Constitutive and Adaptive Detoxification of Nitric Oxide in Escherichia coli

ROLE OF NITRIC-OXIDE DIOXYGENASE IN THE PROTECTION OF ACONITASE*

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Nitric oxide (NO) is a naturally occurring toxin that some organisms adaptively resist. In aerobic or anaerobic Escherichia coli, low levels of NO exposure inactivated the NO-sensitive citric acid cycle enzyme aconitase, and inactivation was more effective when the adaptive synthesis of NO-defensive proteins was blocked with chloramphenicol. Protection of aconitase in aerobically grown E. coli was dependent upon O2, was potently inhibited by cyanide, and was correlated with an induced rate of cellular NO consumption. Constitutive and adaptive cellular NO consumption in aerobic cells was also dependent upon O2 and inhibited by cyanide. Exposure of aerobic cells to NO accordingly elevated the activity of the O2-dependent and cyanide-sensitive NO dioxygenase (NOD). Anaerobic E. coli exposed to NO or nitrate induced a modest O2-independent and cyanide-resistant NO-metabolizing activity and a more robust O2-stimulated cyanide-sensitive activity. The latter activity was attributed to NOD. The results support a role for NOD in the aerobic detoxification of NO and suggest functions for NOD and a cyanide-resistant NO scavenging activity in anaerobic cells.

Nitric oxide (NO) is released by leukocytes and functions as an antibiotic (1–3). It may also be produced endogenously by bacteria during the reduction of NO2 by nitrate reductase (4). In addition, bacteria may encounter NO released by competing microorganisms (5). Regardless of the source, NO produced at sufficient levels directly or indirectly damages critical cell processes (3). Indeed, NO is bacteriostatic toward some bacteria (6), and NO or NO-derived species may display bactericidal activities in vitro and in infected animals (1–3, 7–10).

Various organisms may benefit from adaptive mechanisms for NO detoxification. Denitrifying bacteria (11, 12) and fungi (13) are known to produce NO-inducible (14) NO-detoxifying NORs. NORs catalytically reduce NO to produce nitrous oxide (N2O). NORs also increase the anaerobic energy production capacity of denitrifiers by catalyzing an essential step in the reduction of nitrate (NO3) and nitrite (NO2) to N2 (15). Considerably less is known of the adaptations of nondenitrifying bacteria to NO or of their normal exposures to NO. An adaptation of Escherichia coli to NO under the transcriptional control of the antioxidant regulators SoxRS and OxyR has been suggested, since these global antioxidant regulators provide some survival and growth benefits against NO (16, 17) or nitrosothiols (18), respectively. Yet, it remains unclear how these regulators protect bacteria. E. coli does not appear to produce a typical NOR activity, but it does produce a multifunctional nitrite reductase with NO-reducing capacity (19) and a nitric oxide dioxygenase (NOD) that has been proposed to function in NO detoxification (20).

We have observed, and now report, an increased susceptibility to NO of the NO-sensitive citric acid cycle enzyme aconitase (21, 22) in aerobic or anaerobic E. coli inhibited for de novo protein synthesis. Protection of aconitase activity correlated with an increased rate of cellular NO consumption and correlated with an increased NOD activity in cell-free extracts. The results support the proposed function of NOD in the constitutive and adaptive detoxification of NO in aerobic E. coli. The possible role of the NO-induced NOD activity in aerobic E. coli is discussed.

MATERIALS AND METHODS

Cells and Reagents—E. coli strain DH5α was from Life Technologies, Inc. Mutants deficient in the terminal oxidases ECL3936 (Δcyo), ECL3937 (Δcyd), and the parent ECL933 were kindly provided by E. C. C. Lin (23). Compressed gas cylinders containing 1200 ppm (± 5%) NO in ultrapure N2, 99.998% N2, and 99.993% O2 were obtained from Praxair (Bethlehem, PA). NO-saturated water was prepared by stirring N2-equilibrated water under 98.5% NO gas (Aldrich), which was first bubbled through 1 N NaOH. Saturated NO2 was stored at 4 °C in a rubber septum-sealed glass tube. Sodium cyanide, NADP⁺, FAD, chloramphenicol, nitric oxide from Aspergillus niger, glucose-6-phosphate dehydrogenase from bakers’ yeast, sodium nitrite, sodium nitrate, succinic acid, D-glucose, glucose-6-phosphate, l-arginine-HCl, thiamine HCl, Tris, and MnCl2 were from Sigma. Nitrate reductase from Aspergillus niger and bovine liver catalase were purchased from Boehringer Mannheim. Tryptone and yeast extract were obtained from Difco.

Media, Growth of Bacteria, and Extracellular Preparation—The minimal salts medium was made up with tap water and contained 60 mM K2HPO4, 33 mM KH2PO4, 7.6 mM (NH4)2SO4, 1.7 mM sodium citrate, 1 mM MgSO4, 10 μM MnCl2, 10 μM thiamine Cl, 40 μg/ml L-arginine, and 10 mM sodium succinate or 10 mM glucose as indicated. The phosphate-buffered LB medium was prepared with 10 g of tryptone, 5 g of yeast extract, and 10 g of NaCl per liter of 66 mM KH2PO4, 33 mM KH2PO4, and 10 μM MnCl2. The pH of the phosphate-buffered LB and minimal salts media were adjusted to 7.0 with HCl or NaOH. MnCl2 was routinely added to media to ensure full expression of the inducible O2-scavenging manganese-containing superoxide dismutase, maximal activity of the O2-sensitive aconitase, and thus optimal growth on citric acid cycle-dependent substrates (32). To achieve maximal gas exchange, cultures were routinely grown in a geyrorotary water bath shaking at >200 rpm at 37 °C with a medium flask volume ratio of at most 1:5, and growth was monitored by following the turbidity at 550 nm. Bacterial densities were determined by dilution, plating, and colony counting. An absorbance of 1.0 at 550 nm corresponded to 7 × 10⁶ bacteria/ml when bacteria were grown in the minimal medium. Anoxic growth of cultures

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‡ The abbreviations used are: NO, nitric oxide; NOR, nitric-oxide reductase; NOD, nitric-oxide dioxygenase; LB, Luria-Bertani.
was achieved by incubating cultures at 37 °C in static stopper-sealed 50-ml Erlenmeyer flasks filled with 50 ml of medium. To minimize the disturbance of head space gases, culture aliquots were removed from gas-equilibrated culture flasks using a 1-ml tuberculin syringe connected via small tubing. Culture aliquots were immediately transferred to 1.5-ml Eppendorf tubes and were quickly centrifuged at 20,000 × g for 25 s, the supernatant was aspirated, and the cell pellet was frozen on dry ice. Cell pellets were resuspended and lyzed by sonicating in 0.1 ml of buffer containing 50 mM Tris-Cl, pH 7.4, 0.6 mM MnCl₂, and 20 μM barium dl-fluorocitrate, and the lysate was frozen on dry ice. Cell lysates were stored at −70 °C for up to 2 weeks without noticeable loss of aconitase activity. Lysates were thawed quickly in a 25 °C water bath and clarified by centrifugation for 25 s at 20,000 × g immediately prior to the assay of aconitase activity. Cell lysates were prepared for the assay of NOD activity essentially as described for the assay of aconitase except that the lysis buffer contained 50 mM potassium phosphate, pH 7.8, and 0.1 mM EDTA.

Gas Exposures—A three-way gas proportioner (Cole-Parmer Instrument Co.) was used to produce various mixtures of O₂, N₂, and NO in a constant flow rate of 30 ml/min, and gas mixtures were passed through a trap containing NaOH pellets to remove higher oxides of nitrogen.

Assay of Aconitase, Protein, NO₂, and NO₃—Aconitase activity and protein were assayed as described previously (21). For the measurement of NO₂ and NO₃, cultures were clarified by centrifugation and supernatants were incubated at 37 °C for 2 h in a 0.1-ml reaction mixture containing 7 milliliters of nitrate reductase and 40 μM NADPH in 100 mM Tris-Cl, pH 7.5. Samples were assayed for NO₂ with the Griess reagent (24) using sodium nitrite as a standard.

NO₂ and O₂ Consumption Measurements—NO consumption was measured at 37 °C with an NO microelectrode (Diamond General Inc.) fitted in a water-jacketed glass-stoppered 2-ml capacity cell (Gilson Inc.) equipped with a magnetic stirrer. One microliter of NO was delivered to the cell with a Hamilton syringe from a saturated solution (2 mM) prepared in water. Rates of NO consumption by bacteria were measured in minimal medium salts containing chloramphenicol and glucose or succinate as indicated. Rates of NO consumption were determined for initial rates and were corrected for the background rate of NO decomposition. O₂ consumption was measured at 37 °C with a Clark-type O₂ electrode (Yellow Springs Instrument Co.) in a water-jacketed cell in a total volume of 2.0 ml of minimal succinate medium. The O₂ concentration for media saturated with air at normal atmospheric pressure and 37 °C was taken to be 200 μM (25).

Assay for NOD Activity—Cell-free extracts were assayed for NOD activity at 37 °C in a 2-ml reaction mixture containing 50 mM potassium phosphate buffer, pH 7.8, 0.1 mM EDTA, 1 μM FAD, 0.2 mM NAD⁺, 0.5 units/ml glucose-6-phosphate dehydrogenase, 2.5 mM glucose 6-phosphate, and 1 μM NO. Initial rates of NO disappearance from reaction mixtures were followed amperometrically with an NO electrode and were corrected for the background rate of NO decomposition. Where indicated, O₂ was removed by incubating the mixture with 10 mM glucose, 2 units/ml glucose oxidase, and 130 units/ml catalase for 5 min prior to the addition of NO and extract. O₂ removal was followed amperometrically with an O₂ electrode.

Data Analysis—Results are representative of two or more independent experiments.

RESULTS

Growth-inhibitory Effects of NO—We measured the effects of NO on the growth of E. coli in order to gauge the capacity of cells to adapt to NO under various growth conditions. Exposure of log phase cultures to an atmosphere containing 960 ppm NO had little effect on the aerobic growth of E. coli in either the minimal succinate or the rich LB medium (Fig. 1, A and B, compare lines 1 and 2). Similarly, 480 ppm NO exerted no discernible effect on the anaerobic growth of E. coli in a minimal glucose medium supplemented with 10 mM nitrate (data not shown). Interestingly, however, NO exposure caused a small, but highly significant, decrease in the aerobic growth rate and the yield of E. coli in the LB medium at the end of the log phase (Fig. 1B, compare lines 1 and 2). The effect of growth phase on NO inhibition was explored further. Exposure of late stationary phase E. coli to 960 ppm NO strongly inhibited growth (Fig. 1, C and D, compare lines 1 and 2), and the growth-inhibitory effects of NO were more pronounced in the LB medium (Fig. 1D). Growth inhibition was readily reversible, as indicated by the ability of NO-treated cells to recover normal growth following NO removal (Fig. 1, C and D, compare lines 2 and 3). The susceptibility of stationary phase cultures to NO-mediated growth inhibition suggests a requirement for nutritional resources, protein synthesis, or growth competency for NO resistance. Moreover, the ability of cells to grow normally at NO levels that were previously shown to potently inactivate the citric acid cycle enzyme aconitase (21) suggests that NO resistance is due to the presence of adaptive mechanisms.

Adaptive Protection of Aconitase against NO-mediated Inactivation in Aerobic and Anaerobic E. coli—The NO-sensitive aconitase (21, 22) was used to explore the mechanism of adaptation of E. coli to NO. Thus, we supposed that adaptation to NO should correlate with the protection of aconitase from inactivation. Indeed, aconitase activity was more sensitive to NO inactivation in aerobic cultures (Fig. 3, compare lines 1 and 2) and, interestingly, chloramphenicol had no apparent effect on the susceptibility of aconitase to NO-mediated inactivation in the absence of O₂ (Fig. 2, compare lines 3 and 4). The data clearly indicate inducible protective mechanisms for aconitase and demonstrate a role of O₂ in the adaptive mechanism.

We also measured the effects of chloramphenicol on NO-mediated aconitase inactivation in anaerobic E. coli, since aerobic succinate-adapted E. coli are dependent upon O₂ for growth, respiration, and ATP production, which may have affected the ability of cells to adapt. Exposure of anaerobic glucose-adapted E. coli to 240 ppm NO in N₂ caused a greater decline of aconitase activity in the presence of chloramphenicol than in its absence (Fig. 3, compare lines 1 and 2). It is noteworthy that the inducible protection of aconitase was relatively less effective under anaerobic than aerobic conditions. Thus, a ~20% loss of aconitase was observed following 60 min of 240 ppm NO exposure in anaerobic cultures (Fig. 3, line 1), whereas 480 ppm NO did not affect the aconitase activity in
aerobic cultures (Fig. 2, line 1). Thus, anaerobic as well as aerobic *E. coli* protect aconitase from NO-mediated inactivation. Moreover, while O₂ was not absolutely essential for adaptive protection, it did increase the apparent NO⁻ detoxification capacity of cells.

**Dioxygen Dependence and Cyanide Sensitivity of Aconitase Protection**—We suspected that the decreased sensitivity of aconitase to NO⁻ in chloramphenicol-treated *E. coli* in the presence of O₂ might be due, at least in part, to a lower exposure to NO⁻ since the O₂-mediated oxidation of NO⁻ to form NO₂⁻ and N₂O₃ would be expected to decrease the steady-state NO⁻ levels and increase NO₂⁻ and NO₃⁻ formation (26). Alternatively, the O₂-dependent protection may be due to an O₂⁻ metabolic pathway, which was directly or indirectly dependent upon O₂. For example, the protective effect of O₂ may be linked to the respiration of *E. coli*. Thus, the homologous mitochondrial terminal oxidase, cytochrome c oxidase, is thought to metabolize NO⁻ (27, 28).

To evaluate the contribution of O₂-mediated NO⁻ oxidation to the protection, we measured the concentration of O₂ required for aconitase protection in *E. coli* exposed to NO⁻ and compared it with that required for NO⁻ oxidation as detected by the formation of NO₂⁻ and NO₃⁻ in the culture medium. Surprisingly, aconitase was near maximally protected from the inactivating effect of 240 ppm NO⁻ by the lowest O₂ concentration tested (−17 µM O₂) (Fig. 4, closed circles). However, at this O₂ level, NO₂⁻ and NO₃⁻ formation was only a fraction of that achievable via NO⁻ autoxidation (Fig. 4, open circles). Thus, the O₂-mediated decomposition of NO⁻ does not appear to account for the protective effects of O₂.

The role of respiration and the terminal respiratory oxidases in the O₂-dependent protection of aconitase was assessed by measuring the effects of the inhibitor cyanide. The addition of cyanide (25 µM) to aerobic cultures completely blocked the protection of aconitase by O₂ (Fig. 5A, compare lines 1 and 2), while cyanide was without effect in the absence of O₂ (compare lines 3 and 4). Importantly, cyanide was effective at decreasing aconitase protection at much lower concentrations than those required for the inhibition of respiration. Thus, while −5 µM NaCN was saturating in its effect on the O₂-dependent protection of aconitase (Fig. 5B), half-maximal inhibition of respiration required >50 µM NaCN (Fig. 5B, inset). The results clearly demonstrate a cyanide-sensitive mechanism of aconitase protection; however, the difference in cyanide sensitivities indicates a mechanism of inhibition independent of cell respiration and the terminal respiratory oxidases.

We also investigated the effects of O₂ and cyanide on the NO⁻ sensitivity of aconitase in naive aerobic cultures and compared these effects with those in NO⁻-treated cultures to determine whether the adaptive protection displayed a similar O₂ dependence and cyanide sensitivity. Indeed, the induced protection of aconitase was O₂⁻-dependent; however, this protection appeared less sensitive to cyanide than the constitutive activity (Fig. 6). We were unable to test the effects of higher cyanide concentrations, because aconitase activity was sensitive to cyanide at >25 µM. This may be understandable in light of the ability of cyanide to stimulate O₂⁻ production by respiring *E. coli* membranes (29) and the ability of O₂⁻ to inactivate aconitase (30).
Effects of cyanide on the O2-dependent protection of aconitase and respiration (Resp). The effects of various concentrations of NO on the aconitase activity in DH5α cells were measured in the presence or absence of cyanide (A). Controls were incubated with 25 μM NaCN and various concentrations of NO under an atmosphere containing either 21% O2 in N2 (line 1) or N2 (line 3). The effects of various concentrations of NO on aconitase activity in the absence of NaCN in cultures incubated under 21% O2 in N2 (line 2) or N2 (line 4) from Fig. 2 are presented for comparison. The effect of NaCN concentration on the sensitivity of aconitase to inactivation during a 60-min exposure to 240 ppm NO (panel B) and on the respiration rate of DH5α cultures (inset) was measured and were measured as described under “Materials and Methods.” 100% aconitase activity and respiration corresponded to 234 ± 27 milliunits/mg protein and 10.4 ± 0.1 nmol of O2/min/108 bacteria, respectively. Culture growth was as described in the legend to Fig. 2.

FIG. 5.

O2 dependence and cyanide sensitivity of the constitutive and the induced protection. Growing cultures of DH5α were preincubated for 20 min with vigorous shaking at 37 °C under an atmosphere containing 21% O2 or no O2 and in the presence or absence of 25 μM NaCN as indicated. Cultures were harvested, and extracts were prepared and assayed for aconitase activity and protein as described under “Materials and Methods.” Culture growth was as described in the legend to Fig. 2. The percentage of aconitase activity was calculated from the activity in extracts prepared from cells immediately following the incubation with chloramphenicol for each trial. For all trials, 100% aconitase activity was equal to 372 ± 30 and 364 ± 53 milliunits/mg protein in control and NO-treated cultures, respectively. Results represent the mean ± S.D. of three independent trials.

FIG. 6.

Inducible NO consumption by naive and adapted E. coli. Cultures of DH5α (A590 = 0.4) were either maintained under air (A) or exposed to an atmosphere containing 960 ppm NO in 21% O2 balanced with N2 (B). Following 120 min of incubation, chloramphenicol was added to the cultures at 200 μg/ml. Cells were counted, harvested, washed, and assayed for NO consumption in minimal succinate medium with no additions (Control), with 25 μM NaCN (+low CN) or 250 μM NaCN (+high CN), or in the absence of O2 (+O2) as described under “Materials and Methods.” Cultures were grown aerobically in minimal succinate medium as described in the legend to Fig. 2. Results are the mean ± S.D. of three measurements and are representative of two or more trials.

Induction of NOD by Nitric Oxide—Prompted by the aforementioned results, we identified an O2-dependent cyanide-sensitive NO-converting activity in extracts of E. coli that was NOD/flavohemoglobin and has been described elsewhere (20). Further, we supposed that this NOD activity might account for the NO-dependent, cyanide-sensitive, and O2-dependent protection of aconitase and NO consumption by cells. As shown by the data in Table II, NOD activity in DH5α cells was induced ~36-fold following an exposure to 960 ppm NO. Extract NOD activity was dependent upon O2, was potently inhibited by cyanide, and displayed cofactor requirements consistent with the flavohemoglobin/NOD activity (20). NOD activity was also measured in anaerobic cultures exposed to NO or O2 as described in the legend to Table I. NOD activity was increased 33-fold (588 milliunits/mg versus 18 milliunits/mg) following exposure to 960 ppm NO and 40-fold (644 milliunits/mg versus 16 milliunits/mg) during anaerobic growth with NO3. Thus, NO-induced NOD activity levels correlate with the NO-induced aconitase protection and NO consumption in both aerobic and anaerobic cells.

Inducible NO consumption in aerobic and anaerobic cultures of E. coli—To determine whether aconitase protection in E. coli was associated with an increased rate of NO metabolism, we measured the rate of NO consumption by aerobic and anaerobic cells. We also measured the effects of O2 and cyanide on these rates. As shown by the data in Fig. 7A, aerobic E. coli consumed NO. Moreover, NO consumption was sensitive to cyanide and dependent upon O2. Further, NO consumption was induced approximately 13-fold in aerobic cultures exposed to 480 ppm NO for 60 min (Fig. 7B). The induced rate of NO consumption was also sensitive to cyanide and dependent upon O2. Control anaerobic cultures did not express an NO consumption activity, whereas anaerobic cells exposed to 960 ppm NO for 60 min produced an NO-consuming activity that did not require O2 and that was insensitive to cyanide (Table I, Experiment A). Interestingly, however, the presence of O2 in the assay revealed a cryptic NO consumption activity that was cyanide-sensitive and was also induced ~12-fold by the NO exposure. NO3 (10 μM) also induced an NO-consuming activity in anaerobic cultures with similar properties as that induced by gaseous NO (Table I, Experiment B).

Discussion

Our results demonstrate aerobic and anaerobic pathways for NO detoxification that appear to differ from the NORs described in denitrifiers (11–13, 14, 15). Foremost among these differences was the requirement for O2. Moreover, the similar NO inducibility, O2 dependence, and cyanide sensitivity between the NOD activity in extracts (Table II) and the inducible and constitutive aconitase protection and NO consumption by intact E. coli strongly supports a role for the recently described NOD (20) in the observed aerobic NO removal and detoxification pathway. We can estimate that the NOD activity measured in cell extracts is within range of the NO consumption activity of intact aerobic E. coli. Thus, assuming roughly 10−13 g of soluble protein per E. coli, we can calculate the NOD...
Anaerobic cultures of DH5α (Experiment A) were grown overnight in stoppered Erlenmeyer flasks filled to capacity with minimal glucose medium supplemented with amino acids. Cells were washed and resuspended at their original density in fresh medium and were incubated under an atmosphere of N₂ for 30 min prior to a 60-min exposure to 960 ppm NO in N₂ or to N₂ alone. For Experiment B, static overnight anaerobic cultures of DH5α were grown under similar conditions except that amino acids were omitted and 10 mM NaN₃ was added as indicated. Cells were harvested and washed, and NO consumption was measured in minimal glucose medium as described under "Materials and Methods." Growth was initiated with 2% inocula from aerobic log phase cultures grown in phosphate-buffered LB medium. Results represent the average ± S.D. of three measurements.

### Table I

| Treatment | Final growth | Without O₂ | With O₂ |
|-----------|--------------|------------|---------|
|           | A₃₅₀ | Basal | + NaCN | Basal | + NaCN |
| Expt. A   |       |       |        |       |        |        |
| N₂ control| 0.72 | 0.0±0.0 | 0.0±0.0 | 2.0±0.1 | 0.0±0.0 |
| + 960 ppm NO| 0.63 | 2.2±0.3 | 2.4±0.3 | 24.4±1.5 | 0.0±0.0 |
| Expt. B   |       |       |        |       |        |        |
| Anoxic control | 0.56 | 0.0±0.0 | 0.0±0.0 | 1.1±0.1 | 0.0±0.0 |
| + 10 mM NaN₃| 0.65 | 0.5±0.0 | 0.6±0.1 | 15.9±0.5 | 0.0±0.0 |

*Expt., experiment.

### Table II

| Sample | Assay | Specific activity | Activity |
|--------|-------|------------------|----------|
|        |       | nmol/min/mg | %       |
| Air control | Complete | 5.8 | 100 |
| NO-exposed | Complete | 209.5 | 100 |
| - FAD | 2.3 | <2 |
| - NADP⁺ | 2.4 | <2 |
| - G-6-P⁺ | 1.1 | <1 |
| - G-6-PD | 0.0 | 0 |
| - O₂ | 7.1 | <4 |
| + NaCN | 27.3 | <15 |

*Glucose 6-phosphate.

Glucose-6-phosphate dehydrogenase.

Activity in extracts from control and NO-exposed E. coli to equal 0.1 and 2.1 nmol of NO/min/10⁸ cells, respectively. These values are comparable with the respective values of 0.2 and 3.2 measured for intact aerobic DH5α grown under similar conditions (Fig. 7). Furthermore, NO consumption by cells does not appear to involve the terminal respiratory oxidases as proposed for mitochondria (27, 28), since cyanide was without effect on the respiration of E. coli at levels that inhibited NO detoxification (Figs. 5 and 7). Moreover, E. coli strains deficient in either of the terminal respiratory oxidases (23) expressed normal levels of the constitutive and inducible aerobic NO consumption activity (data not shown), whereas NOD flavohemoglobin-deficient E. coli express essentially no constitutive or inducible aerobic NO consumption activity (20).

The identity of the inducible anaerobic pathway for NO metabolism and detoxification is less clear, since there is no evidence for a typical NOR in E. coli. The demonstration of NOD induction and a cryptic O₂-stimulated and cyanide-sensitive NO consumption activity in anaerobically grown E. coli exposed to NO or NO₂ suggests an additional role of NOD in anaerobic NO detoxification. However, both the failure of cyanide to inhibit the anaerobic activity in vivo (Table I) and the failure of the induced NOD to metabolize NO in the absence of O₂ (Table II) suggests a limited role of NOD/flavohemoglobin in anaerobic NO metabolism and detoxification. Nevertheless, NOD may have other anaerobic protective functions or may prepare the cell for more efficient NO detoxification upon exposure to O₂. It will now be important to assess the overall contributions of NOD and other potential NO-metabolizing systems, such as the NO-reducing multiheme nitrite reductase (19), to NO detoxification in E. coli using deletion strains and overexpressors under various growth conditions.

Finally, it is hoped that greater knowledge of the function and regulation of NO detoxification systems, including the E. coli NOD, may reveal the true antibiotic potential of NO toward a variety of organisms. For example, the greater ability of NO to inhibit the growth of stationary phase E. coli (Fig. 1) suggests that latent or dormant pathogens will be more susceptible to the antibiotic action of NO than healthy growing microorganisms simply because they are unable to induce their NO detoxification systems. Indeed, this may account for the activation of latent Mycobacterium tuberculosis and Leishmania spp. infections in nitric-oxide synthase-deficient mice (1, 2). The ability of O₂ to increase the NO detoxification capacity of cells through the action of an NOD-like activity, like that of the (flavo)hemoglobin (20), may also explain the sensitivity of intraerythrocytic malaria parasites to NO at low O₂ tensions (31). Furthermore, greater knowledge of the NODs, NORs, and other NO detoxification systems may facilitate the design of new drugs that target these systems and allow the expression of the full potential of NO as a natural broad spectrum antibiotic (3).

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