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Forum Review

Compartmentalized Nitrosation and Nitration in Mitochondria

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ABSTRACT

A wide spectrum of the biological actions of nitric oxide and its oxidizing metabolites are mediated via mitochondria. Mitochondria are highly compartmentalized organelles consisting of three distinct compartments: the matrix, the intermembrane space, and the membranes. These compartments are different in their electrochemical properties, redox state, pH, enzymes, and ion content. Nitric oxide and its reactive species react within these compartments in distinct manners. The mitochondrial intermembrane space provides an environment that favors *S*-nitrosation, whereas nitration occurs largely within the matrix. This article will review some of the interactions of these species with certain mitochondrial respiratory chain complexes, apoptotic proteins, and enzymes. The reversibility and the suborganelle preference of these reactions will be discussed.
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INTRODUCTION

THE DISCOVERY THAT NITRIC OXIDE (NO) is the endothelium-derived relaxing factor (EDRF) (26, 46) changed our understanding of NO from that of a noxious gas to a molecule with a unique broad spectrum of biological activities. In the last two decades, several biological functions of NO in the cardiovascular, nervous, gastrointestinal, and immune systems have been revealed (for review, see 42). Many of these actions are due to the reaction of NO with the soluble guanylate cyclase and subsequent elevation of cytosolic cyclic GMP. Yet a considerable portion of these actions are cyclic GMP-independent. Increasing evidence suggests that mitochondria are the foremost cycli GMP-independent biological mediators of NO. These membranous cytoplasmic organelles consume >90% of oxygen and produce >90% of ATP of the eukaryotic cells. Alteration of mitochondrial oxygen or ATP homeostasis affects many cellular functions. NO reacts with hemoproteins, thiols, and free radicals such as superoxide anion (O_2^-). Mitochondria possess several hemoproteins, such as cytochrome oxidase, and thiols, such as reduced glutathione (GSH) or cysteine-containing proteins. These organelles are also the main cellular sources of O_2^-. The reactions of NO with these mitochondrial targets are distinct in a number of ways. For example, the reaction of NO with hemoproteins, such as cytochrome oxidase, is O_2 concentration-dependent, whereas with thiol-containing molecules, such as the mitochondrial caspase-3, it is pH- and redox-sensitive. The reaction of NO with O_2^- to produce the powerful oxidizing adduct peroxynitrite ($ONOO^-$) is nearly diffusion-controlled. However, it requires equal fluxes of NO and O_2^-, is favored in higher pH, and might be limited by strong mitochondrial redox barriers, such as manganese superoxide dismutase (MnSOD).

MITOCHONDRIAL TARGETS OF NO

Hemoproteins

NO readily reacts with heme, and mitochondria contain several hemoproteins, such as cytochrome oxidase, the terminal enzyme of the respiratory chain. The mitochondrial respiratory chain consists of four complexes functionally arranged in a redox potential (also called midpoint potential) hierarchy. Electrons enter the chain from complex I or II and flow to the downstream complexes, following the redox potential hierar-

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chy. At the terminal respiratory complex, the complex IV, they reduce O_2 to water. Coupled to this electron flow, protons are pumped from the mitochondrial matrix into the mitochondrial intermembrane space (Fig. 1). The pioneering work of Mitchell in the 1950s (40; for review, see 41), which introduced the chemiosmotic principle, postulates two immediate consequences for this proton extrusion: (a) An electrochemical gradient across the mitochondrial inner membrane, the transmembrane potential ($\Delta\Psi$), that polarizes the inner membrane negative inside. The $\Delta\Psi$ varies in mitochondria of different cells. In succinate-energized rat liver mitochondria, it is generally about −180 mV, much higher than the cell membrane potential. The $\Delta\Psi$ is the driving force for mitochondria to participate in the cellular homeostasis of cations such as Ca^{2+}. Although the role of mitochondria in cellular Ca^{2+} homeostasis was overlooked until recently, several studies have now revealed the importance of these organelles in phasic Ca^{2+} oscillation (49). (b) A proton gradient across the coupling membrane, the ΔpH, that is alkaline inside. Inhibition of the mitochondrial electron transport chain *e.g.,* at the level of cytochrome oxidase, would logically decrease the $\Delta\Psi$ and the ΔpH. The O_2 binding site of cytochrome oxidase is highly specialized for O_2; however, NO exerts similar physicochemical properties that allow it to bind to this binding site and subsequently inhibit the O_2 consumption (1). The inhibition by NO of O_2 consumption occurs at physiologically relevant concentrations of NO and is competitive, reversible, and dose-dependent in a manner resembling a pharmacological competitive antagonism (22). Thus, NO causes a reversible decrease in $\Delta\Psi$ (50) and ΔpH (17).

SH moieties

NO can react with a reduced thiol, *i.e.,* R–S–H, to produce a nitrosothiol *i.e.,* R–S–N=O, such as *S*-nitrosocysteine or *S*-nitrosoglutathione. This reaction, *S*-nitrosation, is reversible,

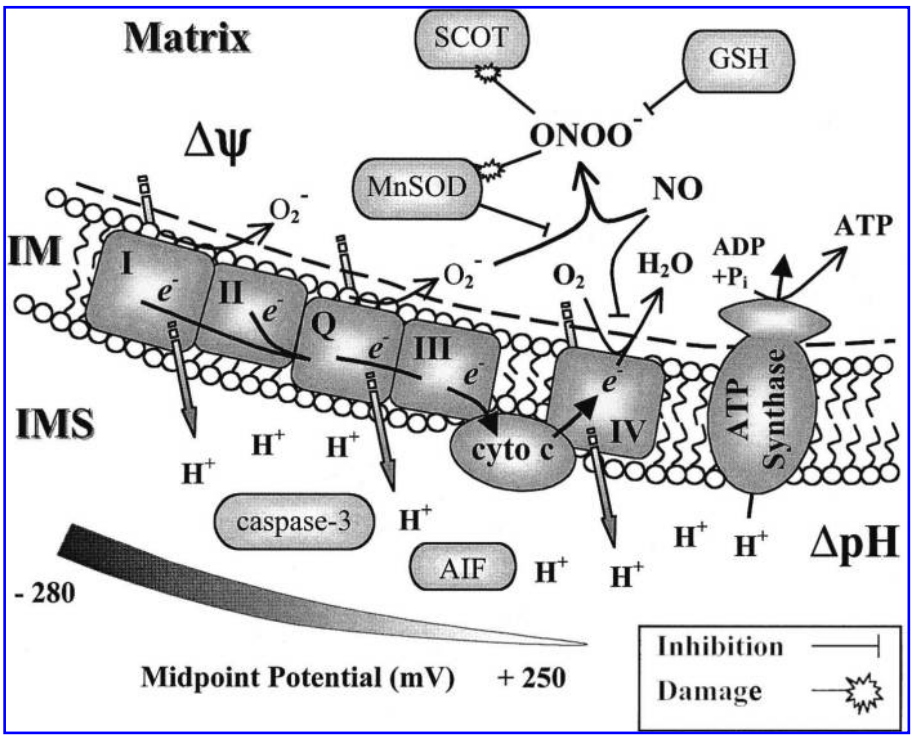

FIG. 1. Mitochondria consist of three distinct suborganelle compartments: the inner membrane (IM), the matrix, and the intermembrane space (IMS), which are different in composition, electrochemistry, and redox state. ***IM:*** Carries the respiratory chain. The chain consists of four complexes (I–IV), which are embedded in the IM, the coenzyme Q (ubiquinone; Q), and the ATP synthase. These complexes are functionally arranged in an electrochemical hierarchy based on their redox potentials. The respiratory chain provides a unique broad spectrum of redox potentials varying from −280 mV (complex I) to +250 mV (complex IV). Electrons flow down the chain to complex IV and reduce O_2 to H_2O. Coupled to the electron flow, protons are pumped from the matrix into the IMS. The proton extrusion establishes a transmembrane potential ($\Delta\Psi$, negative inside) and an electrochemical gradient (ΔpH, alkaline inside) across the coupling membrane. The IM is impermeable to H^+, which can reenter the matrix through the ATP synthase machinery. Mitochondrial respiratory chain is one of the main cellular sources of O_2^-. NO potently reacts with O_2^- to produce $ONOO^-$. Mitochondrial redox barriers, such as MnSOD, may affect the rate of $ONOO^-$ formation. ***Matrix:*** Major mitochondrial redox defense members MnSOD and GSH are located in the matrix. Some matrix proteins, such as MnSOD and SCOT, are susceptible to $ONOO^-$ induced damage. The matrix environment favors tyrosine nitration. ***IMS:*** Cytochrome c (cyto c) is the only respiratory chain member located in this compartment. Many mitochondrial apoptogenic proteins, such as caspase 3 and apoptosis-inducing factor (AIF), are also located in the IMS. The IMS environment favors the *S*-nitrosation reactions.

light-sensitive, and redox-dependent. *In vitro,* many proteins can undergo *S*-nitrosation by stoichiometric substitution of the proton with the nitrosonium, and *S*-nitrosated proteins are relatively stable. However, some conditions *in vivo* may hinder the *S*-nitrosation or destabilize the *S*-nitrosated products. The highly compartmentalized structure of mitochondria with different electrochemistry, redox state, and enzyme content would complicate studying a possible *S*-nitrosation of the mitochondrial components. For example, inorganic phosphate, which is present in high concentrations in the matrix, inhibits the *S*-nitrosation reaction (11). In the presence of nitrite, which is abundant in mitochondria, the *S*-nitrosation requires a low pH (53), which is provided only in the intermembrane space. Electron flow within the respiratory chain is accompanied by pumping protons from the matrix into the intermembrane space. The inner membrane is impermeable to almost all small ions, particularly to H^+, which can reenter mitochondria only through the F_0F_1 ATP synthase. In intact tightly coupled mitochondria, the pH is in the range of 7.5–7.8 in the matrix (2, 3, 17) and 6.9–7.0 in the intermembrane space (10). This ΔpH would favor *S*-nitrosation in the intermembrane space, rather than the matrix. Moreover, the matrix environment may accelerate the degradation of *S*-nitrosothiols, *e.g.,* by enzymatic reactions. Glutathione peroxidase is located in the matrix and decomposes the *S*-nitrosothiols (25). Thioredoxin reductase, which is found in the matrix (47), also cleaves the *S*-nitrosothiols (44). Thus, high concentrations of inorganic phosphate, the relatively alkaline environment, and the presence of enzymes capable of breaking down the *S*-nitrosothiols in mitochondrial matrix would not favor formation or stabilization of reaction of NO with reduced thiols such as GSH (56) in this compartment.

In contrast to the matrix, *S*-nitrosation of proteins in the intermembrane space is favored. Several mitochondrial apoptogenic proteins that are released during apoptosis are located within this compartment (20, 28, 35). Caspase-3 is located in the intermembrane space and induces apoptosis once released from mitochondria. Caspase-3 is *S*-nitrosated while within the intermembrane space (36), which keeps it apoptotically silent. This might be a protective mechanism for mitochondria against the proteolytic activity of caspase, and for cells against unwanted apoptosis. Upon release from the intermembrane space, the higher pH and the reduced environment of cytoplasm would denitrosate and, accordingly, activate the caspase. Thus, it seems that within mitochondria, the intermembrane space is the preferred suborganelle site for *S*-nitrosation.

Protein nitrosation is also favored in lipophilic membranous environments (43), such as in mitochondria (24). For example, the mitochondrial respiratory chain complex I, which is embedded in the inner membrane, can undergo *S*-nitrosation in a reversible and redox-sensitive manner (9). Whether *S*-nitrosation of complex I occurs at its intra- or intermembrane sites is not yet clear.

Taken together, by competing with O_2 at the level of cytochrome oxidase or by *S*-nitrosating the complex I, NO keeps mitochondrial respiration lowered in a transient and reversible manner. Presently, there is no general consensus as to the relative importance and biological significance of regulating the respiration at the level of complex IV compared with complex I. Whereas the former mechanism is O_2 concentration-dependent and requires relatively lower concentrations of NO, the latter is pH- and redox-sensitive and normally requires prolonged exposure of mitochondria with NO. Nevertheless, inhibition of endogenously produced NO increases respiration in many models ranging from the intact conscious animal (52) to isolated mitochondria (16; for reviews, see 15, 18).

MITOCHONDRIAL TARGETS OF NITRATING NO SPECIES

The reaction of NO with O_2^- is nearly diffusion-limited, and the product, $ONOO^-$, is a powerful oxidizing congener. The reaction is stoichiometric and requires equal fluxes of NO and O_2^-, and the product is more stable in higher pH (39, 62). Although difficult to measure directly, $ONOO^-$ formation has been reported for mitochondria (21, 61). Other oxidizing species can also be generated within the mitochondria, including hypervalent metal-oxo compounds formed by the reaction of the heme groups with peroxides (57). In contrast to the reactions of NO with mitochondria, the reactions of oxidizing NO species with mitochondrial components are mostly irreversible. With the sole exception of cytochrome *c,* all of the mitochondrial respiratory chain components have matrix faces and thus would be exposed to the higher pH environment of the matrix, as well as the release of O_2^- from the matrix face of the inner mitochondrial membrane (55). Oxidative inactivation of mitochondrial respiratory chain complexes I to IV has been reported by many groups (7, 48, 51; for review, see 19). Recent reports support the idea of limited O_2^- production by the respiratory chain during normal respiration (14, 30, 54, 55); however, mitochondria are still the main cellular sources of O_2^- and H_2O_2, particularly during inhibition of complex I. In 1997, a membrane-associated Ca^{2+}-dependent NO synthase in mitochondria (mtNOS) that continuously produces NO was found (16). This finding was later confirmed by the same group and by many others (27, 31; for reviews, see 15, 22). The continual basal production of NO provides a "sink" for the small amounts of O_2^- released from the mitochondria during normal respiration, thus helping to control the redox environment of the mitochondria. In addition, the presence of nitrite and H_2O_2 and its potential interaction with mitochondrial peroxidases, as well as the potential presence of $ONOO^-$, can lead to formation of nitrotyrosine residues on proteins, a footprint of oxidative NO species. Although tyrosine nitration of cytochrome *c,* which is located in the low pH intermembrane environment, does not seem favorable, oxidizing NO species can release cytochrome *c* from mitochondria (4, 21).

Oxidative stress and mitochondria play a role in the pathology of neurodegenerative diseases (6, 12, 13, 32, 45, 58). Oxidative NO species inactivate MnSOD by nitrating its critical tyrosines (34), and this inactivation is important in the pathology of certain diseases (33). MnSOD is a major detoxifying enzyme protecting the organelles against oxidative injury, such as in ischemia/reperfusion (8). Other mitochondrial matrix enzymes are also subject to damage by $ONOO^-$ or other oxidative NO species. Tyrosine nitration of succinyl-CoA:3-oxoacid CoA-transferase (SCOT), the mitochondrial rate-

limiting enzyme in ketolysis, is involved in the pathology of streptozotocin-induced diabetes (59). Thus, the mitochondrial matrix seems to be the preferred suborganelle site for the formation of oxidizing NO species, such as $ONOO^-$ (60). Intramitochondrially formed oxidizing NO species such as $ONOO^-$ increase lipid peroxides, release apoptogenic proteins from mitochondria (21), and are involved in oxidative stress-related conditions such as aging (61). Interestingly, in aged humans with neurodegenerative diseases, a selective nitration of mitochondrial respiratory complex I has been observed (63).

The higher mitochondrial matrix pH is also important for apoptosis. During apoptosis, mitochondria operate a permeability transition pore (23, 37). Alkalinization of the matrix facilitates the operation of this pore, whereas acidification prevents it (2). Matrix alkalinization is necessary for many forms of apoptosis (29), such as that induced by Bax (38).

Taken together, the effect of reactive nitrogen and reactive oxygen species on mitochondrial targets will vary in a distinct way for which different microenvironments within the organelle play decisive roles. The mitochondrial intermembrane space provides an environment that would favor the reaction of NO with thiols to form *S*-nitrosothiols. Several apoptogenic proteins, such as caspase-3, are located within the intermembrane space. *S*-Nitrosation of these proteins keeps them apoptotically silent while within mitochondria. The matrix fraction provides an environment that would favor the formation of oxidizing species, including those that can initiate tyrosine nitration. Integral mitochondrial redox defense barriers, such as MnSOD, which is susceptible to irreversible inhibition by oxidation, are located within this compartment. How mitochondria balance the reversible nitrosation versus irreversible nitration reactions needs to be further elucidated. However, mtNOS located within mitochondria will play a critical role in regulating these processes.

ABBREVIATIONS

$\Delta\Psi$, mitochondrial transmembrane potential; GSH, reduced glutathione; MnSOD, manganese superoxide dismutase; mtNOS, mitochondrial nitric oxide synthase; NO, nitric oxide; O_2^-, superoxide anion; $ONOO^-$, peroxynitrite; SCOT, succinyl-CoA:3-oxoacid CoA-transferase.

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