 consumption was arrested by the addition of $^{\bullet}NO$ as a saturated aqueous solution, and the inhibition ended upon depletion of $^{\bullet}NO$. Depletion of cellular glutathione levels prior to Fe^{2+} addition resulted in a more rapid initial rate of O_2 depletion and a shorter time for the $^{\bullet}NO$-induced inhibition of O_2 consumption. Conclusion from those studies suggested that $^{\bullet}NO$ protects HL-60 human leukemia cells from lipid peroxidation and that this protection ameliorates the toxicity of the oxidation processes initiated by Fe^{2+} and dioxygen [12].

Lipid peroxidation has three major components: initiation, propagation, and termination [12]:

$$LH + \text{oxidant}^{\bullet} \rightarrow L^{\bullet} + \text{oxidant-H} \quad \text{(initiation)} \quad (6)$$

$$L^{\bullet} + O_2 \rightarrow LOO^{\bullet} \quad \text{(propagation cycle)} \quad (7)$$

$$LOO^{\bullet} + LH \rightarrow L^{\bullet} + LOOH \quad \text{(propagation cycle)} \quad (8)$$

$$L^{\bullet} + L^{\bullet} \rightarrow \text{non-radical products} \quad \text{(termination)} \quad (9)$$

$$L^{\bullet} + LOO^{\bullet} \rightarrow \text{non-radical products} \quad \text{(termination)} \quad (10)$$

It is in the propagation and termination process that $^{\bullet}NO$ has been proposed to play a crucial role as an antioxidant in lipid peroxidation [11]:

$$L^{\bullet} + {}^{\bullet}NO \rightarrow LNO \quad (11)$$

$$LO^{\bullet} + {}^{\bullet}NO \rightarrow LONO \quad (12)$$

$$LOO^{\bullet} + {}^{\bullet}NO \xrightarrow{k = 1.0\text{-}3.0 \times 10^{9}\ M^{-1}s^{-1}} LOONO \quad (13)$$

Nitric oxide reacts with $LO^{\bullet}$ and $LOO^{\bullet}$ at near diffusion-limited rates (for $LOO^{\bullet}$, k = 1.0-3.0 $x10^{9}\ M^{-1}s^{-1}$) [13], effectively limiting less facile radical chain propagation reactions.

Studies showed that when LOOH species prepared by soybean lipoxygenase (SLO) oxidation of either linoleic or linolenic acid were incubated with bovine serum albumin (BSA), fluorescent products having excitation and emission spectra similar to those of oxidized LDL were observed (Figure 2) [14]. From this, it has been proposed that after hemolytic cleavage of LOOH to the more reactive $LOO^{\bullet}$, a concerted reaction occurs between $LOO^{\bullet}$ and polypeptide amino groups to yield fluorescent adducts without prior LOOH fragmentation to aldehydes or other more stable products [15]. During LDL or cell membrane oxidation this mechanism could also occur in concert with lipid aldehyde-mediated Schiff's base formation. The observed $^{\bullet}NO$ inhibition of Schiff's base fluorescent

conjugate formation between BSA and oxidized linoleic acid (Figure 2) supports the contention that LOO•, rather than aldehydic intermediates, was the principal and proximal species responsible for fluorescent adduct formation. Therefore, it can be concluded that •NO can play a potent oxidant-protective role in the vessel wall by inhibiting lipoxygenase-dependent lipid and lipoprotein oxidation. This occurs *via* termination of lipid radical chain propagation reactions catalyzed by LO• and LOO• intermediates of lipid peroxidation rather than by inhibition of lipoxygenase-catalyzed initiation reactions [15].

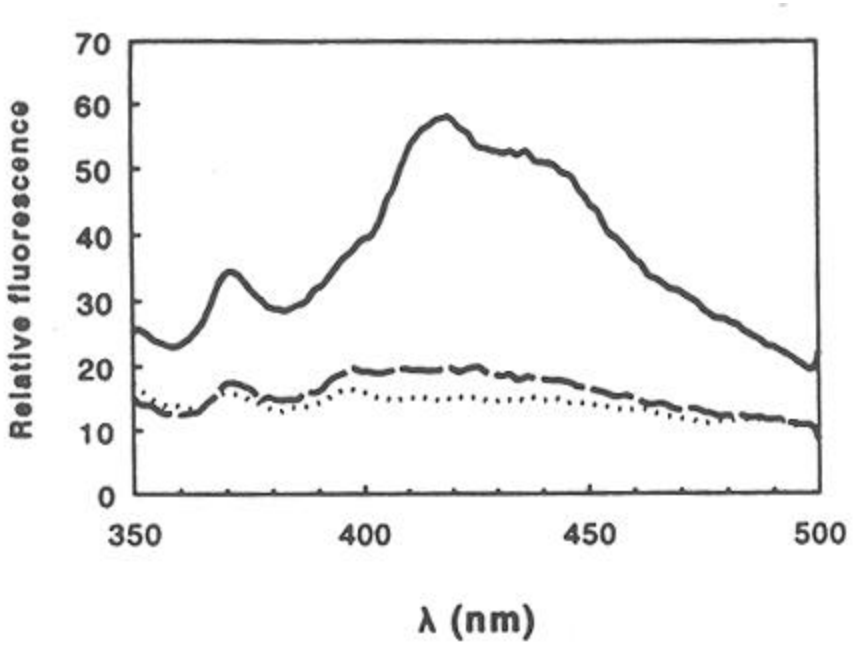

Figure 2. The effect of S-nitrosoglutathione on the generation of fluorescent products. Fluorescence emission spectra were obtained by coincubation of LOOH generated from 0.1 mg/ml linoleic acid plus 100 U/ml SLO in the absence (—) and presence of 0.5 mM (---) and 1mM (…) GSNO. BSA(0.1 mg/ml) was added after oxidation and these mixtures were incubated at room temperature for 3 h. Adapted from [15].

Summary

Collectively these research data demonstrate that •NO can protect cells from the deleterious effects of peroxide and the resulting ROS formed. It appears that at a chemical level •NO has a protective property, especially in LDL peroxidation. Studies utilizing NOS

inhibitors *in vivo* showing protection may suggest that there are physiological mechanisms rather than chemical which could be important as well.

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