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REACTIONS OF PEROXYNITRITE IN THE MITOCHONDRIAL MATRIX

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Abstract—Superoxide radical ($O_2^{\cdot-}$) and nitric oxide (NO) produced at the mitochondrial inner membrane react to form peroxynitrite ($ONOO^-$) in the mitochondrial matrix. Intramitochondrial $ONOO^-$ effectively reacts with a few biomolecules according to reaction constants and intramitochondrial concentrations. The second-order reaction constants (in M^{-1} s^{-1}) of $ONOO^-$ with NADH (233 ± 27), ubiquinol-0 (485 ± 54) and GSH (183 ± 12) were determined fluorometrically by a simple competition assay of product formation. The oxidation of the components of the mitochondrial matrix by $ONOO^-$ was also followed in the presence of CO_2, to assess the reactivity of the nitrosoperoxocarboxylate adduct ($ONOOCO_2^-$) towards the same reductants. The ratio of product formation was about similar both in the presence of 2.5 mM CO_2 and in air-equilibrated conditions. Liver submitochondrial particles supplemented with 0.25–2 μM $ONOO^-$ showed a $O_2^{\cdot-}$ production that indicated ubisemiquinone formation and autooxidation. The nitration of mitochondrial proteins produced after addition of 200 μM $ONOO^-$ was observed by Western blot analysis. Protein nitration was prevented by the addition of 50–200 μM ubiquinol-0 or GSH. An intramitochondrial steady state concentration of about 2 nM $ONOO^-$ was calculated, taking into account the rate constants and concentrations of $ONOO^-$ coreactants. © 2000 Elsevier Science Inc.

Keywords—Peroxynitrite, Mitochondria, NADH-oxidation, Ubiquinol oxidation, GSH oxidation, Superoxide production, Mitochondrial protein nitration, Free radicals

INTRODUCTION

The intramitochondrial formation of peroxynitrite ($ONOO^-$) follows to the primary production of both superoxide anion ($O_2^{\cdot-}$) and nitric oxide (NO) at the mitochondrial inner membrane. Nitric oxide and $O_2^{\cdot-}$ react to produce $ONOO^-$ in a diffusion-controlled reaction ($k = 1.9 \times 10^{10}$ M^{-1} s^{-1}) [1]. This free radical termination reaction, in which both reactants have one unpaired electron in their external orbitals, is now recognized as the main pathway of intramitochondrial NO utilization [2] and was proposed 10 years ago by Beckman as the molecular mechanism of ischemic injury [3]. At physiological pH, $ONOO^-$ protonates to yield peroxynitrous acid (ONOOH; pKa = 6.8), which rearranges and decomposes to yield nitrate (NO_3^-) and radical species with a global half life of about 1 s [4–7].

Superoxide radical is a well-established mitochondrial metabolite produced vectorially into the matrix by the autooxidation of ubisemiquinone ($UQH^{\cdot}$) [8, 9], bound to the Q-binding protein; these radicals are confined to the mitochondrial matrix by its impermeability through the inner membrane and kept at a steady state level of about 10^{-10} M by intramitochondrial Mn-SOD [10].

The mitochondrial production of NO by a nitric oxide synthase (mtNOS) located at the inner membrane was recently recognized [11–13]. When its substrate, L-arginine, is added to isolated mitochondria, NO production is detected as a decrease in the respiratory rate due to cytochrome oxidase inhibition [14].

Peroxynitrite is a powerful univalent oxidant ($E^{o\prime}_{ONOO^-}$ as $ONOO^-$ = 1.40 V; [6]) that readily oxidizes the sulfhydryl group of cysteine and glutathione

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(GSH) [15], the sulfur atom of methionine [16], ascorbate [17], and the purine and pyrimidine bases of DNA [18]. It is also able to start the lipoperoxidation process in biomembranes and liposomes [19] and in isolated LDL [20]. There is evidence indicating $ONOO^-$ formation in different cell types, such as macrophages [21], neutrophils [22], Kupffer cells [23], and cultured endothelial cells [24].

This study addresses the intramitochondrial pathways of $ONOO^-$ utilization and provides the apparent second order rate constants for the reactions of $ONOO^-$ with NADH, ubiquinol-0 (UQ_0H_2) and GSH that were determined fluorometrically by a simple competition assay of product formation. It also gives evidence of $UQH^{\bullet}$ and O_2^- formation after supplementation of submitochondrial particles with relatively low $ONOO^-$ concentrations and of nitrated proteins after addition of relatively high $ONOO^-$ concentrations.

MATERIALS AND METHODS

Chemicals

Ascorbic acid, diethylenetriaminepentaacetic acid, glutathione, hydrogen peroxide, melatonin, reduced nicotinamide adenine dinucleotide (NADH), sodium bicarbonate, sodium nitrite, ubiquinone-0, and uric acid were purchased from Sigma Chemical Co. (St. Louis, MO, USA). Acrylamide solutions, PVDF membranes, and goat anti-rabbit IgG were purchased from Bio-Rad (Hercules, CA, USA). Specific anti-nitrotyrosine polyclonal antibody was a gift from Dr. A. Estevez and Dr. J. Beckman (University of Alabama at Birmingham, AL, USA). Other reagents used were of analytical grade.

Peroxynitrite preparation

Peroxynitrite was prepared by reacting an 0.6 M H_2O_2 solution in 0.7 M HCl with a solution of 0.6 M $NaNO_2$, followed by stabilization of the product with 1.2 M NaOH, as described by Reed et al. [25]. Peroxynitrite solution was treated with solid manganese dioxide to eliminate excess H_2O_2, and its concentration was determined spectrophotometrically at 302 nm (ϵ = 1.67 mM^{-1} cm^{-1}). Dilutions in 1 M NaOH were made to prepare work solutions (usually 2–5 mM).

NADH oxidation in the presence or absence of reductants

The reaction of NADH with $ONOO^-$ was followed fluorometrically at 37°C in a reaction medium consisting of 100 mM phosphate buffer (pH 7.0), 0.1 mM DTPA, 100 μM NADH, and 100–700 μM $ONOO^-$. The reaction extent was determined by measuring NADH fluorescence in a Hitachi F-3010 fluorescence spectrophotometer using 340 and 463 nm as excitation and emission wavelengths. Fluorescence intensity was measured after 30 s and 3 min after $ONOO^-$ addition to the reaction mixture. Control experiments were conducted to test eventual effects of $ONOO^-$ decomposition products. For this purpose, $ONOO^-$ was first incubated for 5 min in phosphate buffer to allow for its complete decomposition, and NADH was then exposed to the decomposed $ONOO^-$; the fluorescence change was less than 1% of the one produced by $ONOO^-$ addition.

Other reductants, such as ascorbic acid (0–300 μM), ubiquinol-0 (UQ_0H_2, 0–50 μM, from a 2 mM ethanol solution), glutathione (GSH, 0–150 μM), uric acid (0–80 μM), and melatonin (0–300 μM) were used in competition with NADH. In addition, NADH oxidation by 200 μM $ONOO^-$ was also followed in the presence of 13 mM $NaHCO_3$ (2.5 mM CO_2 at pH 7.0) and the effect of the reductants, ascorbic acid, ubiquinol and glutathione, was assayed.

Treatment of kinetic data

An indirect fluorometric technique was used to calculate the apparent rate constants of the reactions of $ONOO^-$ with NADH and various other reductants. The method is based on a model of simple competition kinetics of product formation that involves the participation of $ONOO^-$; the target molecule, NADH; and competitive reductants [26]. The decrease of NADH fluorescence was measured after a constant addition of 200 μM $ONOO^-$ and taken as NAD production, in the absence and presence of other reductant scavengers (AH_2). The treatment of data is based in the occurrence of the following main and global reactions:

$$ONOO^- + H^+ \rightleftarrows ONOOH \xrightarrow{ka} NO_3^- + H^+ \quad (1)$$

$$2ONOO^- + NADH \xrightarrow{kb} NAD^+ + 2NO_2 + H_2 + H_2O \quad (2)$$

$$2ONOO^- + AH_2 \xrightarrow{kc} A + 2NO_2 + H_2O \quad (3)$$

In alternative reactions 1 to 3, product yield is proportional to the ratio of each rate to the sum of the rates of the involved reactions, which in each case are the result of the product of each rate constant by the corresponding reactant concentration [27]. Thus, the rate of $ONOO^-$

utilization by reactions 1, 2, and 3 can be expressed by differential Eqn. 4:

$$d[ONOO^-]/dt = [ONOO^-] (k_a + k_b [NADH] + k_c [AH_2]) \quad (4)$$

The simple competition kinetics of alternative reactions 2 and 3 predict the following:

$$[NAD]_o/[NAD] = k_c [AH_2]/k_b [NADH] + 1 \quad (5)$$

in which $[NAD]_o$ is the NAD produced after $ONOO^-$ addition; [NAD] is the NAD produced in the presence of $ONOO^-$ and AH_2; and k_b and k_c are the apparent rate constants of the reaction of $ONOO^-$ with the NADH or AH_2, respectively. Thus, plotting the left-hand term of equation 5 versus $[AH_2]/[NADH]$ will result in a straight line whose slope is k_c/k_b.

Isolation of submitochondrial particles

Swiss mice (20–25 g) were killed by cervical dislocation, and the livers were immediately excised. Livers were homogenized in a medium consisting of 0.23 M mannitol, 70 mM sucrose, 10 mM Tris-HCl, and 1 mM EDTA, pH 7.4. Mouse liver homogenates were centrifuged at 700 × *g* for 10 min to discard nuclei and cell debris, and the pellet was washed to enrich the supernatant that was centrifuged at 7000 × *g* for 10 min. The pellet was washed and resuspended in 100 mM phosphate buffer and 80 mM KCl, pH 7.4. The operations were carried out at 0–2°C. Submitochondrial particles (SMP) were obtained by freezing and thawing (three times) the mitochondria preparation [28]. Protein was assayed by the method of Lowry et al. [29] using bovine serum albumin as standard.

Mitochondrial superoxide anion production

Superoxide anion production by mouse liver submitochondrial particles was measured by the superoxide dismutase-sensitive cytochrome *c* reduction at 550–540 nm (ϵ = 19 mM^{-1} cm^{-1}) [28]. The reaction medium consisted of submitochondrial particles (0.2 mg protein/ml) suspended in 0.23 M mannitol, 70 mM sucrose, 30 mM Tris-HCl, 1 mM EDTA (pH 7.4), 7 mM succinate, 20 μM cytochrome *c*, 2 μM myxothiazol, and 1 mM cyanide.

Western blot analysis

Mouse liver submitochondrial particles were exposed to 200 μM $ONOO^-$, in the absence or presence of 50–200 μM reductants (ascorbic acid, UQ_0H_2, GSH, NADH, uric acid, and melatonin) or 2 mM CO_2. The reactions were initiated by adding a small drop of $ONOO^-$ with a Hamilton syringe on the test tube wall above the assay solution and then rapidly mixing with the solution by vortexing. Submitochondrial particles were separated by electrophoresis on 7.5% SDS–polyacrylamide gel and transferred to a PVDF membrane. The membranes were incubated with a rabbit anti-3-nitrotyrosine polyclonal antibody (1:250). Bound antibodies were visualized with a goat antibody anti rabbit IgG (1:3000) conjugated to alkaline phosphatase and detected by chemiluminescence. Bovine serum albumin (BSA) was exposed to 1 mM $ONOO^-$ as positive control.

Statistical analysis

Results are expressed as mean values ± SEM and are the average of at least three independent experiments.

RESULTS

NADH oxidation by peroxynitrite

Exposure of NADH to $ONOO^-$ causes the immediate oxidation during mixing time of reduced pyridine nucleotide, as shown by the decrease of the 463 nm fluorescence peak (Fig. 1). The addition of up to 400 μM $ONOO^-$ produced an almost linear NADH oxidation at a ratio of about 0.25 NAD formed/$ONOO^-$ added. The relatively low oxidation yield is explained by the two-electron donation required to oxidize NADH and by the relatively large contribution of spontaneous decomposition of $ONOO^-$ (reaction 1).

Determination of rate constants

The supplementation of the reaction medium with various concentration of ascorbic acid decreased the extent of NADH oxidation by $ONOO^-$ (Fig. 2). The data, plotted as described in the Methods section, yielded a straight line whose slope, taking as reference k_c = 569 M^{-1} s^{-1} for the reaction of ascorbic acid and $ONOO^-$ at 37°C [17], gave a rate constant of k_b = 233 M^{-1} s^{-1} for the reaction of NADH with $ONOO^-$ (Table 1).

Similar experimental procedures and analysis were used with the other reductants. In these cases, the corresponding rate constants were calculated from the slope of the graphs and from the apparent second-order rate constant for the reaction of NADH and $ONOO^-$ as referred to in Table 1. The highest values of the rate constants were the ones corresponding to the reactions of $ONOO^-$ with UQ_0H_2 and uric acid. The values of the rate constants for all the assayed reductants are in agreement

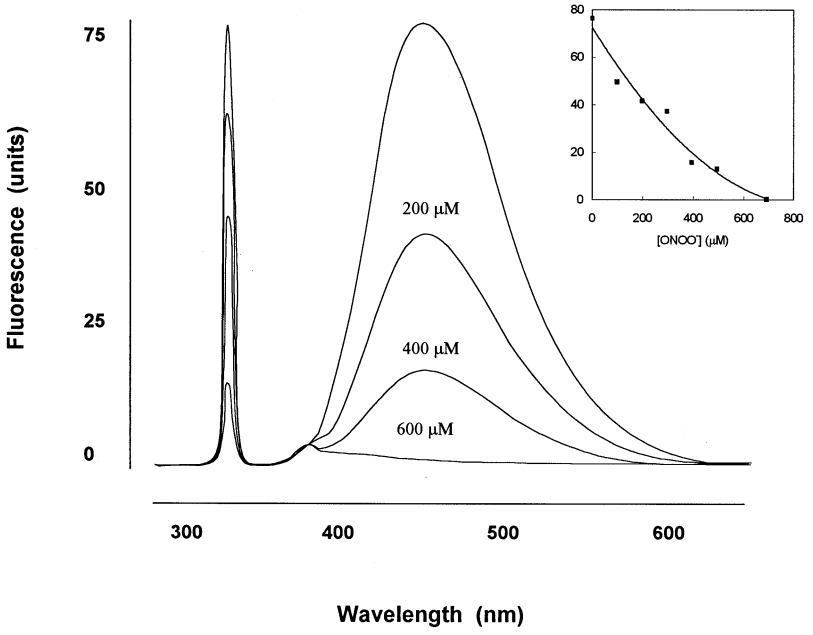

Fig. 1. NADH oxidation by peroxynitrite. Changes in NADH fluorescence emission spectrum after addition of 0, 200, 400, and 600 μM $ONOO^-$. Inset: Decrease of NADH fluorescence produced by the addition of 0–800 μM $ONOO^-$.

with the reductant concentrations that 50% inhibited (IC_{50}) NAD formation (Table 1).

When the effect of $ONOO^-$ on NADH oxidation was studied in the presence of CO_2, the determined ratios of the apparent rate constants k_c/k_b for the reaction of the adduct $ONOOCO_2^-$ with ascorbic acid, UQ_0H_2, and GSH obtained as described in the Methods section (Table 2). The ratio of the apparent reaction constants were

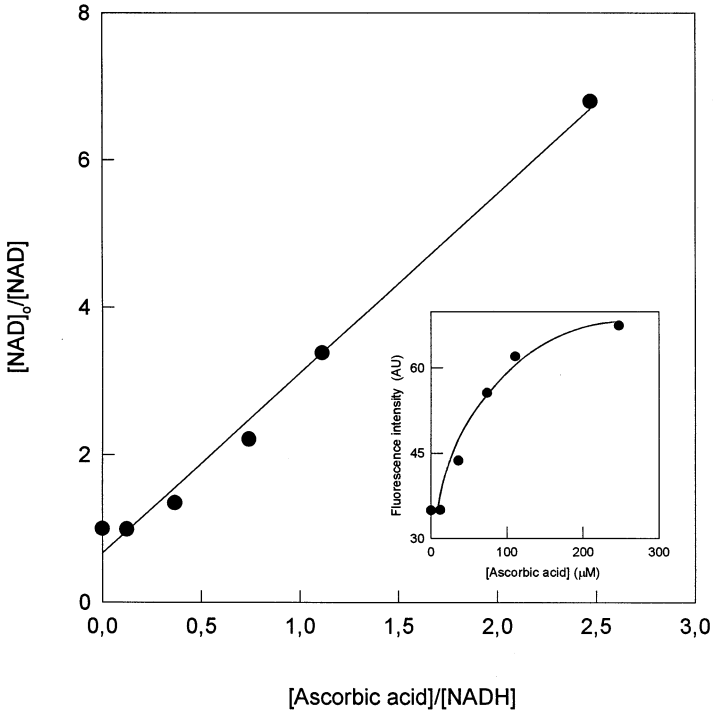

Fig. 2. Effect of ascorbic acid on NADH oxidation by peroxynitrite. Plot of $[NAD]_o/[NAD]$ as a function of [ascorbic acid]/[NADH]. Inset: Inhibition of NAD production by ascorbic acid.

Table 1. Second-Order Rate Constants and IC_{50} of the Reactions of $ONOO^-$ and Reductants

Reductants	IC_{50} (μM)	k_c/k_b	k (M^{-1} s^{-1})
NADH		—	233 ± 27
Ascorbic acid	55 ± 6	2.44 ± 0.28	569[a]
Ubiquinol-0	48 ± 5	2.08 ± 0.23	485 ± 54
Uric acid	114 ± 11	0.89 ± 0.04	207 ± 18
Glutathione	129 ± 13	0.78 ± 0.03	183 ± 12
Melatonin	519 ± 21	0.19 ± 0.01	43 ± 3

[a]Bartlett et al. [17].

in the same magnitude range both in the presence of CO_2 and in air-equilibrated conditions. It appears that the adduct oxidizes ubiquinol, NADH, ascorbic acid and GSH in a way that is similar to the way by which $ONOO^-$ oxidized the same reductants.

Superoxide production

The addition of low concentrations of $ONOO^-$ to SMP supplemented with mixothiazole and succinate elicited O_2^- production (Fig. 3), which is consistent with an oxidation of membrane bound ubiquinol to the corresponding ubisemiquinone and its subsequent autooxidation to generate O_2^-.

Tyrosine nitration by peroxynitrite

Nitration of proteins was observed in mitochondrial membranes after $ONOO^-$ addition (Fig. 4A). Supplementation with 2 mM CO_2 did not affect the nitration observed when $ONOO^-$ was added alone. No significant protection was obtained when melatonin was used. The other reductants minimized the tyrosine nitration produced by $ONOO^-$ in the following sequence: ascorbic acid > UQ_0H_2 ≅ uric acid > NADH ≅ GSH (according to their effect at the same concentrations of 100 μM). The effect of different concentrations of UQ_0H_2 and GSH on tyrosine nitration by $ONOO^-$ are shown in Fig. 4B. Both UQ_0H_2 and GSH inhibited tyrosine nitration in a concentration-dependent manner. Ubiquinol-0 was more effective than GSH in protecting mitochondrial proteins from nitration.

Table 2. Effect of Carbon Dioxide on NADH Oxidation by $ONOO^-$ in the Presence of Ascorbic Acid, Ubiquinol-0 or Glutathione

	Air-equilibrated		CO_2	
	k_c/k_b	IC_{50} (μM)	k_c/k_b	IC_{50} (μM)
Ascorbic acid	2.4 ± 0.3	55 ± 6	2.1 ± 0.4	48 ± 5
Ubiquinol-0	2.1 ± 0.2	48 ± 5	0.57 ± 0.05	178 ± 21
Glutathione	0.78 ± 0.03	129 ± 13	0.14 ± 0.01	735 ± 78

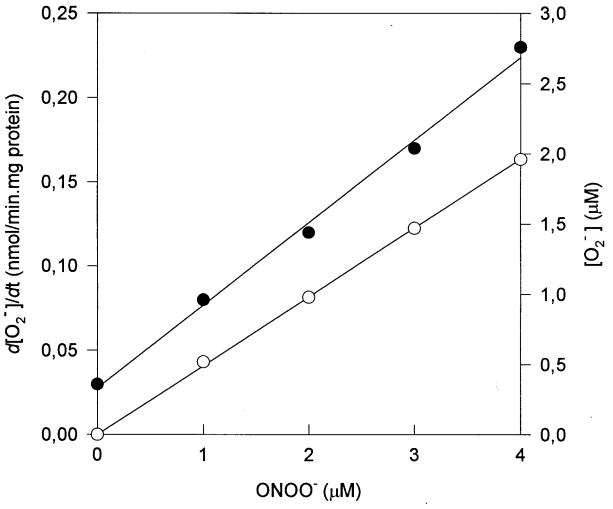

Fig. 3. Effect of $ONOO^-$ addition on O_2^- production rate (●) and on O_2^- concentration (○) by mouse liver SMP.

DISCUSSION

The mitochondrial matrix is a metabolically differentiated intracellular space concerning NO, O_2^-, and $ONOO^-$ metabolism because of the impermeability of the mitochondrial inner membrane to O_2^-, H^+, and $ONOO^-$ and because of the specific presence of about 3 μM Mn-superoxide dismutase [10].

The rate of intramitochondrial $ONOO^-$ formation can be calculated by considering the steady-state concentrations of NO and O_2^- in the mitochondrial matrix and the second-order rate constant $k = 1.9 \times 10^{10}$ M^{-1} s^{-1} [1] as follows:

$$d[ONOO^-]/dt = k\,[NO]\,[O_2^-] = k \times 50\ nM \times 0.1\ nM = 9.5 \times 10^{-8}\ M\ s^{-1} = 95\ nM\ s^{-1}$$

The accepted steady state concentration of O_2^- is 0.1 nM [10]. Nitric oxide is a highly diffusible molecule that when produced by mtNOS can diffuse equally to either the mitochondrial matrix or the cytosol depending on the NO concentrations in those compartments. Superoxide radical acts as an intramitochondrial trap for NO because the product $ONOO^-$, a charged species, is badly diffusible to the extramitochondrial space. The considered NO steady-state concentration (50 nM) is a mean value obtained from estimated values of 50 nM for rat liver mitochondria [14], 20 nM for brain mitochondria [30], 20 nM for isolated rat diaphragm [31], and 100 nM for bradykinin-stimulated perfused beating rat heart [32].

Several biomolecules constitute potential targets for $ONOO^-$. However, only a few are expected to be preferential targets due to the kinetic conditions. The rate of

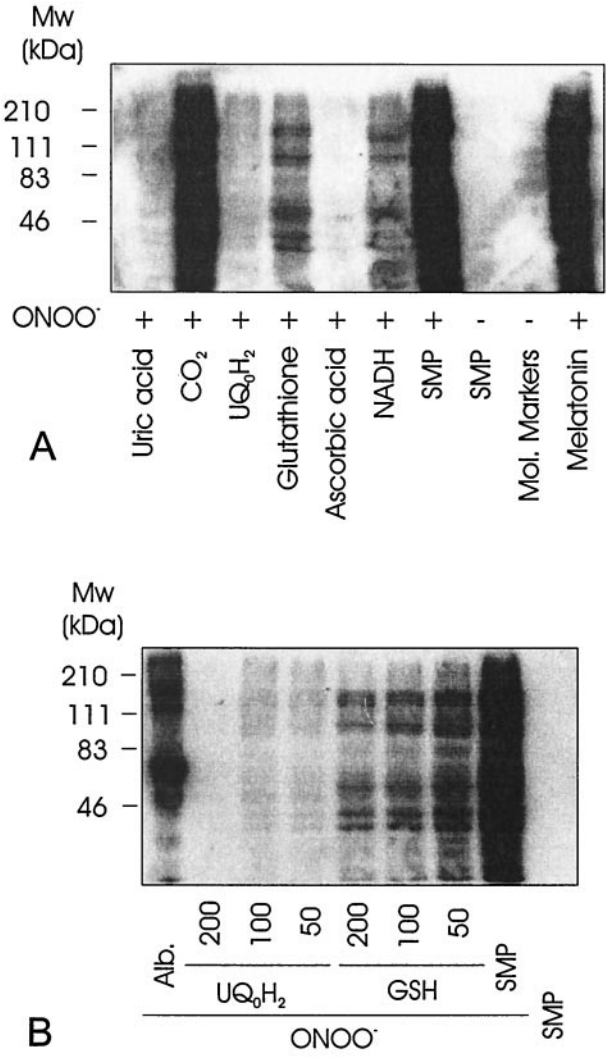

Fig. 4. Western blot analysis of 3-nitrotyrosine residues. (A) Effect of 200 μM $ONOO^-$ on SMP (3 mg/ml) in the absence or presence of 100 μM reductants. (B) Effect of 200 μM $ONOO^-$ on SMP (3 mg/ml) in the absence or presence of 50–200 μM GSH or UQ_0H_2. Lanes in each panel were loaded with equivalent amounts of SMP protein (60 μg).

mitochondrial $ONOO^-$ utilization can be estimated by considering the steady-state approach in which the rate of NO production ($+ d[ONOO^-]/dt$) equals the rate of NO utilization ($-d[ONOO^-]/dt$) and by applying differential Eqn. (6):

$$-d[ONOO^-]/dt = [ONOO^-]\,(k_1 + k_2\,[NADH] + k_4\,[UQ_0H_2] + k_5\,[GSH] + k_6\,[CO_2]) \quad (6)$$

The five terms in the parentheses describe the main metabolic pathways of mitochondrial $ONOO^-$. Rat liver intramitochondrial concentrations of 0.64 mM NADH, 0.42 mM UQ_0H_2, and 0.50 mM GSH were calculated from the mitochondrial contents of 3.8 [33], 2.5 [34], and 3.0 [35] nmol/mg protein, respectively, and a mitochondrial matrix volume of 6 μl/mg protein (from 35 mg mitochondrial protein per gram of liver and a 0.2 ratio of mitochondrial volume to total volume [35]). Mitochondrial CO_2 concentration was taken to be 1 mM [36], and the rate constant for the reaction of $ONOO^-$ with CO_2 was $k = 5.8 \times 10^4\ M^{-1}\ s^{-1}$ [37]. The values for the pseudo–first-order rate constants ($k' = k\,[AH_2]$) for the $ONOO^-$ reactions with the mitochondrial components NADH, UQ_0H_2, and GSH were as follows: 0.149 s^{-1} (k_2 [NADH]); 0.204 s^{-1} (k_4 [UQ_0H_2]); and 0.092 s^{-1} (k_5 [GSH]). The rates of the $ONOO^-$ reactions, with the nonmitochondrial components ascorbic acid, uric acid, and melatonin included for comparative purposes, do not appear quantitatively relevant after considering their pseudo–first-order rate constants: 0.011 s^{-1} (k [20 μM ascorbic acid]); 0.004 s^{-1} (k [20 μM uric acid], [38]); and $0.43 \times 10^{-6}\ s^{-1}$ (k [10 nM melatonin], [39]). The sum of the various pseudo–first-order rate constants gives a total value for k' of 58.5 s^{-1}, which, by the steady-state approach, allows the estimation of the mitochondrial steady state concentration of $ONOO^-$ as follows:

$$-d[ONOO^-]/dt = d[ONOO^-]/dt = k' \times [ONOO^-]$$

$$d[ONOO^-]/dt/k' = [ONOO^-]$$

$$= 95\ nM\ s^{-1}/58.5\ s^{-1} = 1.6\ nM$$

The oxidation of the various reductants by $ONOO^-$ is treated in Eqn. 6 as a bimolecular process, and the corresponding second-order reaction constants are given in Table 1. Although product formation follows formally the concentration dependence of a second order reaction, the consideration of the reactions as being bimolecular processes has to be taken as a simplification. A series of second-order constants has been reported in the literature for the reaction of $ONOO^-$ with monovalent or bivalent reductants [7]. From the side of the reductant, two successive bimolecular reactions are needed in which $ONOO^-$ oxidized the fully reduced forms (UQ_0H_2, NADH, ascorbic acid, and GSH) with production of an intermediate radical form that (i) is oxidized by $ONOO^-$; (ii) disproportionates ($UQH^\bullet$, $NADH^\bullet$, $Asc^\bullet$); or (c) dimerizes ($GS^\bullet$) in a further reaction. From the side of the oxidant, it is understood that in the isomerization of $ONOO^-$ to NO_3^-, ONOOH is formed upon protonation followed by the formation of an intermediate [HO· · · · ·NO_2] able to undergo rearrangement to yield NO_3^- and H^+ or to yield the two radicals $NO_2^\bullet$ and $HO^\bullet$ after homolysis. The radical yield seems low because generated radicals are extremely reactive with organic substrate ($k \cong 10^9\ M^{-1}\ s^{-1}$).

The $ONOO^-$ dependent production of O_2^- (Fig. 3) indicates the formation of ubisemiquinone (reaction 7) and its subsequent autoxidation to yield O_2^- by the reaction of Boveris-Cadenas (reaction 8 [8,9]). The two reactions point out to a protective role of ubiquinol that detoxifies $ONOO^-$ and prevents nitration by forming O_2^- and H_2O_2:

$$ONOO^- + UQH_2 \rightarrow NO_2 + OH^- + UQH^\bullet \quad (7)$$

$$UQH^\bullet + O_2 \rightarrow UQ + O_2^- + H^+ \quad (8)$$

In the presence of bicarbonate, $ONOO^-$ readily reacts with CO_2 ($k = 5.8 \times 10^4\ M^{-1}\ s^{-1}$ [37]) forming the adduct $ONOOCO_2^-$. It has been shown that this adduct participates in oxidation and nitration processes [37]. According to our results, the adduct oxidizes UQ_0H_2, NADH, ascorbic acid, and GSH in a similar way as $ONOO^-$. It appears that the formation would occur via the homolytic scission of $ONOOCO_2^-$ yielding $NO_2^\bullet$ and carbonate radical, this latter would afford the univalent oxidation similar to $HO^\bullet$. Formation of the adduct decreases the intramitochondrial steady state concentration of $ONOO^-$ without markedly changing product formation (UQ, NAD, or GSSG). It can be calculated from equation 6, that the effect of adduct formation (from the value of the term $k\ [CO_2]\ [ONOO^-]$) is to decrease $ONOO^-$ steady-state level from about 30 nM to 2 nM in the mitochondrial matrix. Then, adduct formation can be understood as another detoxification pathway of intramitochondrial $ONOO^-$ that takes advantage of the relatively high CO_2 concentration in the mitochondrial matrix and the higher instability of the adduct ($t_{1/2} = 1\ \mu s$ [40]) as compared with $ONOO^-$ ($t_{1/2} = 1$ s [1]).

A series of $ONOO^-$ reactions with mitochondrial components has been reported, such as inactivation of mitochondrial aconitase [41], Mn-SOD [42], succinate dehydrogenase, and ATPase [43] usually utilizing $ONOO^-$ concentrations in the μM range, as did the ones used in Fig. 4 to nitrate mitochondrial proteins. It can be estimated in terms of molecular collisions that the effect of a 100 μM pulse of $ONOO^-$ should be equivalent to the effect of $ONOO^-$ steady state of 1.6 nM during 26 hours. The time course of those effects has to be examined now in terms of the physiological level of $ONOO^-$ in the mitochondrial matrix.

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ABBREVIATIONS

$ONOO^-$—peroxynitrite
ONOOH—peroxynitrous acid
NO_3^-—nitrate anion
NO—nitric oxide
$NO_2^{\bullet}$—nitrogen dioxide radical
O_2^-—superoxide anion radical
NADH—reduced form of nicotinamide adenine dinucleotide
NAD—oxidized form of nicotinamide adenine dinucleotide
UQ_0H_2—quinol form of ubiquinone-0
$UQ_0H^{\bullet}$—semiquinone form of ubiquinone-0
$UQH^{\bullet}$—semiquinone form
UQ—quinone form
GSH—reduced glutathione