**Tellurite reductase activity of nitrate reductase is responsible for the basal resistance of *Escherichia coli* to tellurite**

Cécile Avazeri, Raymond J. Turner, Jeanine Pommier, Joel H. Weiner, Gérard Giordano and André Verméglio

Keywords: nitrate, tellurite and selenate reductase, tellurite resistance

**INTRODUCTION**

Tellurium and selenium oxyanions are found as trace components in natural environments as well as in higher concentrations within polluted soils and water. Tellurium compounds can be found in high concentrations near sites of waste discharge, while selenium is present, in the soluble selenate form, in alkaline soils and water. Some of the tellurium and selenium oxyanions are toxic to micro-organisms. The toxicity of potassium tellurite to micro-organisms, particularly Gram-negative bacteria, is well established, yet the mechanism is not fully understood (Summers & Jacoby, 1977; Turner et al., 1995). Some Gram-positive organisms (*Corynebacterium diphtheriae*, *Streptococcus faecalis* and some *Staphylococcus aureus* strains) are naturally resistant to potassium tellurite, but little is known about the mechanisms responsible for this resistance (Summers & Silver, 1978). Many bacteria can acquire high resistance when transformed with multi-drug resistance plasmids, many of which encode tellurite resistance (Summers & Jacoby, 1977; Walter et al., 1991; Turner et al., 1992; Hill et al., 1993; Lloyd Jones et al., 1994; Taylor et al., 1994). Resistance to tellurite is conferred by highly specific genetic determinants (Walter & Taylor, 1992).

Historically, tellurite has been used as an indicator for the localization of reducing activity in bacteria (Barrett & Palade, 1957; Van Iterson & Leene, 1964). However, the enzymes responsible for this tellurite to tellurium reduction were unknown. One possible mechanism to detoxify tellurite (TeO$_4^{2-}$) is to reduce it to metallic tellurium (Te$^0$). Indeed, bacteria grown on media containing potassium tellurite form black colonies due to intracellular deposition of tellurium (Turkey et al., 1962; Taylor et al., 1988; Lloyd Jones et al., 1994; Moore & Kaplan, 1992). Selenium, in the form of either SeO$_4^{2-}$ or SeO$_2^{2-}$, is also reduced to Se$^0$ and deposited along the cell membranes (Gerrard et al., 1974; Macy, 1994). Since tellurium and selenium are within the same...
group in the periodic table as sulfur, they possess some similarities in their chemistry. The possibility that micro-organisms involved in the sulfur cycle can also reduce selenate was investigated by Zehr & Oremland (1987). They concluded that only trace amounts of selenate (picomolar–nanomolar) were reduced via the sulfate reduction pathway. More recently, field studies by Oremland (1994) support the idea that selenate reduction is carried out by one or more of the enzymes involved in dissimilatory nitrate reduction (denitrification).

Complete denitrification (i.e. reduction of nitrate to nitrogen) requires four different enzymes: nitrate, nitrite, nitric oxide and nitrous oxide reductases. The first step in the reduction of nitrate in Escherichia coli is performed by two dissimilatory membrane-bound nitrate reductases (NR) denoted A and Z. The former, NR A, is encoded by the narGHI operon, induced under anaerobic conditions when nitrate is present in the culture medium, repressed by oxygen, and controlled by the fnr global anaerobic regulator (Blasco et al., 1989; Stewart, 1982; Kapraleck et al., 1982; Chippaux et al., 1981). NR A, which contains molybdenum, iron–sulfur centres (Vincent et al., 1978) and cytochrome b, possesses three polypeptide subunits: α (NarG), β (NarH) and γ (NarL) with approximate M₉, 155000, 60000 and 25000, respectively. NarJ is found in the structural operon for NR, but it is not a component of the active enzyme and its function is unclear (Sodergren & DeMoss, 1988). NR Z is encoded by the narZYWV operon and is constitutively expressed at low levels, even in aerobic conditions. The two operons coding for NR A and NR Z are similar and located at 32-5 min and 27 min, respectively, on the E. coli chromosome (Blasco et al., 1990). It is thought that narZYWV and narGHIJ descended from a common ancestor by gene duplication, and so the two enzymes have great structural resemblance (Bonnefoy et al., 1987; Iobbi et al., 1987; Iobbi-Nivol et al., 1990).

In this study, we show that NRs from E. coli also have tellurite and selenate reductase activities and are responsible for the natural resistance of E. coli to tellurite. We also identify a soluble enzyme in anaerobically grown cells which reduces tellurite.

**METHODS**

**Bacterial strains, plasmids and media.** The strains of E. coli used are listed in Table 1. They were grown under anaerobic or aerobic conditions at 37°C in Luria-Bertani (LB) broth medium (Miller, 1992). Potassium tellurite and sodium selenite were added to final concentrations of 10 µM and 100 µM, respectively. For the induction of NR A, potassium nitrate was added to a final concentration of 1 g L⁻¹. Plasmids were maintained by adding 50 µg ampicillin ml⁻¹. For the induction of NR A, potassium tellurite was added to a final concentration of 1 g 1⁻¹. For the induction of NR Z, potassium selenite was added to final concentrations of 10 µM and 100 µM, respectively. For the induction of NR Z, potassium tellurite was added to a final concentration of 1 g 1⁻¹. For the induction of NR A, potassium selenite was added to final concentrations of 10 µM and 100 µM, respectively. For the induction of NR Z, potassium tellurite was added to a final concentration of 1 g 1⁻¹. For the induction of NR Z, potassium selenite was added to final concentrations of 10 µM and 100 µM, respectively.

**Minimal inhibitory concentration (MIC).** The MIC was defined as the lowest concentration of inhibitor preventing growth of E. coli strains at 37°C as previously described (Turner et al., 1995). Once autoclaved, LB agar was cooled to ~50°C and K₂TeO₃ was added to each flask to an appropriate concentration from a stock solution of 10 mg ml⁻¹. When used, nitrate was added to 1 g l⁻¹ and IPTG to 0.1-0.2 mM. Selenium, selenite and tellurite MIC assays were performed similarly using stock solutions of 100 mg Na₂SeO₃ ml⁻¹, 100 mg Na₂S₂O₇ ml⁻¹ and 0.1 mg K₂TeO₃ ml⁻¹. All stock solutions were filter-sterilized.

**Preparation of cell extracts.** All steps were done at 4°C. Cells were harvested, during the exponential phase of growth, by centrifugation at 4000 g for 10 min, washed with 40 mM Tris/HCl (pH 7.6) and then resuspended in the same buffer. Crude extracts were prepared by twice passing the cell suspension through a French pressure cell at 7 MPa. Proteases were inhibited by the addition of 1 mM AEBSF ([4-(2-aminoethyl)-benzenesulfonyl fluoride]). The crude extract was then centrifuged at 18000 g for 25 min in order to sediment unbroken cells. The supernatant fraction obtained was ultracentrifuged at 200000 g for 90 min to separate membrane and soluble fractions. The pellet containing membranes was washed in 40 mM Tris/HCl (pH 7.6) and then ultracentrifuged again.

**Polyacrylamide gels.** Electrophoresis under non-denaturing conditions was carried out as described by Iobbi-Nivol et al. (1990). A separating gel with 7.5% acrylamide containing 0.1% Triton X-100, and a stacking gel (3% acrylamide containing 0.1% Triton X-100) were prepared from a stock solution (30% acrylamide, 0.8% bis-acrylamide). The buffer system was 25 mM Tris (pH 8.5), 192 mM glycine, 0.02% Triton X-100. Gels were stained with Coomassie brilliant blue R250 for protein detection.

**Enzyme assays.** Nitrate, tellurite and selenate reductase activities were assayed spectrophotometrically with reduced benzyl viologen as the electron donor (Jones & Garland, 1977). The reaction mixture (3.5 ml) contained 40 mM Tris/HCl buffer (pH 7.6) degassed and sparged with high-purity argon, and 0.6 mM benzyl viologen reduced with enough Na₂S₂O₇ to give an A₅₅₀ of ~1.0. The different substrates introduced were KNO₃ (40 mM), K₂TeO₃ (160 mM) or Na₂SeO₃ (160 mM).

The enzymic activities were also visualized on non-denaturing polyacrylamide gels (Lund & DeMoss, 1976; Iobbi et al., 1987). The gels were incubated in 40 mM Tris/HCl buffer (pH 7.6) containing 0.6 mM benzyl viologen reduced with enough Na₂S₂O₇. The enzyme activity was visualized by the appearance of a clear band on the gel, impregnated with reduced methyl viologen (which results in a dark-blue-stained gel), after introduction of substrates KNO₃ (40 mM), K₂TeO₃ (160 mM) or Na₂SeO₃ (160 mM).

**Rocket immunoelectrophoresis.** This was performed as described by Graham et al. (1980). Agarose plates (4 × 4 cm; 6 ml) containing 1% agarose in which 100 µl antiserum was incorporated were used. The protein concentration loaded in the agarose gel was 4 mg ml⁻¹ for strains MC4100, LCB 2048 and TP100, and 1 mg ml⁻¹ for LCB 333/pVAT70. The protein migrated overnight at 2 mA in a solution of 20 mM sodium barbital (pH 8.6) and Triton X-100 (1%).

**Growth on oxygenians.** The wild-type strain MC4100 was grown in a minimal salts medium with 1% (v/v) glycerol as the non-fermentable carbon source in 9 ml screw-top culture tubes. The oxygenians were added at subinhibitory concentrations as follows: K₂TeO₃, 1 and 4 µg ml⁻¹; K₂TeO₃, 2 µg ml⁻¹; Na₂SeO₃, 100 µg ml⁻¹; 1 and 10 mg ml⁻¹; and Na₂SeO₃, 100 µg ml⁻¹; 1 and 10 mg ml⁻¹. HB101/pVAT70 was grown in the presence of 8 and 16 µg K₂TeO₃ ml⁻¹. MC4100 and HB101 were also transformed with tellurite-resistance determinants tehAB (Taylor et al., 1994) and klaAkalatBtelB (Turner et al., 1995).
 Tellurite activity of NR from *E. coli*

### Table 1. Bacterial strains and plasmids

<table>
<thead>
<tr>
<th>Plasmid/strain</th>
<th>Description/genotype</th>
<th>Phenotype</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>pVA70</td>
<td>narGHIJ regulated by p-nar, Amp’</td>
<td>NR A overexpressed</td>
<td>Guigliarelli <em>et al.</em> (1992)</td>
</tr>
<tr>
<td>pVA700</td>
<td>narGHIJ regulated by p-tac, Amp’</td>
<td>NR A expressed by addition of IPTG</td>
<td>Guigliarelli <em>et al.</em> (1996)</td>
</tr>
<tr>
<td>pLCB14</td>
<td>narZYWV constitutively expressed, Amp’</td>
<td>NR Z overexpressed</td>
<td>Bonnefoy <em>et al.</em> (1987)</td>
</tr>
<tr>
<td>pTWT100</td>
<td>tehAtehB constitutively expressed, Amp’</td>
<td>Tellurite resistance to 128 μg ml⁻¹</td>
<td>Taylor <em>et al.</em> (1994)</td>
</tr>
<tr>
<td>pDT1558</td>
<td>klaaklaBtelB constitutively expressed, Amp’</td>
<td>Tellurite resistance to 256 μg ml⁻¹</td>
<td>Walter <em>et al.</em> (1991)</td>
</tr>
<tr>
<td>MC4100</td>
<td>araD139 (lacIPOZYA-argF) rpsL thi</td>
<td>Parental strain</td>
<td>Casadaban (1976)</td>
</tr>
<tr>
<td>HB101</td>
<td>F’hsdS20(r_m) leu supE44 ara14 galK2 lacY1 proA2 rpsL20</td>
<td></td>
<td>Boyer &amp; Roulland-Dussoix (1969)</td>
</tr>
<tr>
<td>LCB2048</td>
<td>LCB333 ΔnarZYWV</td>
<td>NR A⁻ NR Z⁻</td>
<td>Blasco <em>et al.</em> (1992)</td>
</tr>
<tr>
<td>LCB333</td>
<td>thi-1 leu-6 thr-1 tonA21 lacY1 supE44 Δnar-25</td>
<td>NR A⁻</td>
<td>Blasco <em>et al.</em> (1992)</td>
</tr>
<tr>
<td>LCB79</td>
<td>MC4100 897(nar-lac)</td>
<td>NR A⁻</td>
<td>Pascal <em>et al.</em> (1982)</td>
</tr>
<tr>
<td>TP100</td>
<td>MC4100 ΔmobAB</td>
<td>NR present but inactive</td>
<td>Palmer <em>et al.</em> (1996)</td>
</tr>
</tbody>
</table>

1994) using plasmids pTWT100 and pDT1558, respectively. In the cases of growth with tellurite-resistance determinants, K₂TeO₃ was added at concentrations of 50 and 100 μg ml⁻¹. Control cultures were grown with 1 mg ml⁻¹ and 100 μg ml⁻¹ KNO₃. The degree of growth was assessed after 48 h as OD₆₀₀.

**RESULTS**

**Nitrate, tellurite and selenate reductase activities in *E. coli* membrane extracts**

The tellurite (TeO₃⁡⁻⁻), tellurate (TeO₂⁻⁻), selenite (SeO₃⁻⁻) and selenate (SeO₄⁡⁻⁻) reductase activities were measured spectrophotometrically at 595 nm, by following the oxidation of reduced benzyl viologen (BV⁻) acting as electron donor. No BV⁺ oxidation activity was observed with tellurate or selenate as electron acceptors. The tellurite and selenate reductase activities obtained for different mutants and growth conditions are compared in Table 2. For all strains studied, the tellurite or selenate reductase activities were roughly proportional to the NR activity. For example, these activities were increased in the mutants overexpressing NR A (LCB333/pVA70, LCB79/pVA700) compared to the parental strain, MC4100. Additionally, no BV⁺ oxidation activity was observed with nitrate, tellurite or selenate as electron acceptors for membranes of strain.

### Table 2. Nitrate, tellurite and selenate reductase specific activities in membrane fractions from wild-type and nar mutants

<table>
<thead>
<tr>
<th>Strain</th>
<th>Culture conditions</th>
<th>Nitrate (μmol oxyanion reduced (mg protein)⁻¹ min⁻¹)</th>
<th>Tellurite (μmol oxyanion reduced (mg protein)⁻¹ min⁻¹)</th>
<th>Selenate (μmol oxyanion reduced (mg protein)⁻¹ min⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>MC4100</td>
<td>-O₂ (control)</td>
<td>0.27 0.10 0.17</td>
<td></td>
<td></td>
</tr>
<tr>
<td>LCB2048</td>
<td>-O₂ + KNO₃</td>
<td>0.68 0.26 0.25</td>
<td></td>
<td></td>
</tr>
<tr>
<td>LCB333/pVA70</td>
<td>-O₂ (control)</td>
<td>&lt;0.002 &lt;0.002 &lt;0.002</td>
<td></td>
<td></td>
</tr>
<tr>
<td>LCB79/pLCB14</td>
<td>-O₂ (control)</td>
<td>1.45 0.48 0.30</td>
<td></td>
<td></td>
</tr>
<tr>
<td>LCB79/pVA700</td>
<td>-O₂ (control)</td>
<td>2.97 0.98 0.58</td>
<td></td>
<td></td>
</tr>
<tr>
<td>TP100</td>
<td>-O₂ (control)</td>
<td>2.08 0.76 0.45</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>-O₂ + KNO₃</td>
<td>2.05 0.72 0.42</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>-O₄ (control)</td>
<td>&lt;0.002 &lt;0.002 &lt;0.002</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>-O₄ + KNO₃</td>
<td>&lt;0.002 &lt;0.002 &lt;0.002</td>
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</tr>
</tbody>
</table>

Cultures were grown under anaerobic (−O₂) or aerobic (+O₂) conditions in LB medium as described in Methods. Strain LCB79/pVA700 was grown in the presence of 0.2 mM IPTG. The specific activities are expressed as μmol oxyanion reduced (mg protein)⁻¹ min⁻¹. Variation of specific activities was within ±10% of the mean (n = 5).
LCB2048 (deleted in NR A and NR Z) or strain TP100 (altered in the biosynthesis of the molybdenum cofactor) whatever the culture conditions. The addition of nitrate during anaerobic growth induces an increase of nitrate, tellurite and selenate reductase activities in the parental strain (MC4100) and the mutant overexpressing NR A (LCB333/pVA70). NR Z (LCB79/pLCB14) is constitutively expressed, but does not accumulate to very high levels (Iobbi et al., 1987). However, there is still significant BV oxidation activity with selenate and tellurite compared to the deletion mutant (LCB2048). In plasmid pVA700, NR A synthesis is under the control of the tac promoter and is induced by addition of IPTG. Similar activities were observed under aerobic or anaerobic conditions (plus or minus nitrate). We conclude from this series of experiments that NR catalyses the tellurite and selenate reductase activities.

Nitrate, tellurite and selenate reductase activities from membrane extracts in non-denaturing gels

We monitored tellurite and selenate reductase activities using non-denaturing gels of membrane fractions of the parental strain, MC4100, grown under anaerobic conditions. The reductase activities in this case were assessed with reduced methyl viologen (MV\(^2\)) as electron donor. A single clear band, due to the oxidation of the MV\(^2\), appeared immediately after addition of tellurite or selenate, as electron acceptor, to non-denaturing gels of the membrane fraction. This is shown in Fig. 1(b, c) for membranes of E. coli grown under different conditions. Fig. 1 also shows that the enzymes responsible for the tellurite and selenate reductase activities migrated at an R\(_p\) of 0.2, identical to the R\(_p\) observed for NR activity, NR A. When the cells were grown under anaerobic conditions and in the presence of nitrate, the synthesis of NR was enhanced and, as expected, the NR activity observed on the gel was more intense (Fig. 1a, lane 2). Under these growth conditions, both tellurite (Fig. 1b, lane 2) and selenate (Fig. 1c, lane 2) reductase activities were also enhanced. Growth of E. coli cells in the absence of nitrate but in the presence of...
tellurite (10 μM) or selenate (100 μM) did not induce the appearance of novel bands on non-denaturing gels of either soluble (data not shown) and membrane fractions (Fig. 1). Addition of tellurite (10 μM) or selenate (100 μM) induced a slight decrease (20 to 30%) in the synthesis of NR (Fig. 1, compare lane 1 to lanes 3 and 4). This effect has been confirmed by measurements of NR concentration by immunoprecipitation and of β-galactosidase activities with a narG::lacZ transcriptional fusion (unpublished results). These experiments using MV²⁺ as the electron donor indicate that NR has both tellurite and selenate reductase activities. The results also indicate that tellurite and selenate do not induce the expression of other reductases which can use MV²⁺ to reduce oxyanions.

We analysed the nitrate, tellurite and selenate reductase activities for membrane fractions of different mutants grown anaerobically (Fig. 2). Strain LCB2048 is deleted in NR A and NR Z, while strain TP100 is altered in the synthesis of molybdenum cofactor and synthesizes NR A and NR Z in an inactive form. For both mutants, no decoloration of MV²⁺ appeared upon addition of nitrate, tellurite or selenate (Fig. 2, lanes 4 and 5). For strain LCB333/pVA70, overexpressing NR A, the activities of nitrate, tellurite or selenate reductases were very intense, as shown by the strong decoloration band observed on the gel upon addition of the different substrates (KNO₃, K₂TeO₃ and Na₂SeO₄) (Fig. 2, lane 2). When NR Z is overproduced, using pLCB14, it has been shown that NR Z accumulates to levels which give a clear band with a different Rₚ value from NR A (Iobbi et al., 1987). When the gel containing LCB333/pLCB14 was exposed to tellurite and selenate, a unique band was observed with the same Rₚ value as NR Z (Fig. 2, lane 3).

**Nitrate reductase is responsible for the tellurite and selenate reductase activities**

Further evidence for the involvement of NRs A and Z in tellurite and selenate reduction was obtained by rocket immunoelectrophoresis. Since the antigenic sites of the NRs are not localized at the catalytic site, NR activity is retained after immunoprecipitation, and it can be detected on the rocket immunoelectrophoresis stained with methyl viologen as the electron donor. Fig. 3 shows that the oxidation of methyl viologen upon addition of nitrate, tellurite or selenate as electron acceptor strictly coincides with precipitation arcs of MC4100 and LCB333/pVA70. For strain LCB2048, devoid of NR A and Z, no immunoprecipitated line or reductase activity was detected. For strain TP100, where the NR is present but inactive, the precipitation arc was present, but no nitrate, tellurite or selenate activities could be observed. Similarly, experiments performed using NR Z antiserum showed that this enzyme also has tellurite and selenate reductase activities (data not shown). This series of
C. AVAZÉRI and OTHERS

![Figure 4](image)

**Fig. 4.** Identification of a tellurite reductase that is expressed only anaerobically. Soluble extracts from the NR A- and NR Z-deleted mutant strain (LCB2048) were loaded onto a 7.5% non-denaturing polyacrylamide gel and stained with reduced methyl viologen. Lanes 1 and 2, cells grown under anaerobic conditions without and with potassium nitrate (1 g l⁻¹), respectively. Lanes 3 and 4, cells grown aerobically without and with potassium nitrate (1 g l⁻¹), respectively.

Experiments clearly show that, in E. coli, the major enzymes responsible for both tellurite and selenate reduction, mediated by MV⁻ or BV⁻, are the membrane NRs A and Z.

In addition to tellurite and selenate we also investigated the other oxyanions of these elements. When selenite or tellurate was added as electron acceptor, no decoloration of MV⁺ was observed in the rockets (data not shown).

**Nitrate, selenate and tellurite reduction activities in the soluble fraction from anaerobic cultures**

When grown under anaerobic conditions, the wild-type strain and the NR A overexpressing strain (LCB79/pVA700) showed a low level of reductase activity in the soluble fraction. This activity was about 5% of the reductase activities observed in membranes from the same culture. This activity was identified as NR A by its Rᵥ value in non-denaturing polyacrylamide gels and by rocket immunoelectrophoresis (data not shown).

A strain devoid of NR A and NR Z (LCB2048) grown anaerobically revealed the presence of another enzyme with both nitrate and tellurite reductase activity. Using an extended incubation time (greater than 20 min), a band of decoloration appeared upon addition of tellurite on a MV⁻ stained non-denaturing polyacrylamide gel, which had an Rᵥ value unique from NR A and NR Z (Fig 4).

It is important to note that this newly identified tellurite reductase activity is very difficult to detect even when low levels of NR A and Z are present in the supernatant. This activity is not present under aerobic conditions. We conclude that in E. coli, a soluble enzyme, present in low concentration, also has MV⁻/tellurite oxidoreductase activity.

**Minimal inhibitory concentrations**

The MICs of tellurite were determined for different NR mutants of E. coli under both aerobic and anaerobic conditions. The results are summarized in Table 3. MC4100 showed a low-level resistance to TeO₃⁻⁻, which is typical of wild-type E. coli strains (Summers & Jacoby, 1977; Jobling & Ritchie, 1987; Moore & Kaplan, 1992; Taylor et al., 1994). As previously shown by Turner et al. (1995), the NR deletion mutant (LCB2048) is hypersensitive to TeO₃⁻⁻ under aerobic conditions. We find that when the NRs are inactive (mutant TP100), the culture is also hypersensitive under aerobic conditions. Overexpression of NR A by LCB79/pVA700 mediates significant tellurite-resistance levels under both aerobic and anaerobic conditions. Going from aerobic to anaerobic conditions in the wild-type induces a tenfold increase of NR A synthesis. However, we observed only a twofold increase in tellurite resistance. Mutant LCB333 harbouring the NR Z plasmid, pLCB14, did not express tellurite resistance, most likely due to the low level of expression.

Under anaerobic growth conditions, mutants deleted in NR activity do not show increased sensitivity and have the same level of resistance as wild-type. This lack of hypersensitivity may be due to the other tellurite reductase activity, observed at a different Rᵥ to NR A and Z, present in the soluble fraction under anaerobic growth conditions (see above). Additionally, there may be additional enzymes which are not detectable using our BV⁻ and MV⁻ based assays.

The MIC of selenate for all strains tested was greater than 33 mg ml⁻¹, showing that selenate is not toxic for E. coli. The absence of NR does not cause hypersensitivity, contrary to what is observed with tellurite.

**Growth on tellurium and selenium oxyanions**

To determine whether the observed reductase activities are bioenergetically linked, wild-type MC4100 was grown anaerobically on minimal medium with
Tellurite activity of NR from *E. coli*

Oxyanions as the sole terminal electron acceptor. No growth was observed for tellurite (1 and 4 μg ml⁻¹), tellurate (2 μg ml⁻¹), selenite (100 μg ml⁻¹, 1 and 10 mg ml⁻¹) or selenate (100 μg ml⁻¹, 1 and 10 mg ml⁻¹), whereas growth was observed in control cultures where nitrate (100 μg ml⁻¹, 1 and 10 mg ml⁻¹) was provided. Additionally, cultures harbouring plasmids containing the tellurite-resistance determinants *tehAB* and *klaAklakBtelB* could not grow with tellurite (50 and 100 μg ml⁻¹) as the terminal electron acceptor. Nevertheless, mutant LCB79/pVA700, which synthesizes NR A in the presence of IPTG, is able to grow in the presence of tellurium and selenium oxyanides (the same concentrations as used above) upon addition of 0·2 mM IPTG. These results suggest that *E. coli* can use these oxyanions for anaerobic respiration only when NR is induced in large quantities.

**DISCUSSION**

Using different biochemical approaches and mutants of *E. coli*, we have shown that NRs A and Z also catalyse tellurite and selenate reduction. In the case of tellurite, we were not able to check whether NR is responsible for the total reduction of Te⁴⁺ into Te⁰ or whether this reaction is the result of two enzymic steps. The only +II valence intermediate possible in the reduction of tellurite to tellurium is TeO. This oxyanion is very unstable. It has been reported that reduction of TeO₂⁻ at a mercury electrode undergoes reduction to Te⁰ in a single step or through a short-lived Te⁷⁺⁻ species (Cooper, 1971). Therefore, it is possible that NR could reduce TeO₂⁻ directly to Te⁰. However, NR activity is not the only process responsible for the deposition of Te⁰ in *E. coli*, since mutants of NR exposed to tellurite still turn black as a result of tellurite reduction. It is clear that other biochemical processes are present under both aerobic and anaerobic conditions which give rise to tellurite reduction. These other reductive processes were not identified by our BV⁻ and MV⁻ dependent assays. They are likely to involve the thiol redox components of the cell (Turner et al., 1995; unpublished results).

The tellurite reductase activity of NR has an important effect on the resistance of *E. coli* to this oxyanion. Mutants of NR are hypersensitive to tellurite under aerobic conditions (Table 3). This hypersensitivity was absent when the resistance was assessed anaerobically. On rich medium, under aerobic conditions, wild-type *E. coli* synthesizes a low level of NR A. The data imply that the minor amount of NR expressed aerobically is responsible for the wild-type tellurite-resistance levels, and that the absence of NR has direct consequences. A low threshold level of NR A is required for increased resistance; we observed that overexpression of NR A gives a higher level of tellurite resistance either aerobically or anaerobically in the mutant LCB79/pVA700. These results imply that tellurite reduction via NR is a mechanism used by the bacteria to detoxify this oxyanion. Under anaerobic conditions, the NR mutants are not hypersensitive to tellurite. It is likely that this lack of hypersensitivity to tellurite is due to the contribution of the additional enzyme we detected in the soluble fraction under anaerobic growth conditions which possesses tellurite reduction activity.

In addition to the reduction of tellurite into tellurium, several other possible mechanisms, such as reduced uptake and increased efflux, sequestration, and the repair of cellular damage can explain the resistance of *E. coli* to this oxyanion (see Turner et al., 1995, for a detailed discussion). Although the exact biochemical mechanisms have still to be identified for *tehAB* (Taylor et al., 1994) and *klaAklakBtelB* (Turner et al., 1994) tellurite-resistance determinants, the resistance level for these determinants was lowered when introduced into the mutant LCB2048 (deleted in NR A and NR Z) and their MIC decreased by a factor of 8–16 (Turner et al., 1995). These results also imply the important function of NR in the tellurite resistance.

*Thauera selenatis* has been reported to use selenate as an electron acceptor for anaerobic respiration (Macy, 1994). This bacterium possesses a specific selenate reductase different from NR as well as a single nitrite reductase able to reduce both nitrite and selenite. The ability of *E. coli* to reduce tellurite and selenate opens the possibility that these compounds serve as electron acceptors for anaerobic respiration. However, growth of *E. coli* with tellurium and selenium oxyanions as electron acceptors was only obtained under anaerobic conditions in a mutant where NR is induced by IPTG. Therefore, the reduction of tellurite or selenate by *E. coli* (wild-type) does not play an important role in the bioenergetic process of this organism, but appears to provide a defence against oxyanions for the cell.

The ability of the membrane-bound NRs of *E. coli* to reduce chlorate or bromate is a well-known phenomenon (Iloffi et al., 1987; Morpeth & Boxer, 1985). We have shown here that these enzymes possess tellurite and selenate reductase activity. This is apparently a common feature of different types of NRs. Indeed, we have observed that the membrane NR of *Alcaligenes eutrophus*, *Paracoccus denitrificans* and the soluble periplasmic NR of *Rhodobacter sphaeroides* subsp. *denitrificans* present high tellurite and selenate reductase activities (Avazéri et al., 1995; unpublished results).

Our observation that different types of NR are able to reduce selenate explains the field observation of Oremland (1994). This author reported that nitrate and selenate reductions in situ give a similar profile as a function of the depth of the sediment. Different sets of experiments done on irrigation drainage water of the San Joaquin Valley (California) have shown that selenate reduction is inhibited by the presence of oxygen or nitrate (Lundquist et al., 1994). These observations are consistent with our proposal that NR has an important role in selenate reduction. Indeed the synthesis of this enzyme is inhibited by aerobiosis, and nitrate is a much better substrate than selenate (T. Montarges, unpublished results).
In this study we found that the membrane-bound NRs A and Z of _E. coli_ possess tellurite and selenate reductase activities. We also identified tellurite reductase activity that is expressed only under anaerobic conditions. The reduction activities are not used as bioenergetic processes but help the bacteria through detoxification of the oxyanions.

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REFERENCES


Tellurite activity of NR from *E. coli*


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