

NOTES

Reduction of Chromate by *Desulfovibrio vulgaris* and Its c_3 Cytochrome

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Washed cell suspensions of *Desulfovibrio vulgaris* rapidly reduced Cr(VI) to Cr(III) with H₂ as the electron donor. The c_3 cytochrome from this organism functioned as a Cr(VI) reductase. *D. vulgaris* may have advantages over previously described Cr(VI) reducers for the bioremediation of Cr(VI)-contaminated waters.

The ability of some microorganisms to reduce highly soluble and highly toxic Cr(VI) to less toxic, less soluble Cr(III) has led to the suggestion that Cr(VI)-reducing microorganisms might be useful agents for remediating Cr(VI)-contaminated waters and soils (1, 5, 7, 11, 12, 22, 26). A wide range of microorganisms are capable of enzymatic Cr(VI) reduction (13). In no instance has it been conclusively demonstrated that Cr(VI) reduction yields energy to support growth (13), and it has been suggested that Cr(VI) is fortuitously reduced by enzymes that have other, as yet unidentified, natural substrates (2, 8). An enzyme which enhances the rate of NADH reduction of Cr(VI) has been purified from *Pseudomonas ambigua* (24).

Cr(VI) reduction by whole cells. The finding that several *Desulfovibrio* species can enzymatically reduce Fe(III) and U(VI) (3, 14-16) led us to investigate whether they might also act as Cr(VI) reducers. *Desulfovibrio vulgaris* (Hildenborough) (ATCC 29579) was obtained from the American Type Culture Collection, Rockville, Md. Suspensions of whole cells that had been grown with lactate as the electron donor and sulfate as the electron acceptor were prepared in bicarbonate buffer (10 ml) under N₂-CO₂ (80:20) in serum bottles (25 ml) as previously described (15), with the exception that the buffer also contained 500 μ M potassium chromate. The pH was 6.8. Incubations were at 30°C. For determination of protein concentrations, the cells were first digested in 1 N NaOH for 5 min at 100°C, and then protein was determined by the Lowry method (18).

Cr(VI) was analyzed by the diphenylcarbazide method (25). Subsamples (0.2 ml) were withdrawn with a syringe and needle and added to 9.8 ml of 0.2 N H₂SO₄. Then 0.5 ml of diphenylcarbazide reagent (0.025 g of *sym*-diphenylcarbazide in 10 ml of acetone) was added. Samples were filtered (0.2- μ m-pore-diameter Acrodisc filter), and the A₅₄₀ was measured.

When H₂ (10 ml) was added to cell suspensions, there was a steady loss of Cr(VI) over time (Fig. 1). There was no loss of Cr(VI) in the presence of H₂ in the absence of cells (not shown). There was also little disappearance of Cr(VI) in the presence of cells when H₂ was omitted (Fig. 1). This result indicated that the decrease of Cr(VI) in the presence of H₂ was not due to adsorption of Cr(VI) onto the cells. The H₂-

dependent loss of Cr(VI) was associated with a change in the color of the cell suspension from the yellow characteristic of dissolved Cr(VI) to a white colloidal suspension characteristic of Cr(III) hydroxide. If the N₂-CO₂ was replaced with air, then the capacity for Cr(VI) reduction was lost. No attempts were made to grow *D. vulgaris* with Cr(VI) as the electron acceptor because of our previous inability to grow sulfate reducers with either Fe(III) or U(VI) as the sole electron acceptor (15, 16).

To determine the stoichiometry of H₂ uptake and Cr(VI) reduction, less H₂ (0.17 ml) was added to cell suspensions so that H₂ uptake could be monitored along with Cr(VI) reduction. When corrected for endogenous reduction of Cr(VI) in cell suspensions without added H₂, the ratio of H₂ uptake to Cr(VI) loss was 1.74 ± 0.13 (mean \pm standard deviation; $n = 5$), which suggests that Cr(VI) was primarily reduced according to the formula $3\text{H}_2 + 2\text{Cr(VI)} \rightarrow 2\text{Cr(III)} + 6\text{H}^+$.

Cr(VI) reduction by soluble fraction and cytochrome c_3 . To localize the H₂-dependent, Cr(VI)-reducing activity, cells were broken in a French pressure cell and fractionated into soluble and membrane-bound protein fractions through differential centrifugation as previously described (17). The soluble protein fraction reduced Cr(VI) faster than the membrane fraction did (Fig. 2). With 4 mg of protein in 10 ml of buffer, the first-order rate constants for loss of Cr(VI) in the soluble and membrane fractions were 0.0772 and 0.0236 min⁻¹, respectively. Soluble protein accounted for 86% of the cell protein that was recovered from the broken cells. Thus, as was previously found for U(VI) reduction (17), most (ca. 95%) of the H₂-dependent Cr(VI)-reducing activity was in the soluble protein fraction.

Cytochrome c_3 catalyzes U(VI) reduction in *D. vulgaris* (17). As previously shown for the U(VI) reductase activity, when cytochrome c_3 was selectively removed from the soluble protein fraction by passing it over a cation-exchange column, the H₂-dependent Cr(VI) reductase activity was completely lost (data not shown). When cytochrome c_3 was added back to the soluble protein that was not retained on the cation-exchange column, the ability to reduce Cr(VI) was restored. Like U(VI) (17), Cr(VI) oxidized reduced cytochrome c_3 . These results suggest that cytochrome c_3 can also function as a Cr(VI) reductase.

When cytochrome c_3 is combined with hydrogenase, its physiological electron donor, then U(VI) is reduced in the presence of H₂ (17). To determine whether a similar enzyme system could reduce Cr(VI), pure cytochrome c_3 (0.06 mg) was

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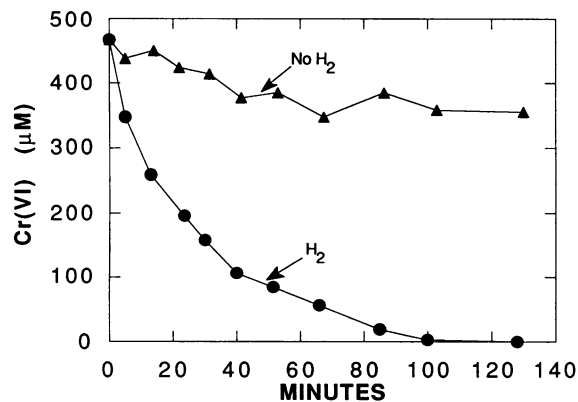


FIG. 1. Loss of Cr(VI) over time in the presence of H_2 and *D. vulgaris*. There was 11.1 mg of cell protein in 10 ml of buffer.

combined with an excess of hydrogenase activity that had been purified from the soluble fraction of *D. vulgaris* (17). In the presence of H_2 , the hydrogenase fraction, and cytochrome c_3 , there was a steady loss of Cr(VI) over time (Fig. 3). There was no loss of Cr(VI) in the absence of H_2 (Fig. 3). Neither cytochrome c_3 alone nor the hydrogenase fraction alone could reduce Cr(VI) (Fig. 3). Therefore, cytochrome c_3 is a Cr(VI) reductase.

Environmental and bioremediation implications. These results demonstrate that *D. vulgaris* is an effective Cr(VI)-reducing organism. Previous studies have demonstrated that sulfide produced by sulfate-reducing bacteria can reduce Cr(VI) (23). However, sulfide production did not account for the Cr(VI) reduction observed in the sulfate-free systems studied here. In addition to sulfide, a variety of organics and Fe(II) can also nonenzymatically reduce Cr(VI) (4, 19, 21). Thus, in comparison with nonenzymatic mechanisms for Cr(VI) reduction, enzymatic Cr(VI) reduction is probably of minor importance in most natural environments. The greater significance of enzymatic Cr(VI) reduction is in its potential application for removing Cr(VI) from contaminated waters and waste streams.

D. vulgaris may be more desirable than some other Cr(VI)-

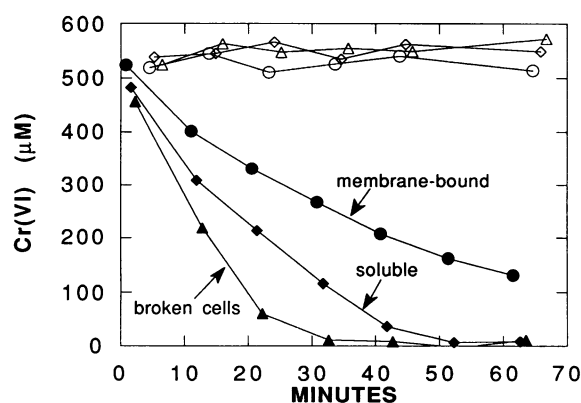


FIG. 2. Cr(VI) reduction by broken cells, soluble fraction, or membrane fraction of *D. vulgaris*. There was 4 mg of protein of each of the three fractions in 10 ml of buffer. Closed symbols, fractions with added H_2 ; open symbols, the corresponding protein fractions without added H_2 .

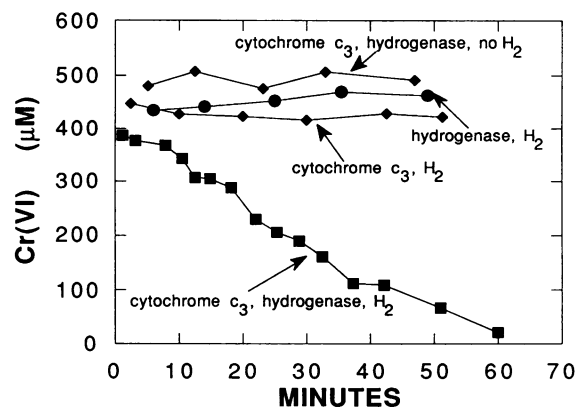


FIG. 3. Cr(VI) reduction with H_2 , hydrogenase fraction, and cytochrome c_3 from *D. vulgaris*.

reducing bacteria for treating Cr(VI) contamination. *Enterobacter cloacae* is the Cr(VI)-reducing microorganism that has been most intensively investigated for its potential application for bioremediation of Cr(VI) (references 6, 9, 10, 20, and 26 and references therein). Cr(VI) removal from industrial effluents with *E. cloacae* has been problematic because heavy metals and sulfate in the waste inhibit Cr(VI) reduction (6, 10, 20). However, sulfate concentrations as high as 50 mM did not inhibit Cr(VI) reduction by *D. vulgaris*. Furthermore, when tested at a concentration of 100 μM , the following metals also had no effect on Cr(VI) reduction by *D. vulgaris*: nickelous chloride, manganous sulfate, cobalt chloride, cupric chloride, cuprous chloride, zinc chloride, magnesium sulfate, vanadyl sulfate, sodium vanadate, sodium molybdate, and sodium selenate. These results are similar to the general lack of effect of these metals and sulfate on U(VI) reduction by *Desulfovibrio desulfuricans*, with the exception that copper inhibited U(VI) reduction in *D. desulfuricans* (14). An additional benefit is that *D. vulgaris* can reduce Cr(VI) in a simple mineral medium, whereas *E. cloacae* requires a rich heterotrophic medium in order to reduce Cr(VI) (20). The use of H_2 as an electron donor for Cr(VI) reduction may be particularly attractive, as it does not leave a residue of soluble organic compounds in the treated water. Like the U(VI) reductase activity in *D. desulfuricans* (14), the Cr(VI)-reducing capability of *D. vulgaris* is quite stable, as cells that have been freeze-dried and stored under air at room temperature retain their ability to reduce Cr(VI).

As previously discussed (17), the finding that cytochrome c_3 can function as a Cr(VI) reductase points out the possibility of developing cell-free systems for treating Cr(VI)-containing wastes. Although detailed kinetic studies have yet to be conducted, the rate of Cr(VI) reduction by cytochrome c_3 in Fig. 3 is 40-fold faster than the V_{max} previously reported for the Cr(VI) reductase from *P. ambigua* (24). Thus, *D. vulgaris* and/or cytochrome c_3 should be considered as potential Cr(VI)-reducing systems in situations for which bioremediation of chromate-contaminated waters and soils is being contemplated.

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