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REDUCTION OF NITRITE TO NITRIC OXIDE BY ENTERIC BACTERIA

Xiao-bing Ji and Thomas C. Hollocher

Department of Biochemistry, Brandeis University, Waltham, MA 02254

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SUMMARY: Seven bacteria representing seven genera of enteric bacteria, in addition to *Escherichia coli*, were shown to reduce nitrite to NO under anaerobic conditions when the cells were grown as nitrate respirers. NO production was inhibited by nitrate and azide and was self limiting, just as was found to be the case previously with *E. coli* and its nitrate reductase. Maximum initial rates of NO production were observed at pH 5.5-6.

Ji and Hollocher (1, 2) found that *Escherichia coli* grown as a nitrate respirer can reduce nitrite to NO and that the respiratory (type A) nitrate reductase is also capable of catalyzing this reaction. For intact cells, the membrane fraction and solubilized nitrate reductase, NO production was inhibited by millimolar nitrate and micromolar azide and was self limiting with respect to the final concentration of NO. It was of interest in view of these results to survey other enteric bacteria for an ability to produce NO from nitrite, and seven additional bacteria of this group were examined (Table 1). Data for *E. coli* are shown for ease of comparison.

METHODS. Bacteria were grown anaerobically on nitrate in media indicated in Table 1 and were harvested and washed at 4°C just before or upon having reached maximum cell density. The medium used for a particular bacterium was selected on the basis of its ability to support growth of the organism as a nitrate respirer. Incubation times were 10-18 h, depending on the bacterium and size of the inoculum. When media contained 10 mM nitrate, $A_{660\ nm}$ values at maximum cell density were 0.25-0.45, except for *A. hydrophila* which read 0.15. In the 14 h generally required for cultures to reach maximum density, nitrate was almost entirely (90% or more) reduced to nitrite, except for *A. hydrophila* for which the conversion was only 50%. The lower cell yields for *A. hydrophila* can therefore be attributed, at least in part, to incomplete reduction of nitrate. In all cases, anaerobic growth on the medium used was very poor over 14 h when nitrate was omitted.

For the assay of NO production (1), the NO-electrode was maintained under argon at 30°C and the reaction was initiated by the injection of anaerobic 5 mM $NaNO_2$ into the bacterial suspension. NO uptake was measured similarly following injection of 0.2 mM NO. The identification N_2O as the product of NO reduction was by gas chromatography (1).

RESULTS AND DISCUSSION. All seven bacteria had an ability to reduce nitrite to NO when grown as nitrate respirers but little or no such ability when grown as aerobes with vigorous aeration. As with *E. coli* (1) and its

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TABLE 1. Production of NO by enteric bacteria

Organism	Growth medium[c]/ temperature (°C)	Assay medium[d]/ pH	Initial rate of NO production (nmol x min^{-1} per mg of protein)	Maximum concentr. of NO (μM)
Aeromonas hydrophila ATCC 7965	1/30	PBS, formate/7.3	2.5	180
Enterobacter aerogenes ATCC 13048	1/30	1 *minus* nitrate/7.3	14.6	400
Escherichia coli K12, RF 1005[a]	2/37	PBS, formate/7.3	10.9	250
Klebsiella pneumonia BS[b]	2/37	PBS, formate/7.3	3.4	190
Proteus mirabilis ATCC 7002	1/37	1 *minus* nitrate/7.3	17.6	315
Salmonella typhimurium ATCC 23564	1/37	1 *minus* nitrate/7.3	25.2	320
Serratia grimesii ATCC 14460	1/30	PBS, formate/6.0	5.4	300
Shigella sonnei ATCC 9290	1/37	1 *minus* nitrate/7.3	12.6	300

[a]Provided by D. Ralt, Massachusetts Institute of Technology.

[b]Provided by C. Fulton, Brandeis University; also available as ATCC 29679.

[c]Media: 1, 3g of yeast extract and 5 g of tryptone per liter with 10 mM $NaNO_3$; 2, 5 g of yeast extract and 10 g of tryptone per liter with 10 mM $NaNO_3$.

[d]PBS, phosphate-buffered saline (1); 10 mM formate.

nitrate reductase (2), NO production was completely prevented by 5 mM nitrate or 5 μM azide and self-limiting in the sense that NO production always stopped prior to the quantitative conversion of nitrite to NO. The more vigorous of the NO producers, such as *Salmonella typhimurium* and *Proteus mirabilis* (in addition to *E. coli* (1)) were also observed to reduce NO to N_2O at measurable rates which were 5-10% of the rates of NO production. *E. coli* and *S. typhimurium* exhibited a maximum for the rate of NO production at pH 5.5 and about 6, respectively. Although the optimum pH was not determined for the other bacteria, it was shown that the rates of NO production were from 1.3 to 2.5 times greater at pH 6 than at pH 7.3. Such results with *Serratia grimesii* and *Aeromonas hydrophila* were not very precise due to the low rates encountered at pH 7.3. In all cases NO production was not detected in the absence of nitrite. Rates of NO production were similar for a given organism irrespective of whether cells were

assayed in fresh growth medium (minus nitrate) or phosphate-buffered saline supplemented with formate or succinate.

The seven bacteria newly studied for NO production represent what may be seven genera among the enteric group. Each showed the pattern of NO production that was first demonstrated for *E. coli* (1), although it is clear that the specific rates can differ substantially from one organism to another. It will be interesting to determine if the different rates correlate with different nitrate reductase activities. Although the bacteria studied do not exhaust the enteric group, we believe that they are sufficiently representative to suggest that many bacteria of the group will prove to be NO producers, at least when grown as nitrate respirers. This possibility may have implications in toxicology.

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