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Characterization of the Reduction of Selenate and Tellurite by Nitrate Reductases

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Preliminary studies showed that the periplasmic nitrate reductase (Nap) of *Rhodobacter sphaeroides* and the membrane-bound nitrate reductases of *Escherichia coli* are able to reduce selenate and tellurite in vitro with benzyl viologen as an electron donor. In the present study, we found that this is a general feature of denitrifiers. Both the periplasmic and membrane-bound nitrate reductases of *Ralstonia eutropha*, *Paracoccus denitrificans*, and *Paracoccus pantotrophus* can utilize potassium selenate and potassium tellurite as electron acceptors. In order to characterize these reactions, the periplasmic nitrate reductase of *R. sphaeroides* f. sp. *denitrificans* IL106 was histidine tagged and purified. The V_{max} and K_m were determined for nitrate, tellurite, and selenate. For nitrate, values of 39 μmol · min^{-1} · mg^{-1} and 0.12 mM were obtained for V_{max} and K_m, respectively, whereas the V_{max} values for tellurite and selenate were 40- and 140-fold lower, respectively. These low activities can explain the observation that depletion of the nitrate reductase in *R. sphaeroides* does not modify the MIC of tellurite for this organism.

Selenium is part of the amino acid selenocysteine present in numerous enzymes and is essential to all living cells (42). Furthermore, selenium can help prevent cancer and other diseases (11). However, at high concentrations this compound, predominantly in the form of selenate and selenite oxyanions, is toxic and can cause some environmental problems; for example, in the San Joaquin Valley in central California, bird malformations due to selenium have been reported (28). Tellurium is not an essential element and is relatively rare in the environment, but it can be found at high concentrations near waste discharge sites. It is also extremely toxic, and the MIC for *Escherichia coli* is approximately 2 μg of potassium tellurite per ml (3). Nevertheless, some gram-negative organisms are resistant to potassium tellurite (24). Different explanations for resistance have been proposed; these include exclusion, increased efflux, and reduction to the less toxic metallic form. Several genetic determinants have been shown to confer tellurite resistance (15, 27, 44, 45, 48, 50). Although physiological functions can be attributed to some of these determinants (for example, *tpm* and *tehB*, which exhibit homology with methyl transferases [1, 8, 19] and *arsRDABC*, which encodes an oxyanion efflux transporter [45]), most of them exhibit no similarity to each other or to the locus encoding any enzyme whose function is known. Therefore, the mechanisms which allow these loci to confer resistance remain largely unknown (46). When reduction occurs, intracellular deposition of tellurium can be observed, and bacteria form black colonies (20, 24). Resistance to selenium oxides is also partially attributed to reduction and accumulation of the red amorphous Se^0 form in the cell (12, 14; M. Bébien, J.-P. Chauvin, J.-M. Adriano, S. Grosse, and A. Verméglio, submitted for publication). For instance, the photosynthetic bacterium *Rhodobacter sphaeroides* accumulates tellurium and selenium after reduction of tellurite and selenite salts (25; Bébien et al., submitted). It is, however, unable to significantly reduce selenate to the metallic form (47; Bébien et al., submitted). For some species, like *Thauera selenatis, Sulfurospirillum barnesii, Bacillus selenitireducens*, or *Bacillus arsenicoselenatis* (21, 30, 43), the reduction of selenate involves a respiratory pathway, generating a proton motive force. In *T. selenatis*, a specific selenate reductase (37) has been purified. This enzyme does not reduce nitrate, nitrite, chlorate, or sulfate.

Reduction of selenium and tellurium oxides has often been associated with denitrification. The association of denitrification enzymes with reduction of selenate is based on the observation that nitrate reduction and selenate reduction in situ have similar profiles as a function of the depth of the sediment (29). Moreover, in *T. selenatis*, selenite reduction is due to the nitrite reductase (11), and in *E. coli*, mutants with mutations that affect nitrate reductase synthesis show a marked hypersensitivity to tellurite (3). Two classes of respiratory nitrate reductases have been identified: membrane-bound enzymes and periplasmic enzymes (6). The occurrence of a membrane-bound nitrate reductase has been shown and studied in detail in a large number of denitrifiers (51). This enzyme, which is synthesized under anaerobic conditions, is composed of three subunits: a 112- to 140-kDa catalytic α subunit (NarG) with a molybdopterin cofactor, a soluble 52- to 64-kDa β subunit (NarH) with one [3Fe-4S] and three [4Fe-4S] centers, and a 19- to 25-kDa membrane diheme *b* quinol-oxidizing γ subunit (NarI). A periplasmic nitrate reductase was isolated and first described in photosynthetic bacteria (36). This enzyme has also been found in many denitrifiers (5, 39, 40). In *R. sphaeroides* f. sp. *denitrificans*, the periplasmic nitrate reductase consists of a 91-kDa molybdenum-containing catalytic subunit (NapA) and

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a 17-kDa diheme cytochrome *c* (NapB). This enzyme, which so far has been only partially purified (35), is responsible for the first reduction step during the denitrification process (18, 34). Previous studies have shown that this enzyme and the membrane-bound *E. coli* nitrate reductases are able to reduce tellurite and selenate in vitro (3, 33).

In the present study, we found that this is a general feature and that nitrate reductases of other denitrifiers are also able to reduce selenate and tellurite. In addition, purification of the periplasmic nitrate reductase of *R. sphaeroides* f. sp. *denitrificans* IL106 after histidine tagging allowed us to characterize this activity and to determine the V_{max} and K_m for each substrate.

MATERIALS AND METHODS

Bacterial strains, plasmids, and growth conditions. *R. sphaeroides* f. sp. *denitrificans* IL106, *Paracoccus denitrificans* DSM 65, *Paracoccus pantotrophus* DSM 2944, and *Ralstonia eutropha* DSM 428 were grown at 30°C in Sistrom minimal medium supplemented with succinate as the carbon source (7) under anaerobic or aerobic conditions (100 ml of culture in 250-ml Erlenmeyer flask, 275 rpm). When necessary, the medium was supplemented with 40 mM KNO_3. *E. coli* strains were grown at 37°C in Luria-Bertani medium. When appropriate, tetracycline, spectinomycin, and streptomycin were added at concentrations of 1, 50, and 50 $\mu g \cdot ml^{-1}$, respectively, for *R. sphaeroides* and at concentrations of 20, 50, and 50 $\mu g \cdot ml^{-1}$ for *E. coli*.

MIC. The MIC was defined as the lowest concentration of inhibitor that prevented growth of *R. sphaeroides* at 30°C on agar plates. Once autoclaved, Sistrom agar was cooled to 50°C, and K_2TeO_3 or Na_2SeO_4 was added to each flask from a stock solution. The plates were incubated under dark aerobic conditions or under phototrophic conditions (75 mol of photons $\cdot m^{-2} \cdot s^{-1}$) in anaerobic jars (GENanaer; BioMérieux).

Preparation of cell extracts and electrophoresis. Cells were resuspended in 50 mM Tris-HCl (pH 8) and disrupted with a French press at 7 MPa. The crude extract was centrifuged at 10,000 × *g* for 10 min at 4°C to remove unbroken cells, and then the supernatant was centrifuged at 200,000 × *g* for 1.5 h at 4°C. The supernatant (soluble fraction) was removed, and the pellet (membrane fraction) was resuspended in the same buffer. Triton X-100 (0.1%) was added to the samples, and proteins were separated on nondenaturing polyacrylamide gels (7.5% acrylamide, 0.1% Triton X-100). The running buffer contained 25 mM Tris-HCl, 192 mM glycine, and 0.02% Triton X-100 (pH 8.3).

Activity staining was done in 50 mM Tris-HCl (pH 8) containing 2 mM methyl viologen for nitrate reductase activity and in 50 mM Tris-HCl (pH 9.1) containing 2 mM benzyl viologen for tellurite and selenate reduction. The viologen dyes were reduced with sodium dithionite before 40 mM KNO_3, K_2TeO_3, or Na_2SeO_4 was added.

Addition of a histidine tag to Nap. A six-histidine tag sequence was introduced at the C terminus of NapB. To do this, we amplified a 3.2-kb fragment with primers M13rev (−48) (Stratagene) and NB6H (5′AAGGTACCTCAGTGATGGTGATGGTGGTGTTCCGCCTCGTTGCTGGCCGG3′) by using pMS538, a pRK415 derivative containing *napABC* (34), as the template. The PCR product was cloned into pGEMT Easy (Promega). The resulting plasmid was then digested with *Eco*RI to obtain a 1.3-kb fragment (containing the terminal 796 bp of *napA* and *napB* modified). This fragment was used to replace the 2.8-kb *Eco*RI fragment of pMS538. The resulting plasmid, pMS611, contained *napAB* genes carrying a His tag sequence fused to the end of the *napB* coding frame. These genes are under the control of *lac* and *tet* promoters of pRK415 (34). To increase the levels of transcription of these genes in *R. sphaeroides*, we introduced the stronger promoter of the *R. sphaeroides puc* operon upstream of *napA*. To do this, the 0.7-kb *Pst*I-*Xba*I fragment of pPS400 (32), containing the *puc* promoter, was cloned into pMS611 digested with *Pst*I and *Xba*I. The resulting plasmid (pMS617) was moved from *E. coli* to *R. sphaeroides nap* mutant MS523 (34) by standard procedures (9).

Purification of the His-tagged periplasmic nitrate reductase. Five 1-liter cultures of *nap* mutant MS523 containing pMS617 in *trans* were grown semiaerobically (1-liter culture in a 2-liter Erlenmeyer flask, 150 rpm) until the end of the exponential phase. A periplasmic extract was prepared as previously described (31), concentrated, and diluted several times with 20 mM phosphate buffer (pH 8)–250 mM NaCl in order to change the final buffer. The resulting extract was loaded on a column containing 2 ml of Ni-NTA agarose resin (Qiagen). The column was washed (0.5 ml/min) with the same buffer and then with 20 mM phosphate buffer (pH 8)–250 mM NaCl–15 mM imidazole to remove nonspecifically bound contaminants. The nitrate reductase was finally eluted with 20 mM phosphate buffer (pH 8)–250 mM NaCl–100 mM imidazole.

Enzyme assay. Nitrate, tellurite, and selenate reductase activities were spectrophotometrically assayed at 600 nm and 30°C by using reduced benzyl viologen as the electron donor ($\varepsilon_{600nm} = 14.8\ M^{-1} \cdot cm^{-1}$). Each reaction mixture (4 ml) contained 50 mM Tris-HCl (pH 7) and 0.5 mM benzyl viologen. The anaerobic cuvette was degassed and sparged with argon prior to addition of 10 μl of freshly prepared 60 mM $Na_2S_2O_4$ to reduce the benzyl viologen. Even under these conditions trace oxygen contamination caused slow oxidation of the viologen dye. To eliminate participation of oxygen, at the beginning of each assay a volume of buffer was injected into the cuvette and the initial rate of oxidation was measured. The value obtained was subtracted from the value obtained after injection of the enzyme in the presence of the substrate. For each assay, 2.3, 70, and 120 μg of purified enzyme were used to measure nitrate, tellurite, and selenate reduction activities, respectively.

RESULTS AND DISCUSSION

Reduction of tellurite and selenate by periplasmic and membrane-bound nitrate reductases of several species. Despite the increased number of studies on the resistance of microorganisms to selenium and tellurium oxides, the precise mechanisms of resistance and reduction remain elusive. We tried to find specific reductases for these compounds in *R. sphaeroides*. Tellurite reduction, selenite reduction, and selenate reduction with methyl viologen as the electron donor were assayed on nondenaturing gels. For tellurite reduction and selenate reduction, only one band of activity was detected; this band had an R_f identical to that of the periplasmic nitrate reductase (2). In *E. coli*, it has been shown that the membrane-bound nitrate reductases NR A and NR Z are also able to reduce these oxyanions (2, 3). In order to determine if this is a general feature, we tested the capacities of nitrate reductases from several denitrifiers to reduce tellurite and selenate. Soluble and membrane extracts were loaded on nondenaturing electrophoresis gels. For soluble extracts, cells were grown under aerobic conditions. Under these conditions, *R. sphaeroides* IL106, *P. denitrificans*, and *P. pantotrophus* express a periplasmic nitrate reductase (Nap) (4, 31, 38, 49). As shown in Fig. 1 (lanes A), these enzymes did not migrate with the same R_f under nondenaturing conditions. The main difference was observed with *R. eutropha* Nap, which remained in the stacking gel under our electrophoresis conditions (data not shown). For each species, the enzyme responsible for tellurite reduction (lanes B) and selenate reduction (lanes C) migrated with the same R_f as Nap. In mutant MS523 of *R. sphaeroides*, which does not synthesize Nap, neither tellurite nor selenate reductase activity was observed.

The capacities of membrane-bound nitrate reductases (Nar) to reduce tellurite and selenate were also tested. To do this, cells were grown under anaerobic conditions in the presence of nitrate. As observed previously for *E. coli* (3), the enzyme responsible for reduction of tellurite (lanes B) and selenate (lanes C) had the same R_f as the nitrate reductase activity (lanes A) in *P. denitrificans*, *P. pantotrophus*, and *R. eutropha*. This shows that the ability of the periplasmic and membrane-bound nitrate reductases to reduce tellurite and selenate is a general feature of different denitrifying species. The capacity of membrane-bound nitrate reductases to reduce various substrates, such as bromate and chlorate, is a well-known phenomenon (13, 26). However, periplasmic nitrate reductases are not

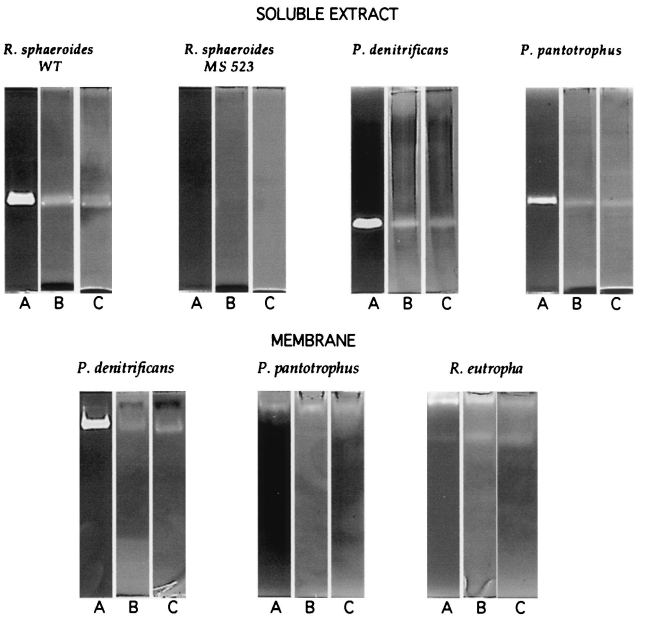

FIG. 1. Nondenaturing electrophoresis of soluble extracts and membranes of *R. sphaeroides* f. sp. *denitrificans* wild type and *nap* mutant MS523, *P. denitrificans*, *P. pantotrophus*, and *R. eutropha*. Gels were stained for nitrate reductase (lanes A), tellurite reductase (lanes B), or selenate reductase (lanes C) activity with dithionite-reduced methyl viologen or benzyl viologen.

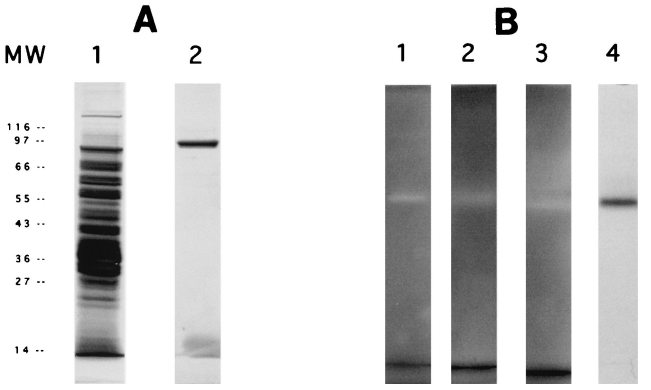

FIG. 2. (A) Silver-stained sodium dodecyl sulfate-polyacrylamide gel. Lane 1, periplasmic extract of *R. sphaeroides* (8 μg of protein); lane 2, His-tagged purified Nap (0.5 μg of protein). MW, molecular weight. (B) Nondenaturing electrophoresis of His-tagged purified Nap. Gels were stained for reductase activity with dithionite-reduced methyl viologen. Lane 1, nitrate reductase activity (1 μg of protein); lane 2, tellurite reductase activity (25 μg of protein); lane 3, selenate reductase activity (25 μg of protein); lane 4, gel stained with Coomassie brillant blue (5 μg of protein).

able to reduce these two compounds and until now seemed to be specific for nitrate (5, 22). The capacity of nitrate reductases to reduce such different substrates is quite surprising since the substrates have different conformations; nitrate is planar, while chlorate and selenate are tetrahedral and tellurite is trigonal.

We recently obtained NapAB crystals from *R. sphaeroides*. Cocrystallization of the enzyme with the different substrates is also in progress. The resulting crystallographic structures should provide a precise image of the binding of the different substrates at the active site.

Purification of the His-tagged nitrate reductase. The capacity of nitrate reductase to reduce selenate and tellurite has been observed previously only with cell extracts. Thus, it was interesting to test this property with a purified enzyme. Periplasmic nitrate reductases of several species have been purified previously (5, 22, 40). However, the purification procedure required three or four different chromatrographic steps, and there was loss of activity at each step. In *R. sphaeroides* IL106, Nap has a high specific activity, but a small amount is synthesized (31). We therefore decided to add a six-histidine tag to the cytochrome subunit NapB to facilitate purification of the enzyme by immobilized-metal affinity chromatography. Plasmid pMS538 containing *napABC* (34) was modified by adding six codons for histidine to the 3′ end of *napB* (see Materials and Methods), resulting in plasmid pMS611. This plasmid was introduced into *nap* mutant MS523 so that only the tagged enzyme (NapAB-6His) would be synthesized. To increase the level of synthesis, we cloned the *puc* promotor upstream of *napA* in pMS611 (the *puc* operon encodes the LHIII light harvesting proteins). With the resulting plasmid (pMS617), the level of expression of NapAB-6His was 10-fold higher than the level of expression with pMS611. Different growth conditions were tested to obtain the largest amount of synthesized Nap. A better yield was obtained under dark semiaerobic conditions than under phototrophic conditions. This is quite surprising since *puc* operon expression is greater under phototrophic conditions (17). Expression of the enzyme was maximal at the end of the exponential phase. A periplasmic extract was prepared and loaded onto an Ni-NTA agarose column (see Materials and Methods). From 5 liters of culture, around 5 mg of purified protein with a specific activity of 30 μmol of nitrate reduced · min^{-1} · mg^{-1} was obtained. The purity of the enzyme was tested by gel electrophoresis (Fig. 2). Analysis of the purified enzyme preparation by using a sodium dodecyl sulfate-polyacrylamide gel electrophoresis gel stained with silver (Fig. 2A) revealed only two bands; these bands had apparent molecular weights of 91,000 and 17,000, which corresponded to the molecular weights of the NapA and NapB subunits, respectively. As observed previously for nitrate reductases from other species (5), NapB does not stain with Coomassie blue and stains very weakly with silver. Nondenaturing gels loaded with purified enzyme were stained for tellurite and selenate reductase activity (Fig. 2B). The results obtained with the soluble cell extract were confirmed with the purified enzyme. This implies that Nap does not require the presence of a possible product present in the cell extract that could comigrate on a nondenaturing gel. However, the amount of enzyme necessary to see significant tellurite or selenate reductase activity on the gel was greater than the amount necessary for nitrate. This suggests that the affinity for the substrate and/or the rate of reduction of the enzyme was higher for nitrate than for tellurite or selenate. We therefore determined the V_{max} and K_m values of Nap for each substrate.

Determination of K_m and V_{max} values for reduction of tellurite and selenate by Nap. The reduction activities were measured spectrophotometrically in an anaerobic cuvette by measuring the oxidation of benzyl viologen. In order to obtain significant activities, 2, 70, and 120 μg of purified enzyme (4.7

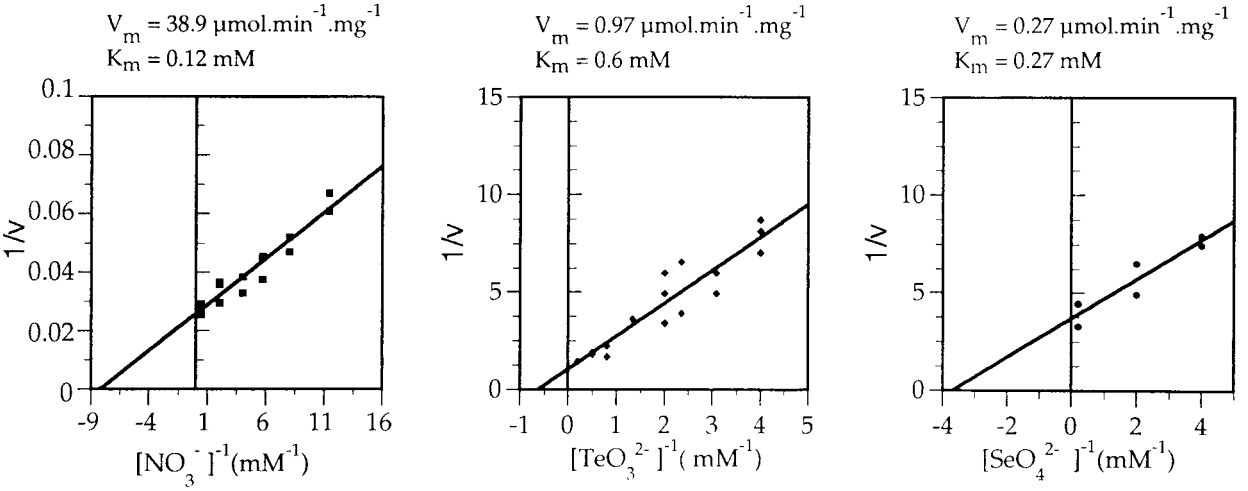

FIG. 3. Lineweaver-Burk plots: kinetics of reduction of nitrate, tellurite, and selenate by purified Nap when methyl viologen is the electron donor. V_{max} is expressed in micromoles of substrate reduced per minute per milligram of enzyme. The correlation coefficients for the nitrate, tellurite, and selenate plots are 0.92, 0.88, and 0.88, respectively.

mg · ml^{-1}) were used for nitrate reduction, tellurite reduction, and selenate reduction, respectively. From Lineweaver-Burk plots (Fig. 3) the V_{max} and K_m values were calculated for each substrate. The K_m for nitrate was 0.12 mM, and the V_{max} was 39 µmol · min^{-1} · mg of protein^{-1}. These values are very similar to the values obtained for *P. pantatrophus* or *R. eutropha* (5, 40), which is consistent with the high level of sequence homology between the Nap enzymes. For selenate reduction, the K_m and V_{max} values were 0.27 mM and 0.27 µmol · min^{-1} · mg^{-1}, respectively. These values can be compared only to the values obtained for the only selenate reductase purified so far, the selenate reductase of *T. selenatis*, which has K_m and V_{max} values of 0.016 mM and 40 µmol · min^{-1} · mg^{-1}, respectively (37). The large difference between the selenate reductase and Nap specific activities shows that Nap is not an efficient selenate reductase; both the affinity of the enzyme for the substrate and the substrate turnover rate are extremely low compared to those of the *T. selenatis* enzyme. The Nap affinity for tellurite is also very low, with a K_m value of 0.6 mM. The catalytic efficiency (V_{max}/K_m) of Nap, which takes into account the affinity and the turnover rate of the enzyme, is 200 times lower for tellurite and 300 times lower for selenate than for nitrate.

The very low selenate reductase activity of Nap in vitro could explain the finding that in *R. sphaeroides*, even when a significant amount of Nap is synthesized, the selenate is not reduced to elemental selenium, while selenite is reduced (47; Bébien et al., submitted). Also, the reduction of tellurite by the bacterium is not due to the Nap reductase alone since an *R. sphaeroides nap* mutant (MS523) is still able to reduce tellurite (data not shown). To determine the possible role of Nap in the resistance of the bacteria to tellurite, we determined the MICs for the wild type and the *nap* mutant.

MICs. The MICs of tellurite for the wild type and *nap* mutant MS523 were determined on plates. The plates were incubated at 30°C under dark aerobic conditions or phototrophic conditions. As observed previously for several species of photosynthetic bacteria (24), the MIC depends on the growth conditions; the MICs were 100 and 280 ppm for aerobic and phototrophic conditions, respectively. However, under both types of conditions, the MICs of tellurite were identical for the wild type and the *nap* mutant. Deletion of the gene encoding the periplasmic nitrate reductase in *R. sphaeroides* IL106 did not, therefore, modify its resistance to tellurite. However, this situation is different from that encountered in *E. coli*, in which mutants depleted in the two membrane-bound nitrate reductases are hypersensitive to tellurite; the MIC is 0.03 µg · ml^{-1}, compared to 2 µg · ml^{-1} for the wild type (3). However, even for the wild type, these values are quite low compared to the value for *R. sphaeroides* (100 µg · ml^{-1} under aerobic conditions). This means that even if in *R. sphaeroides* the nitrate reductase contributes to the same extent as the nitrate reductases of *E. coli* to resistance to tellurite, its participation is masked by other reducing pathways and mechanisms which must be present in vivo in *R. sphaeroides* but not in *E. coli*.

This study showed that despite the capacity of Nap to reduce tellurite and selenate in vitro, the catalytic activity of the enzyme for these substrates is low and the resistance of *R. sphaeroides* to these substrates cannot be attributed to their reduction by Nap. Efforts to isolate specific selenate, selenite, or tellurite reductases in this species by using biochemical approaches have been unsuccessful. A genetic study is now in progress, and some mutants that are not able to reduce oxyanions have already been isolated (Bébien et al., submitted).

REFERENCES

1. **Alonso, G., C. Gomes, C. Gonzalez, and V. R. Lemoine.** 2000. On the mechanism of resistance to channel-forming colicins (PacB) and tellurite, encoded by plasmid Mip233 (IncH13). FEMS Microbiol. Lett. **192:**257–261.
2. **Avazéri, C., J. Pommier, G. Giordano, F. Blasco, and A. Verméglio.** 1995. Reduction of oxyanions by photosynthetic bacteria and *Escherichia coli*: role of the nitrate reductase in the reduction of tellurite and selenate, p. 423–426. *In* P. Mathis (ed.), Photosynthesis: from light to biosphere. Kluwer, Dordrecht, The Netherlands.
3. **Avazéri, C., R. J. Turner, J. Pommier, J. H. Weiner, G. Giordano, and A. Verméglio.** 1997. Tellurite reductase activity of nitrate reductase is responsible for the basal resistance of *Escherichia coli* to tellurite. Microbiology **143:**1181–1189.
4. **Bell, L. C., D. J. Richardson, and S. J. Ferguson.** 1990. Periplasmic and membrane-bound nitrate reductases in *Thiosphaera pantotropha*: the

periplasmic enzyme catalyses the first step in aerobic denitrification. FEBS Lett. **265:**85–87.
5. **Berks, B. C., D. J. Richardson, C. Robinson, A. Reilly, R. T. Aplin, and S. J. Ferguson.** 1994. Purification and characterization of the periplasmic nitrate reductase from *Thiosphaera pantotropha*. Eur. J. Biochem. **220:**117–124.
6. **Berks, B. C., S. J. Ferguson, J. W. B Moir, and D. J. Richardson.** 1995. Enzymes and associated electron transport systems that catalyse the respiratory reduction of nitrogen oxides and oxyanions. Biochim. Biophys. Acta **1232:**97–173.
7. **Cohen-Bazire, G., W. R. Sistrom, and R. Y. Stanier.** 1956. Kinetic studies of pigment synthesis by non-sulfur purple bacteria. J. Cell. Comp. Physiol. **49:**25–68.
8. **Cournoyer, B., S. Watanabe, and A. Vivian.** 1998. A tellurite-resistance genetic determinant from phytopathogenic pseudomonads encodes a thiopurine methyltransferase: evidence of a widely-conserved family of methyltransferases. Biochim. Biophys. Acta **1397:**161–168.
9. **Davis, J., T. J. Donohue, and S. Kaplan.** 1988. Construction, characterization, and complementation of a *puf* mutant of *Rhodobacter sphaeroides*. J. Bacteriol. **170:**320–329.
10. **Demoll-Decker, H., and J. M. Macy.** 1993. The periplasmic nitrite reductase of *Thauera selenatis* may catalyse the reduction of selenite to elemental selenium. Arch. Microbiol. **160:**241–247.
11. **Ganther, H. E.** 1999. Selenium metabolism, selenoproteins and mechanisms of cancer prevention: complexities with thioredoxin reductase. Carcinogenesis **20:**1657– 1666.
12. **Gerrard, T. L., J. N. Telford, and H. H. Williams.** 1974. Detection of selenium deposits in *Escherichia coli* by electron microscopy. J. Bacteriol. **119:**1057–1060.
13. **Iobbi, C., C. L. Santini, V. Bonnefoy, and G. Giordano.** 1987. Biochemical and immunologicol evidence for a second nitrate reductase in *Escherichia coli* K-12. Eur. J. Biochem. **168:**451–459.
14. **Kessi, J., M. Ramuz, E. Wehrli, M. Spycher, and R. Bachofen.** 1999. Reduction of selenite and detoxification of elemental selenium by the phototrophic bacterium *Rhodospirillum rubrum*. Appl. Environ. Microbiol. **65:**4735–4740.
15. **Kormutakova, R., L. Klucar, and J. Turna.** 2000. DNA sequence analysis of the tellurite-resistance determinant from clinical strain of *Escherichia coli* and identification of essential genes. Biometals **13:**135–139.
16. **Laüchi, A.** 1993. Selenium in plants: uptake, function and environmental toxicity. Bot. Acta **106:**455–468.
17. **Lee, J. K., and S. Kaplan.** 1995. Transcriptional regulation of *puc* operon expression in *Rhodobacter sphaeroides*. J. Biol. Chem. **270:**20453–20458.
18. **Liu, H.-P., S. Takio, T. Satoh, and I. Yamamoto.** 1999. Involvement in denitrification of the *napKEFDABC* genes encoding the periplasmic nitrate reductase system in the denitrifying phototrophic bacterium *Rhodobacter sphaeroides* f. sp. *denitrificans*. Biosci. Biotechnol. Biochem. **63:**530–536.
19. **Liu, M. F., R. J. Turner, T. L. Winstone, A. Saetre, M. Dyllick-Brenzinger, G. Jickling, L. W. Tari, J. H. Weiner, and D. E. Taylor.** 2000. *Escherichia coli* TehB requires *S*-adenosylmethionine as a cofactor to mediate tellurite resistance. J. Bacteriol. **182:**6509–6513.
20. **Lloyd Jones, G., A. M. Osborn, D. A. Ritchie, P. Strike, J. L. Hobman, N. L. Brown, and D. A. Rouch.** 1994. Accumulation and intracellular fate of tellurite resistant in *Escherichia coli*: a model for the mechanism of resistance. FEMS Microbiol. Lett. **118:**113–120.
21. **Macy J. M., T. A. Michel, and D. A. Kirsch.** 1989. Selenate reduction by a *Pseudomonas* species: a new mode of anaerobic respiration. FEMS Microbiol. Lett. **61:**195–198.
22. **McEwan, A. G., H. G. Wetzstein, O. Meyer, J. B. Jackson, and S. J. Ferguson.** 1987. The periplasmic nitrate reductase of *Rhodobacter capsulatus*; purification, characterisation and distinction from a single reductase for trimethylamine-N-oxide, dimethylsulphoxide and chlorate. Arch. Microbiol. **147:**340–345.
23. **Menendez, C., J. Igloi, H. Heninger, and R. Brandsch.** 1995. A pAO1-encoded molybdeopterin cofactor gene (*moaA* of *Arthrobacter nicotinovorans*): characterization and site-directed mutagenesis of the encoded protein. Arch. Microbiol. **164:**142–151.
24. **Moore, M. D., and S. Kaplan.** 1992. Identification of intrinsic high-level resistance to rare-earth oxides and oxyanions in members of the class *Proteobacteria*: characterization of tellurite, selenite, and rhodium sesquioxide reduction in *Rhodobacter sphaeroides*. J. Bacteriol. **174:**1505–1514.
25. **Moore, M. D., and S. Kaplan.** 1994. Members of the family *Rhodospirillaceae* reduce heavy-metal oxyanions to maintain redox poise during photosynthetic growth. ASM News **60:**17–23.
26. **Morpeth, F., and D. Boxer.** 1985. Kinetic analysis of respiratory nitrate reductase from *Escherichia coli* K12. Biochemistry **24:**40–46.
27. **O'Gara, J. P., M. Gomelsky, and S. Kaplan.** 1997. Identification and molecular genetic analysis of multiple loci contributing to high-level tellurite resistance in *Rhodobacter sphaeroides* 2.4.1. Appl. Environ. Microbiol. **63:**4713–4720.
28. **Ohlendorf, H. M., and G. M. Santolo.** 1994. Kesterson Reservoir—past, present and future: an ecological risk assessment, p. 69–117. *In* J. R. Frankenberger and S. Benson (ed.), Selenium in the environment. Marcel Dekker, New York, N.Y.
29. **Oremland, R. S.** 1994. Biogeochemical transformation of selenium in anoxic environments, p. 389–420. *In* J. R. Frankenberger and S. Benson (ed.), Selenium in the environment. Marcel Dekker, New York, N.Y.
30. **Oremland, R. S., J. Switzer Blum, C. W. Culbertson, P. T. Visscher, L. G. Miller, P. Dowdle, and F. E. Strohmaier.** 1994. Isolation, growth, and metabolism of an obligately anaerobic, selenate-respiring bacterium, strain SES-3. Appl. Environ. Microbiol. **60:**3011–3019.
31. **Sabaty, M., J. Gagnon, and A. Verméglio.** 1994. Induction by nitrate of cytoplasmic and periplasmic proteins in the photodenitrifier *Rhodobacter sphaeroides* forma sp. *denitrificans* under anaerobic or aerobic condition. Arch. Microbiol. **162:**335– 343.
32. **Sabaty, M., and S. Kaplan.** 1996. *mgpS*, a complex regulatory locus involved in the transcriptional control of the *puc* and *puf* operons in *Rhodobacter sphaeroides* 2.4.1. J. Bacteriol. **178:**35–45.
33. **Sabaty, M., M. Bébien, C. Avazéri, V. Yurkov, P. Richaud, and A. Verméglio.** 1998. Reduction of tellurite and selenite by photosynthetic bacteria, pp. 4123–4128. *In* G. Garab (ed.), Photosynthesis: mechanisms and effects. Kluwer, Dordrecht, The Netherlands.
34. **Sabaty, M., C. Schwintner, S. Cahors, P. Richaud, and A. Verméglio.** 1999. Nitrite and nitrous oxide reductase regulation by nitrogen oxides in *Rhodobacter sphaeroides* f. sp. *denitrificans* IL106. J. Bacteriol. **181:**6028–6032.
35. **Satoh, T.** 1981. Soluble dissimilatory nitrate reductase containing cytochrome c from a photodenitrifier, *Rhodopseudomonas sphaeroides* forma sp. *denitrificans*. Plant Cell Physiol. **22:**443–452.
36. **Sawada, E., and T. Satoh.** 1980. Periplasmic location of dissimilatory nitrate and nitrite reductases in a denitrifying phototrophic bacterium. Plant Cell Physiol. **21:**205–210.
37. **Schröder, I., S. Rech, T. Krafft, and J. M. Macy.** 1997. Purification and characterization of the selenate reductase from *Thauera selenatis*. J. Biol. Chem. **272:**23765–23768.
38. **Sears, H., S. Spiro, and D. J. Richardson.** 1997. Effect of carbon substrate and aeration on nitrate reduction and expression of the periplasmic and membrane-bound nitrate reductases in carbon-limited continuous cultures of *Paracoccus denitrificans* Pd1222. Microbiology **143:**3767–3774.
39. **Sears, H. J., S. J. Ferguson, D. J. Richardson, and S. Spiro.** 1993. The identification of a periplasmic nitrate reductase in *Paracoccus denitrificans*. FEMS Microbiol. Lett. **113:**107–112.
40. **Siddiqui, R. A., U. Warnecke-Eberz, A. Hengsberger, B. Schneider, and B. Freidrich.** 1993. Structure and function of a periplasmic nitrate reductase in *Alcaligenes eutrophus* H16. J. Bacteriol. **175:**5867–5876.
41. **Solomon, P. S., A. L. Shaw, I. Lane, G. R. Hanson, T. Palmer, and A. G. McEwan.** 1999. Characterization of a molybdenum cofactor biosynthetic gene cluster in *Rhodobacter capsulatus* which is specific for the biogenesis of dimethylsulfoxide reductase. Microbiology **145:**1421–1429.
42. **Stadtman, T. C.** 1996. Selenocysteine. Annu. Rev. Biochem. **65:**83–100.
43. **Switzer Blum, J., A. Burns Bindi, J. Buzzelli, J. F. Stolz, and R. S. Oremland.** 1998. *Bacillus arsenicoselenatis*, sp. nov., and *Bacillus selenitireducens*, sp. nov.: two haloalkaliphiles from Mono Lake, California that respire oxyanions of selenium and arsenic. Arch. Microbiol. **171:**19–30.
44. **Taylor, D. E., Y. Hou, R. J. Turner, and J. H. Weiner.** 1994. Location of a potassium tellurite resistance operon (*tehA tehB*) within the terminus of *Escherichia coli* K-12. J. Bacteriol. **176:**2740–2742.
45. **Turner, R. J., Y. Hou, J. H. Weiner, and D. E. Taylor.** 1992. The arsenical ATPase efflux pump mediates tellurite resistance. J. Bacteriol. **174:**3092–3094.
46. **Turner, R. J., J. H. Weiner, and D. E. Taylor.** 1995. The tellurite-resistance determinants tehAtehB and klaAklaBtelB have different biochemical requirements. Microbiology **141:**3133–3140.
47. **Van Fleet-Stalder, V., T. G. Chasteen, I. J. Pickering, G. N. George, and R. C. Prince.** 2000. Fate of selenate and selenite metabolized by *Rhodobacter sphaeroides*. Appl. Environ. Microbiol. **66:**4849–4853.
48. **Walter, E. G., C. M. Thomas, J. P. Ibbotson, and D. E. Taylor.** 1991. Transcriptional analysis, translational analysis and sequence of the KilA-tellurite resistance region of plasmid RK2Te^r. J. Bacteriol. **173:**1111–1119.
49. **Warnecke-Eberz, U., and B. Friedrich.** 1993. Three nitrate reductase activities in *Alcaligenes eutrophus*. Arch. Microbiol. **159:**405–409.
50. **Whelan, K. F., E. Colleran, and D. E. Taylor.** 1995. Phage inhibition, colicin resistance, and tellurite resistance are encoded by a single cluster of genes on the IncH12 plasmid R478. J. Bacteriol. **177:**5016–5027.
51. **Zumft, W. G.** 1997. Cell biology and molecular basis of denitrification. Microbiol. Mol. Biol. Rev. **61:**533–616.