Nitric Oxide Evokes an Adaptive Response to Oxidative Stress by Arresting Respiration*§

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Aerobic metabolism generates biologically challenging reactive oxygen species (ROS) by the endogenous autoxidation of components of the electron transport chain (ETC). Basal levels of oxidative stress can dramatically rise upon activation of the NADPH oxidase-dependent respiratory burst. To minimize ROS toxicity, prokaryotic and eukaryotic organisms express a battery of low-molecular-weight thiol scavengers, a legion of detoxifying catalases, peroxidases, and superoxide dismutases, as well as a variety of repair systems. We present herein blockage of bacterial respiration as a novel strategy that helps the intracellular pathogen Salmonella survive extreme oxidative stress conditions. A Salmonella strain bearing mutations in complex I NADHdehydrogenases is refractory to the early NADPH oxidase-dependent antmicrobial activity of IFNγ-activated macrophages. The ability of NADH-rich, complex I-deficient Salmonella to survive oxidative stress is associated with resistance to peroxynitritite (ONOO−) and hydrogen peroxide (H2O2). Inhibition of respiration with nitric oxide (NO) also triggered a protective adaptive response against oxidative stress. Expression of the NDH-II dehydrogenase decreases NADH levels, thereby abrogating resistance of NO-activated Salmonella to H2O2. NADH antagonizes the hydroxyl radical (OH) generated in classical Fenton chemistry or spontaneous decomposition of peroxynitrous acid (ONOOH), while fueling AhpCF alkylhydroperoxidase. Together, these findings identify the accumulation of NADH following the NO-mediated inhibition of Salmonella’s ETC as a novel antioxidant strategy. NO-dependent respiratory arrest may help mitochondria and a plethora of organisms cope with oxidative stress engendered in situations as diverse as aerobic respiration, ischemia reperfusion, and inflammation.

Oxidative stress engendered by the sustained synthesis of NO mediates cytotoxicity against a variety of eukaryotic and prokaryotic cells (1–3). Because of its unpaired electron, NO directly reacts with metal prosthetic groups of cytochromes in the electron transport chain (ETC) and [Fe–S] clusters of dehydrogenases (4, 5). Alternatively, reactive nitrogen species (RNS) generated through the interaction of NO with O2 and superoxide (O2−) indirectly mediate cytotoxicity of this diatomic radical. The autooxidation of NO with O2 gives rise to RNS such as NO2− and N2O3 with potent oxidative and nitrosative activity. Independently, NO reacts with O2− to generate ONOO−, a species capable of oxidizing amino acids, [Fe–S] clusters, and DNA (6, 7). Despite its well-documented pro-oxidant functions, low concentrations of NO can paradoxically be cytoprotective. NO has been shown to both prevent oxidative damage of cardiomyocytes undergoing ischemia reperfusion and lessen the oxidative stress endured by mammalian host cells exposed to a variety of inorganic or organic peroxides (8–11). The mechanisms by which NO serves antioxidant roles remain, however, poorly understood.

Investigations using prokaryotic microorganisms have elucidated several mechanisms by which NO protects against oxidative stress. In enteric bacteria, the OxyRS and SoxRS regulatory systems coordinate the expression of antioxidant defenses against H2O2 and O2− (12, 13). In addition to responding to oxyradicals, the [Fe–S] prosthetic group of SoxR and redox active cysteines of OxyR can be modified by NO, thereby activating a signal transduction cascade that stimulates transcription of antioxidant defenses such as Mn-superoxide dismutase, endonuclease IV, and hydperoxidase I (14–16). Alternatively, NO can trigger instant cytoprotection without evoking de novo protein synthesis. For instance, in the Gram-positive bacterium Bacillus subtilis, NO promotes antioxidant defenses by: 1) depleting free cysteine and thus limiting the availability of a Fenton fuel and 2) enhancing H2O2 consumption through the activation of KatA catalase (17).

The ETC transfers reducing equivalents from a variety of substrates to respiratory cytochrome oxidases for the reduction of O2 to H2O. Discrete enzymatic components of the ETC such as NADH dehydrogenases and cytochromes couple oxidation reactions with transport of H+ across the membrane, generating a proton gradient that drives ATP biosynthesis by F0/F1 ATPases and energizes transporters and organelles.

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The on-line version of this article (available at http://www.jbc.org) contains supplemental Table S1.

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assembled in the membrane. The collapse of membrane potential and the subsequent fall in ATP synthesis appear to underlie the pathological effects associated with persistent inactivation of ETC redox centers (5, 18, 19). Transient inhibition of respiration, on the other hand, can serve physiological roles by diverting O2 to alternative cellular and tissue usages (20, 21). In the process of reducing quinones and pumping H+ across the membrane, NADH dehydrogenases comprising the ETC complex I consume NADH. We hypothesize herein that inhibition of ETC can serve additional physiological roles by conveying rapid, on the one hand, can serve physiological roles by diverting O2 to alternative cellular and tissue usages (20, 21). In the process of reducing quinones and pumping H+ across the membrane, NADH dehydrogenases comprising the ETC complex I consume NADH. We hypothesize herein that inhibition of ETC can serve additional physiological roles by conveying rapid

### EXPERIMENTAL PROCEDURES

**Bacterial Strains**—Salmonella enterica serovar Typhimurium strain ATCC 14028s was used throughout this study as the wild type and as the background for the construction of mutant strains (Table 1 and supplemental Table S1). Mutations of the Salmonella chromosome were constructed following the one-step, λ-red-mediated gene replacement method described by Datsenko and Wanner (22). To ensure that the phenotypes of the ETC complex I mutant were specific to the lack of NDH dehydrogenases, Δnuo:km and Δndh::FRT mutations were complemented with the low copy number pWSK29 vector expressing a wild-type ndh allele under the control of its native promoter. Wild-type Salmonella strain AV0554 expresses ndh under the pBAD18 promoter. Stationary phase wild-type Salmonella was tested after 16 h of culture in Luria-Bertani (LB) broth. Because of a delayed lag phase, complex I-deficient Salmonella strain AV0436 was grown for 20 h.

**Macrophage Assays**—C57BL/6 and congenic iNOS−/− (3) or gp91phox−/− (23) mice were bred in our animal facility according to Institutional Animal Care and Use Committee guidelines. The anti-Salmonella activity of macrophages was assessed as previously described (24). Briefly, periodate-elicited peritoneal macrophages were cultured in RPMI 1640 medium (Cellgro, Herndon, VA) supplemented with 10% heat-inactivated fetal bovine serum (BioWhittaker, Walkersville, MD), 15 mM HEPES, 2 mM L-glutamine, 1 mM sodium pyruvate (Sigma-Aldrich), and 100 units/ml of penicillin/streptomycin (Cellgro) as described (24). Selected groups of macrophages were treated with 200 units/ml of IFNγ (Invitrogen, St. Paul, MN) for 20 h before Salmonella infection. Macrophages were challenged with Salmonella at a multiplicity of infection (MOI) of 2 and after 25 min of infection the medium replaced with RPMI+ medium containing 18 μg/ml gentamicin. The cells were lysed at the indicated time points after infection and percent surviving bacteria recorded on LB agar plates.

### Inhibition of ETC Enhances Resistance to ROS

**Synthesis of ROS and RNS by Macrophages**—Macrophages isolated as above were infected with Salmonella at an MOI of 10. The monolayers were washed after 15 min of infection and the medium replaced with RPMI+ medium containing 6 μg/ml gentamicin and 100 μM luminol. Luminol chemiluminescence was used as an indicator of ONOO− production (25) by Salmonella-infected, IFNγ-primed macrophages. Synthesis of H2O2 was assessed by the horseradish peroxidase-mediated, luminol-dependent chemiluminescence in a reaction that contained 20 units/ml horseradish peroxidase and 100 μM luminol. Chemiluminescence was recorded at 37°C on an Lmax® luminometer (Molecular Devices) at 5-min intervals for 1 h with an integration time of 4 s.

**Susceptibility to RNS and ROS in Vitro**—Stationary phase wild-type and ETC complex I mutant Salmonella grown overnight in LB broth as above were diluted in PBS at a concentration of 5 × 10^6 cells/ml. The bacteria were incubated with 500 μM hypoxanthine (HX) (Sigma-Aldrich) and 0.1 units/ml xanthine oxidase (XO) (Roche Applied Science, Indianapolis, IN), 400 μM or 2.5 mM H2O2, or 750 μM ONOO− generator SIN1 (Molecular Probes, Eugene, OR). The HX/XO system generated 0.4 and 0.5 μM/min of O2 and H2O2 as estimated by superoxide dismutase-inhibitable reduction of cytochrome c and horseradish peroxidase-catalyzed oxidation of phenol red, respectively (26). The contribution of O2 and H2O2 to the antimicrobial activity of the XO/HX system was estimated by adding 300 units/ml Cu-Zn superoxide dismutase or 25 units/ml catalase (Sigma-Aldrich), respectively. Percent survival was calculated after various times of exposure by recording the number of bacteria able to form a colony on LB plates. Selected groups of wild-type bacteria diluted at a concentration of 10^6 cells/ml in EG medium (0.811 mM MgSO4, 9.52 mM citric acid, 57.41 K2HPO4, 16.74 mM Na(H2)PO4 and 0.4% glucose, pH 7.0) were pre-adapted for 1 h with 750 μM sperm diene NONOate or SIN1 in the presence of 15 μg/ml of the protein synthesis inhibitor chloramphenicol. Control and RNS- and NADH+catalyzed cells were diluted in PBS and challenged for 2 h with 400 μM H2O2 in the presence of chloramphenicol. About 35 μM NO donor spermine NONOate and SIN1 remained during H2O2 challenge.

**NADH/NAD+ Quantification**—NADH and NAD+ were extracted in 0.2 M NaOH and 0.2 M HCl, respectively, from overnight cultures of Salmonella grown in LB medium as described above. The concentration of NADH/NAD+ was measured by the thiazolyl tetrazolium blue cycling assay (27), and the concentration of nicotinamide was calculated by regression analysis of known standards. Nucleotide concentrations were corrected for bacterial density as estimated spectrophotometrically at

### Table 1

| Strains               | Genotype Source       |
|-----------------------|-----------------------|
| *Salmonella typhimurium* strain 14028s | Wild-type ATCC       |
| AV0201                | Δspc::FRT            | (24)               |
| AV0249                | Δcya::FRT            | This study         |
| AV0436                | Δnuo::km Δndh::FRT   | This study         |
| AV0441                | Δnuo::km Δndh::FRT Δspc::FRT | This study         |
| AV0497                | Δnuo::km Δndh::FRT Δhdp::FRT | This study         |
| AV0552                | Δnuo::km Δndh::FRT pNDH (pWSK29::ndh) | This study         |
| AV0553                | Δnuo::km Δndh::FRT pBNDH (pBAD18::ndh) | This study         |
| AV0554                | pBNDH (pBAD18::ndh)  | This study         |
| AV0559                | pBAD (pBAD18)        | This study         |

| Plasmids              | Source                |
|-----------------------|-----------------------|
| pDK13                 | bla FRT ahp FRT oriR6K | (22)               |
| pCP20                 | bla cat cI857 P6 flp pSC101 oriTS | (52)               |
| pWSK29                | bla lacZs oriSC101    | (53)               |
| pBAD18                | araC bla rrdl oriM13 oripBR322 | (54)               |
Inhibition of ETC Enhances Resistance to ROS

A Salmonella strain lacking respiratory NADH dehydrogenases is resistant to NADPH oxidase-mediated intracellular killing. Survival of WT, Δnuo::km Δndh::FRT complex I-deficient (CI), and CI-deficient Salmonella bearing the pNDH plasmid expressing a wild-type ndh allele was tested in IFNγ-primed macrophages (a). To determine the contribution of ROS and RNS to the anti-Salmonella activity of IFNγ-activated macrophages, bacterial survival was studied in macrophages from immunocompetent C57BL/6 mice (b) or congenic controls lacking the gp91phox (phox KO) or iNOS (nos2 KO) hemoproteins (b). The contribution of SPI2 to the intracellular survival of CI-deficient Salmonella was directly determined by introducing the ΔspiC::FRT mutation into the Δnuo::km Δndh::FRT strain AV0436 (c). The ability of Salmonella to stimulate synthesis of ONOO− by IFNγ-primed and H2O2 by control macrophages was estimated as luminal-dependent and the horseradish peroxidase-catalyzed, luminol-dependent chemiluminescence, respectively (d).

Acridine orange and the intracellular NADH concentration calculated based on a bacterial cell volume of 10¹² liters.

Hydroxyl Radical Formation—Terephthalic acid was used to measure OH• via the fluorescence 2-hydroxyterephthalate adduct (λex = 315 nm; λem = 425 nm). The effect of NADH or NAD+ on the formation of 2-hydroxyterephthalate was monitored in a spectrofluorometer after the addition of 4 mM ONOO− into a solution of 2.5 mM of terephthalate in PBS, pH 7.0. Autofluorescence of NADH and NAD+ were subtracted from the final readings.

Oxygen and H2O2 Consumption—Salmonella grown overnight in LB broth were cultured to 1 OD/ml in aerated EG medium, pH 7.0 at 37 °C. Consumption of oxygen and H2O2 was recorded with specific probes using a free radical analyzer (WPI Inc., Sarasota, FL). Selected samples were treated with the indicated concentrations of authentic ONOO− or 750 µM spermine NONOate or SIN1 1 min before analysis.

Spectroscopy—Salmonella strain AV0429 lacking the cyo ubiquinol oxidase was grown overnight to stationary phase in LB broth. Inner membranes were prepared as described by Miller and Gennis (28) with minor modifications. Briefly, bacterial pellets were resuspended in 10 mM EDTA, 100 mM Tris/HCl buffer, pH 8.5. The cells were lysed by passing the suspension through a French Press Cell Disruptor (Thermo Electron Corporation, Milford, MA) three times at 18,000 psi at a flow rate of 5 ml/min. Cell debris were removed by centrifuging for 20 min at 10,000 × g in a Sorvall SL-50T rotor. The supernatant was centrifuged at 200,000 × g in a Beckman-type 70 Ti rotor for 1 h and the pellet solubilized in 75 mM potassium phosphate, 150 mM KCl, 5 mM EDTA, and 60 mM N-dodecyl-N,N-dimethyl-3-ammonio-1-propane sulfonate (Sigma) buffer, pH 6.4. The solution was centrifuged at 200,000 × g for 1 h. Supernatants containing inner membranes were collected, and the protein concentration assayed using the BCA Protein Assay kit (Pierce). The protein was adjusted to 1.5 mg/ml in 75 mM potassium phosphate, 150 mM KCl, 5 mM EDTA, and 60 mM N-dodecyl-N,N-dimethyl-3-ammonio-1-propane sulfonate buffer, pH 6.4. Absorbance spectroscopy was collected in a Cary 50 Bio UV-Visible spectrophotometer. Selected groups of proteins were treated with 100 µM spermine NONOate for 30 min before the absorption spectra were collected. The spermine base was used as a negative control.

RESULTS

Complex I-deficient Salmonella Is Hyper-resistant to the Oxidative Stress Encountered within IFNγ-primed Macrophages—The complex I-deficient Salmonella strain AV0436 (Δnuo::km Δndh::FRT), but not isogenic strains bearing sin-
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Antioxidant systems are especially varied in intracellular pathogens residing within professional phagocytes capable of generating massive amounts of ROS in the NADPH oxidase-dependent respiratory burst. To test whether the hyper-resistance of complex I-deficient Salmonella might be related to the Salmonella pathogenicity island 2 (SPI2) type III secretion system that ameliorates exposure of Salmonella to reactive species such as H$_2$O$_2$ and ONOO$^-$ (30, 31), a ΔspiC::FRT allele that inactivates SPI2 secretion and effector functions (32–34) was combined with Δnuo::km Δndh::FRT alleles generating strain AV0441 (NDH-I$^{-}$ NDH-II$^{-}$ SPI2$^-$). Strain AV0441 was as resistant to early macrophage oxidative killing as its complex I-deficient isogenic control (Fig. 1c). Poor NADPH oxidase-dependent killing of complex I-deficient Salmonella cannot be explained by reduced synthesis of ONOO$^-$ by IFNγ-primed macrophages (Fig. 1d). Moreover, unstimulated macrophages produced similar amounts of H$_2$O$_2$ in response to wild-type or complex I-deficient Salmonella (Fig. 1d).

Complex I-deficient Salmonella Is Hyper-resistant to a Variety of ROS and RNS Generated in Vitro—Next, we tested whether the marked resistance of complex I-deficient Salmonella to NADPH oxidase-mediated intracellular killing might be associated with resistance to ROS and RNS. Strain AV0436 was found to be refractory to the antimicrobial activity of the O$_2^-$/H$_2$O$_2$-generating HX/XO system (Fig. 2a). Catalase, but not Cu-Zn superoxide dismutase, abrogated the killing of wild-type Salmonella by HX/XO (Fig. 2b), suggesting that complex I-deficient Salmonella is resistant to H$_2$O$_2$. Supporting this notion, complex I-deficient bacteria survived exposure to authentic H$_2$O$_2$, becoming susceptible upon expression of the NDH-II-complementing pNDH plasmid (Fig. 2c). Analogous to the NDH-mediated potentiation of oxidative stress previously associated with respiratory arrest in rapidly growing Escherichia coli (35, 36), the log phase complex I-deficient Salmonella was susceptible to H$_2$O$_2$ (Fig. 2d). Because ONOO$^-$, which is similarly formed in response to wild-type and complex I-deficient Salmonella (Fig. 1d), constitutes an intrinsic component of the early anti-Salmonella arsenal of IFNγ-treated macrophages (26, 31), the susceptibility of Salmonella strain AV0436 to the ONOO$^-$ generator SIN1 was also studied. As for H$_2$O$_2$, the viability of stationary phase, complex I-deficient Salmonella was unaffected after exposure to SIN1 (Fig. 2e). These

Figure 3. In increased NADH levels in complex I-deficient Salmonella antagonize biologically active peroxides. NADH/NAD$^+$ ratios were estimated in cytoplasmic extracts from WT and isogenic Δnuo::km Δndh::FRT complex I-deficient (CI) Salmonella by the triazol tetrazolium blue cycling assay (a). The CI-deficient Salmonella expressing the pNDH plasmid was used as a control. The contribution of the NADH-consuming AhpCF alkylhydroperoxidase to resistance of Δnuo::km Δndh::FRT complex I-deficient Salmonella was studied after exposure to 750 μM SIN1 or 400 μM H$_2$O$_2$ (b). Consumption of NADH by ONOO$^-$ was determined in a 20 min NaOH solution, whereas oxidation of NADH by H$_2$O$_2$ and ONOO$^-$ was determined in PBS, pH 7.0 (c). Consumption of NADH was analyzed by fluorometry (λex 340 nm, λem 440 nm), whereas consumption of H$_2$O$_2$ was measured in a spectrophotometer by the horseradish peroxidase-dependent oxidation of phenol red (d). The ability of NADH or NAD$^+$ to block ONOO$^-$ mediated hydroxylaminic acid (HTA) formation is shown in e.

Aerobic organisms exploit an assortment of mechanisms for detoxification or avoidance of ROS (29). The antioxidant repertoire is especially varied in intracellular pathogens residing...
data demonstrate that Salmonella lacking complex I of the ETC are resistant to a variety of biologically active peroxides.

Antioxidant Activity of NADH—As anticipated, Salmonella strain AV0436 lacking NDH-I and NDH-II contained higher NADH/NAD ratios than wild-type controls (Fig. 3a). The intracellular concentration of NADH in wild-type and complex I-deficient bacteria were 167 and 484 μM, respectively, whereas their NAD⁺ levels were 885 and 887 μM. The complex I-deficient Salmonella complemented with the pNDH plasmid contained 206 μM NADH and 763 μM NAD⁺. NADH could serve antioxidant functions by enhancing peroxidatic activity of alkylhydroperoxidases (37, 38). To test this hypothesis, a ΔahpCF ΔFRT mutation deleting both subunits of the alkylhydroperoxidase reductase was combined with cyo-(nuo)- and ndh alleles inactivating NDH-I and NDH-II NADH dehydrogenases. The lack of ahpCF increased by 5- and 2-fold the killing of complex I-deficient Salmonella by 750 μM SIN1 or 400 μM H₂O₂, respectively (Fig. 3b). NADH may also provide direct antioxidant activity, because NADH was oxidized by ONOO⁻ (Fig. 3c) and a combination of H₂O₂ and Fe²⁺ (Fig. 3d). In contrast, NADH was not consumed by ONOO⁻ or H₂O₂ (Fig. 3c). Together, these data indicate that NADH can scavenge OH⁻ derived from H₂O₂ and ONOOH. Accordingly, NADH, but not NAD⁺, prevented ONOOH-induced synthesis of hydroxyterephthalate (Fig. 3e), which is a specific signature of OH⁻ (39).

Inhibition of the ETC by RNS Elicits an Adaptive Response against Oxidative Stress—Because defects in the ETC promote resistance of Salmonella to oxidative stress following exposure to a variety of ROS and RNS (Fig. 2), we tested whether the pharmacological blockage of respiration following NO treatment may also trigger an antioxidant adaptive response. As expected by its ability to inhibit metal centers of cytochromes of the ETC (35, 36), 750 μM NO donor spermine NONOate arrested Salmonella respiration (Fig. 4a). ONOO⁻, which has...
been shown to inhibit ETC complex I in mitochondria (4), also inhibited the respiration of Salmonella (Fig. 4b). NO conferred instant cytoprotection against 2.5 mM (Fig. 4c) or 400 μM H2O2 (Fig. 4e). The protective effects were nonetheless transitory because the viability of NO-adapted Salmonella started to decline after 50 min of exposure to 2.5 H2O2 (Fig. 4c). The reversible nitrosylation of terminal cytochromes provides a mechanism for the transitory protective effects associated with NO. Because the bd-type ubiquinol oxidase is preferentially expressed by bacteria grown to stationary phase (40), such as those used in these studies, nitrosylation of terminal cytochromes was studied in a Δcyo Salmonella strain that expresses increased levels of the ubiquinol bd-type oxidase. Optical absorption spectroscopy of purified membranes from Δcyo Salmonella showed a shift in the absorption band from 650 to 635 nm upon NO treatment (Fig. 4d), consistent with the nitrosylation of heme d. In contrast, the absorption bands at 560 and 530 nm characteristic of cytochrome b558 increased in intensity after NO treatment. Similar protective responses were induced by the classical respiratory inhibitor KCN (Fig. 4e), lending robust support to the notion that the antioxidant roles of NO seen here are mediated through the arrest of respiration. The immediate adaptive response elicited upon inhibition of ETC is independent of de novo protein synthesis as indicated by the fact that the ribosomal inhibitor chloramphenicol did not prevent the 100-fold protection afforded by pretreatment with the ONOO− generator SIN1 or the NO donor spermine NONOate (Fig. 4f). Therefore, classical SoxR and OxyR transcriptional regulators that coordinate adaptive responses of enteric bacteria to O2−, H2O2, and NO (12–15) are not likely to mediate the immediate adaptive response that follows after RNS treatment under our experimental conditions.

NADH Contribution to the RNS-Mediated Protection against Oxidative Stress—The NO-mediated induction of antioxidant defenses described herein does not appear to be due to increased hydroperoxidase activity seen in Bacillus subtilis (17), because the consumption of H2O2 was actually inhibited in NO-treated Salmonella (Fig. 5a), possibly reflecting inactivation of heme-containing catalases (41). The increased resistance of RNS preadapted Salmonella to H2O2 cannot be emulated with the thiol oxidizer diamide (Fig. 5b) as shown for B. subtilis (17). Instead, the adaptive response elicited by NO is dependent on the accumulation of NADH. SIN1 increased the amount of NADH in Salmonella (Fig. 5c). Expression of NDH-II from the pBNDH plasmid not only reduced NADH levels (Fig. 5c) but also abrogated the increased resistance of SIN1-pretreated Salmonella (Fig. 5d). These findings support a model in which elevated NADH levels following NO inhibition of ETC contribute to the resistance of Salmonella to H2O2.

**DISCUSSION**

The ETC shuffles electrons from substrates such as NADH to cytochrome oxidases that reduce O2 to H2O. In the process, protons are translocated at discrete metalcenters of the NADH dehydrogenase and terminal cytochromes, creating a proton motive force that feeds the F0/F1 ATP synthase. Protracted disruption of the ETC by RNS has been traditionally associated with pathology (5, 18, 19). Our data indicate that transient inhibition of the ETC by NO can, however, serve antioxidant functions. According to this model, classical respiratory inhibitors such as NO and KCN independently triggered an adaptive response that protected Salmonella against H2O2-mediated cytotoxicity. NO-mediated cytoprotection was immediate but transitory, possibly reflecting the temporal nitrosylation of terminal bo and bd cytochromes of the ETC (42, 43). Accordingly, optical spectra showed nitrosylation of the heme d after NO treatment. Our findings are consistent with a model in which the ETC serves as a basic relay system that oscillates between “ON” and “OFF” positions according to energetic and antioxidant demands.
Inhibition of ETC Enhances Resistance to ROS

Our data indicate that respiratory arrest contributes to the antioxidant defenses of Salmonella by redirecting NADH usage. In support of this notion, expression of NDH-II prevented the accumulation of NADH in the cytoplasm of RNS-adapted Salmonella, thereby abrogating the antioxidant advantage evoked by NO. We have identified two mechanisms by which NADH buildup can ameliorate H2O2 cytotoxicity. NADH fuels the peroxidatic activity of AhpCF. In addition, NADH-rich, complex I-deficient Salmonella, which NADH buildup can ameliorate H2O2 cytotoxicity. Inhibition of ETC Enhanced Resistance to ROS. NADH buildup that follows ETC blockade provides three independent mechanisms of protection against ONOO−. NADH buildup that follows ETC blockade provides three independent mechanisms of protection against ONOO−. First, NADH powers AhpCF peroxidatic detoxification of ONOO− (38), thereby limiting [Fe-S] oxidation and the consequent release of the Fenton catalyst Fe2+. Second, it fuels detoxification of endogenous H2O2 via AhpCF alkylhydroperoxide (49), thus minimizing substrate availability for Fenton chemistry. And third, it can directly scavenge OH· derived from the spontaneous decomposition of ONOOH.

Analogous to the NADH-mediated potentiation of oxidative stress described upon respiratory arrest in rapidly growing E. coli and Salmonella (35, 36, 50), log phase complex I-deficient Salmonella were susceptible to H2O2. These data indicate that the antioxidant defenses associated with respiratory arrest are dependent on the growth phase of the bacteria, perhaps reflecting the selective expression of defenses such as AhpCF (51). NADH provides immediate relief to oxidative stress, preceding other adaptive pathways, such as those coordinated by OxyRS and SoxRS transcriptional regulators, which rely on de novo protein synthesis. However, our data do not rule out that in the absence of protein inhibitors NO-induced accumulation of NADH will likely synergize with an increased expression of AhpCF following nitrosylation of OxyR (15). In summary, our findings have exposed an unprecedented antioxidant role for respiratory arrest. The novel physiological role associated with NADH following ETC arrest is likely to be an intrinsic component of the immediate antioxidant arsenal of aerobic microorganisms and might be especially relevant in mitochondria.

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