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Instructors:
GARRY R. BUETTNER, Ph.D.
LARRY W. OBERLEY, Ph.D.

with guest lectures from:
Drs. Freya Q . Schafer, Douglas R. Spitz, and Frederick E. Domann

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Nitric Oxide: An Antioxidant

by

Matt Zimmerman

1-251 BSB
Anatomy and Cell Biology
The University of Iowa
Iowa City, IA 52242-1109

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Abbreviations

$L^{\bullet}$	lipid radical
LDL	low-density lipoprotein
LH	lipid
$LOO^{\bullet}$	lipid peroxyl radical
LOOH	lipid peroxide
NRP	non-radical product
$ONOO^{-}$	peroxynitrite
$R^{\bullet}$	radical species
RNOS	reactive nitric oxide species
ROS	reactive oxygen species
SOD	superoxide dismutase

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Abstract

Nitric oxide ($NO^{\bullet}$) is a free radical with an unpaired electron in the highest orbital, making it a highly reactive molecule. $NO^{\bullet}$ can react rapidly with other reactive oxygen species or transition metals to prevent the formation of radical species or to produce a less reactive molecule; therefore, by definition $NO^{\bullet}$ can act as an antioxidant. In this manner, $NO^{\bullet}$ can protect the cell against the damaging cycle of lipid peroxidation by reacting with lipid peroxyl radicals and yielding a non-radical product. This property of $NO^{\bullet}$ has lead to the hypothesis that $NO^{\bullet}$, as an antioxidant, plays a key role in the pathogenesis of cardiovascular diseases, particularly atherosclerosis.

Introduction

Reactive oxygen species (ROS), such as superoxide radical ($O_2^{\bullet -}$), hydrogen peroxide (H_2O_2) and hydroxyl radical ($HO^{\bullet}$), have traditionally been thought of as toxic byproducts of cellular metabolism resulting in direct cellular damage. More recently, however, it has become accepted that ROS play a major role in physiological and pathophysiological conditions by acting as second messengers in signal transduction pathways. In order to keep the levels of ROS produced in balance, the cell has developed numerous defense mechanisms that collectively are classified as antioxidants. Examples of well-known enzyme antioxidants include superoxide dismutase (SOD), catalase, and glutathione peroxidase. More recently, the diatomic radical nitric oxide ($NO^{\bullet}$) has been shown to be involved in antioxidant mechanisms. Ironically, $NO^{\bullet}$ production can lead to the production of reactive nitric oxide species (RNOS), which can act like ROS and cause direct cellular damage. The unique properties of $NO^{\bullet}$ acting as a pro-oxidant and antioxidant have lead to the hypothesis that $NO^{\bullet}$ plays a key role in numerous redox regulated signaling cascades. The purpose of this review is to summarize the chemistry and biochemistry of $NO^{\bullet}$, while focusing on the role of $NO^{\bullet}$ as an antioxidant.

Nitric Oxide Chemistry

Nitric oxide, an uncharged radical, is composed of seven electrons from nitrogen and eight electrons from oxygen [1]. The unpaired electron of $NO^{\bullet}$ makes the molecule a potent reducing substance and allows the molecule to react rapidly with molecules that have unpaired electrons in their outer orbital, such as other free radicals and transition metals. In the gas phase, $NO^{\bullet}$ rapidly reacts with molecular oxygen (O_2) (Reaction 1, $k = 2.4 \times 10^6\ M^{-1}\ s^{-1}$), a reaction often referred to as $NO^{\bullet}$ autoxidation, to produce nitrogen dioxide ($NO_2^{\bullet}$), which in hydrophobic

layers of cellular membranes reacts with an additional $NO^{\bullet}$ molecule to produce dinitrogen trioxide (N_2O_3) [2].

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

In addition to the autoxidation reaction, $NO^{\bullet}$ will react rapidly with superoxide radical ($O_2^{\bullet-}$) at a near diffusion limiting rate to produce the highly reactive molecule peroxynitrite ($ONOO^-$) (Reaction 2, $k = 6.7 \times 10^9\ M^{-1}\ s^{-1}$) [2]. The scavenging of $O_2^{\bullet-}$ by $NO^{\bullet}$ can reduce the

$$NO^{\bullet} + O_2^{\bullet-} \rightarrow ONOO- \qquad (k = 6.7 \times 10^9\ M^{-1}s^{-1}) \qquad (2)$$

amount of ROS produced from the Haber-Weiss reaction, or the superoxide-driven Fenton reaction (Reactions 3, 4) [3]; however, the production of $ONOO^-$ is an irreversible reaction and has been shown induce cellular and tissue injury by causing DNA damage and lipid

$$O_2^{\bullet-} + Fe^{3+} \rightarrow Fe^{2+} + O_2 \qquad (3)$$

$$Fe^{2+} + H_2O_2 \rightarrow HO^{\bullet} + OH^- + Fe^{3+} \qquad (4)$$

peroxidation. The balance between $O_2^{\bullet-}$ and $NO^{\bullet}$ is crucial as an excess of $O_2^{\bullet-}$ will induce lipid peroxidation, while an excess of $NO^{\bullet}$ will promote inhibition of lipid peroxidation [3].

As an antioxidant, $NO^{\bullet}$ works to prevent oxidant formation or scavenge oxidants to form a less damaging molecule. Nitric oxide can directly react with metals to form relatively stable metal nitrosyl complexes; therefore, reducing the metals availability to participate in the Fenton reaction and subsequently attenuate the production of oxidants [3]. The reaction rate between nitric oxide and metals are dependent on various factors including the ligand associated with the metal and the spin of the metal itself [3]. One of the fastest reactions of $NO^{\bullet}$ with a metal is that with ferrous iron (Fe^{2+}) (Reaction 5, $k = 1.8 \times 10^9\ M^{-1}\ s^{-1}$), a key catalyst in the Fenton reaction

$$Fe^{2+} + NO^{\bullet} \rightarrow Fe^{2+}NO \qquad (k = 1.8 \times 10^9\ M^{-1}\ s^{-1}) \qquad (5)$$

(Reaction 4) [3]. An example of $NO^{\bullet}$ acting as an antioxidant by reacting with Fe^{2+} is in heme complexes which can produce oxidizing species such as Fe^{4+} and Fe^{5+} oxo complexes. $NO^{\bullet}$ will bind to ferrous complexes to form a metal-nitrosyl species, thus inhibiting the reaction with peroxide and the metal, thereby preventing ROS production [3]. In addition to reacting with paramagnetic metals, $NO^{\bullet}$ can act as an antioxidant by scavenging ROS in a competitive manner with potential targets. For example, nitric oxide can terminate the chain propagation reaction of lipid peroxidation, which propagates oxidative stress by forming various lipid-oxy and lipid-peroxy adducts, by producing non-radical species [4]. This concept will be addressed in the following section.

Nitric Oxide Biochemistry

The process of lipid peroxidation results in the formation of alkoxyl or peroxyl radicals that can result in cell membrane damage and eventually cytotoxicity [5]. In the initiation reaction, a hydrogen atom is transferred from a lipid (LH) to a radical species ($R^{\bullet}$) resulting in a alkyl radical ($L^{\bullet}$) (Reaction 6). Following the initiation reaction, the lipid radical can rapidly react with molecular oxygen to yield a lipid peroxyl radical ($LOO^{\bullet}$) (Reaction 7). The lipid-peroxyl radical can react with another lipid to produce a lipid peroxide (LOOH) and another lipid radical, a process known as the propagation reaction, which can restart the deleterious process (Reaction 8). Finally in the termination reaction, two lipid peroxyl radicals or lipid radicals can react to yield a non-radical product (NRP) (Reaction 9). In theory, $NO^{\bullet}$ can react with the alkyl radical formed in the initiation reaction of lipid peroxidation; however, oxygen reacts with $L^{\bullet}$ at a diffusion limited rate (Reaction 7) and given the significantly higher concentration (10-100 times) of oxygen relative to $NO^{\bullet}$, it is not likely that $NO^{\bullet}$ can compete for reaction with the alkyl radical [5]. However, $NO^{\bullet}$ can act as an antioxidant by disrupting the propagation reaction

(Reaction 10) [6], thus protecting the cell against peroxide induced cytotoxicity. The non-radical species (LOONO) produced by this reaction has been shown to have some of the characteristics of peroxynitrite [6]. Thus, this unstable intermediate then reacts with an additional $NO^{\bullet}$ to yield a stable organic nitrate, which explains the finding that two $NO^{\bullet}$ molecules are required to scavenge one $LOO^{\bullet}$ [5]. The process of lipid peroxidation and the role of $NO^{\bullet}$ as an antioxidant

$$LH + R^{\bullet} \rightarrow L^{\bullet} + RH \qquad \text{(hydrogen atom transfer)} \qquad (6)$$

$$L^{\bullet} + O_2 \rightarrow LOO^{\bullet} \qquad (k = 3 \times 10^8\ M^{-1}\ s^{-1}) \qquad (7)$$

$$LOO^{\bullet} + LH \rightarrow L^{\bullet} + LOOH \qquad (k = 10\text{-}50\ M^{-1} s^{-1}) \qquad (8)$$

$$LOO^{\bullet} + LOO^{\bullet} \rightarrow NRP \qquad (k = 10^6\text{-}10^7\ M^{-1}\ s^{-1}) \qquad (9)$$

$$\mathbf{LOO^{\bullet} + NO^{\bullet} \rightarrow LOONO} \qquad \mathbf{(k = 1\text{-}3 \times 10^9\ M^{-1}\ s^{-1})} \qquad (10)$$

in lipid peroxidation are summarized in Figure 1. The termination of the propagation reaction by $NO^{\bullet}$ prevents oxidation of low-density lipoprotein in endothelial cells and macrophages [4]. In addition, it has been suggested that $NO^{\bullet}$ plays an important role in limiting the lipid peroxidation in atherosclerosis [7].

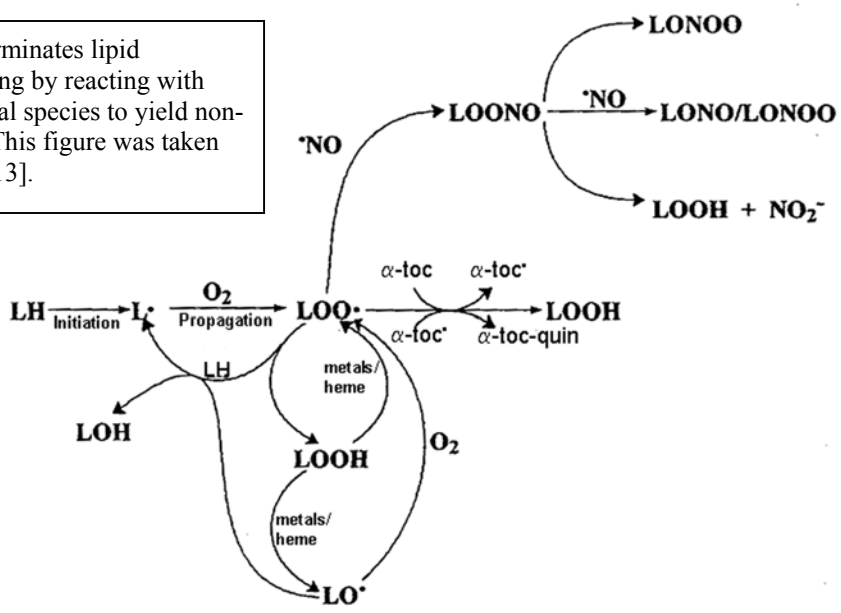

Figure 1. $NO^{\bullet}$ terminates lipid peroxidation cycling by reacting with intermediate radical species to yield non-radical products. This figure was taken from Patel *et al.* [13].

Atherosclerosis is characterized by a significant reduction of vascular relaxation, which is believed to be dependent on the decrease activity and/or synthesis of $NO^{\bullet}$. Within the past decade, it has been shown that oxidation of lipids is key in the atherosclerotic process since the products of lipid peroxidation (discussed above) are found in human atherosclerotic lesions [7]. The identification of lipid peroxides in the lesion has suggested that the process and reactions of lipid oxidation are indeed occurring in the atherosclerotic plaque. The fast rate constant of $NO^{\bullet}$ reacting with the lipid peroxyl radical (Reaction 10) suggests that this reaction will take precedent over the propagation reaction; therefore, the availability of $NO^{\bullet}$ to the plaque is critical if $NO^{\bullet}$ is to act as a beneficial molecule in this disease. This rapid reaction suggests that low concentrations of $NO^{\bullet}$ may be far more efficient in scavenging $LOO^{\bullet}$ than other antioxidants such as Vitamin E, which has a rate constant with $LOO^{\bullet}$ about 10,000 times smaller than $NO^{\bullet}$ [5]. O'Donnell *et al.* demonstrated that steady-state concentration of 30 nM $NO^{\bullet}$ inhibited lipid peroxidation to the same extent as 20 μM Vitamin E, thus providing evidence that $NO^{\bullet}$ is a more powerful antioxidant than Vitamin E [5]. In addition, Goss and colleagues provided evidence for this by demonstrating that in the presence of an $NO^{\bullet}$ donor, loss of Vitamin E is spared in an experimental model of low-density lipoprotein (LDL) oxidation [8]. The ability of $NO^{\bullet}$ to freely diffuse and partition into the liquid phase is an additional advantage of $NO^{\bullet}$ as an antioxidant versus Vitamin E.

More direct evidence establishing a role of nitric oxide in terminating the lipid peroxidation in atherosclerosis has been provided by studies on 15-lipoxygenase [9]. Studies on the time course of lesion formation indicate that lipoxygenase plays a critical role in the initiation of atherosclerosis [9]. In addition, lipoxygenase mRNA was found to be colocalized with oxidized protein-lipid adducts in atherosclerotic lesions [10]. Furthermore, in vitro incubation of

lipoxygenase with LDL results in oxidation of the LDL and this has been postulated to be a mechanism for cell-dependent oxidative damage to the lipoprotein [11]. While these studies provided a correlation between lipoxygenase and atherosclerosis, one must understand the mechanism of lipoxygenase activation to implicate a role for $NO^{\bullet}$ in this process. Schewe *et al*. demonstrated that the activation of 15-lipoxygenase involves the insertion of a peroxide group into a fatty acid substrate [12]. This reaction involves the production of an intermediate alkyl and peroxyl radical bound to the iron atom in the active site [12]. Therefore, $NO^{\bullet}$ has the potential to interact with either the metal center or the fatty acid derived radical intermediates. It is hypothesized that that $NO^{\bullet}$ reacts with the $LOO^{\bullet}$ intermediate, thus returning the enzyme to the inactive form [12].

Summary and Conclusions

Nitric oxide plays a critical role in numerous cellular functions by acting both as a pro-oxidant and antioxidant molecule. The fast kinetics of $NO^{\bullet}$ reacting with other reactive oxygen species, particularly superoxide radical, and paramagnetic metals suggest a role for nitric oxide as a potent antioxidant, thus protecting cells and tissues from oxidative stress. The ability of low concentrations of $NO^{\bullet}$ to disrupt the reactions involved in lipid peroxidation suggest that this molecule plays a critical role in the pathogenesis of numerous diseases, one of which is atherosclerosis. Enhancing the concentration ratio of $NO^{\bullet}$ versus $O_2^{\bullet-}$ may prove critical in the therapy of cardiovascular disease, including atherosclerosis, as a larger concentration of $NO^{\bullet}$ favors lipid peroxidation inhibition. While recent studies have provide valuable insight into nitric oxide as a antioxidant, continued investigation is required to better understand the importance of this molecule in other cardiovascular diseases.

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