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Chromium(VI) reductase activity is associated with the cytoplasmic membrane of anaerobically grown *Shewanella putrefaciens* MR-1

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C.R. MYERS, B.P. CARSTENS, W.E. ANTHOLINE AND J.M. MYERS. 2000. *Shewanella putrefaciens* MR-1 can reduce a diverse array of compounds under anaerobic conditions, including manganese and iron oxides, fumarate, nitrate, and many other compounds. These reductive processes are apparently linked to a complex electron transport system. Chromium (Cr) is a toxic and mutagenic metal and bacteria could potentially be utilized to immobilize Cr by reducing the soluble and bioavailable state, Cr(VI), to the insoluble and less bioavailable state, Cr(III). Formate-dependent Cr(VI) reductase activity was detected in anaerobically grown cells of *S. putrefaciens* MR-1, with highest specific activity in the cytoplasmic membrane. Both formate and NADH served as electron donors for Cr(VI) reductase, whereas L-lactate or NADPH did not support any activity. The addition of 10 μmol l^{-1} FMN markedly stimulated formate-dependent Cr(VI) reductase, and the activity was almost completely inhibited by diphenyliodonium chloride, an inhibitor of flavoproteins. Cr(VI) reductase activity was also inhibited by *p*-chloromercuriphenylsulphonate, azide, 2-heptyl-4-hydroxyquinolone-*N*-oxide, and antimycin A, suggesting involvement of a multi-component electron transport chain which could include cytochromes and quinones. Cr(V) was detected by electron paramagnetic resonance (EPR) spectroscopy, suggesting a one-electron reduction as the first step.

INTRODUCTION

While chromium (Cr) is widespread in the environment in trace amounts, large amounts of Cr are used in a variety of industries, including the production and use of chromate pigments, stainless steel and other high tensile structural steels, zinc chromate primer paints used on aircraft, chrome plating, offset printing, photography, certain cleaning agents and leather tanning. Significant Cr discharge into the environment can be associated with these various industries.

In aqueous environments, there are two stable oxidation states of Cr, Cr(VI) and Cr(III). Cr(VI) is very water-soluble and bioavailable (Buttner and Beyersmann 1985; Bauthio 1992), and is the form used in many industrial applications. In contrast, Cr(III) compounds are generally quite insoluble, kinetically relatively inert (Bartlett and James 1988) and much less bioavailable (Kortenkamp *et al.* 1987). Exposure to Cr compounds is associated with a wide array of toxic effects (Tandon 1982; Langaard and Norseth 1986), and Cr compounds are also mutagenic (Venitt and Levy 1974) and teratogenic (Bauthio 1992). The bulk of *in vitro* studies have implicated exposure to Cr(VI) compounds as the predisposing factor to Cr-induced genotoxicity (Tsapakos *et al.* 1983; Cantoni and Costa 1984; Christie *et al.* 1984; Levy and Venitt 1986). Factors which influence the reduction of Cr(VI) in the environment therefore have important implications for Cr bioavailability and toxicity.

Shewanella putrefaciens is a Gram-negative facultative anaerobe that can reduce a number of metals at high rates, including manganese(III/IV), iron(III), uranium(VI) and technetium(VII) (Myers and Nealson 1988a,b, 1990a; Lovley

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et al. 1989, 1991; Lloyd and Macaskie 1996). *Shewanella putrefaciens* MR-1 can generate energy for growth via the respiratory-linked reduction of certain metals including Fe(III) and Mn(IV) (Myers and Nealson 1990b). This work describes preliminary studies on the Cr(VI) reductase activity of this bacterium, knowledge of which is essential to understanding its potential effects on the fate of Cr(VI) in contaminated environments and its potential utility for the bioremediation of Cr(VI)-contaminated industrial effluents.

MATERIALS AND METHODS

Organism, medium and growth conditions

Shewanella putrefaciens MR-1 (Myers and Nealson 1988a; Myers and Myers 1993a, 1994) cells were grown in an anaerobic chamber (Myers and Myers 1992b) to mid-logarithmic phase at 23–25 °C in defined medium (Myers and Nealson 1990b) supplemented with 15 mmol l^{-1} lactate and vitamin-free Casamino acids (0·1 g l^{-1}); 24 mmol l^{-1} fumarate was added to the medium as the electron acceptor. It has been previously demonstrated that MR-1 has the ability to reduce various metals, including Fe(III) and Mn(IV), when grown under these anaerobic conditions (Myers and Nealson 1990b).

Isolation and purification of subcellular fractions

Cytoplasmic membrane (CM), intermediate membrane (IM), outer membrane (OM) and soluble fractions (periplasm plus cytoplasm) were purified using an EDTA-lysozyme-Brij protocol described and utilized previously (Myers and Myers 1992ab, 1993a, 1997abc); final dialysis of purified fractions was against 0·5 mmol l^{-1} HEPES pH 7·5/0·5 mmol l^{-1} phenylmethylsulphonyl fluoride (PMSF). The IM is a hybrid of CM and OM and has been previously characterized from MR-1 (Myers and Myers 1992b); such intermediate hybrid membrane fractions have been observed in other bacteria (Osborn *et al*. 1972; Kent and Wisnieski 1983; Barbas *et al*. 1986; Myers and Collins 1987, 1988). The separation and purity of these subcellular fractions was assessed by spectral cytochrome content (Myers and Myers 1992b), NADH oxidase activity (Osborn *et al*. 1972), membrane buoyant density (Myers and Myers 1992b) and SDS-PAGE gels (Laemmli 1970; Myers and Collins 1986). SDS-PAGE gels were stained for protein with ISS ProBlue (Owl Separation Systems, Portsmouth, NH, USA) or for heme as previously described (Myers and Myers 1992b). Protein was determined by the Lowry method, modified as described (Collins and Hughes 1983), with bovine serum albumin as the standard.

Enzyme assays and analytical procedures

Cr(VI) reductase activity was determined using a protocol modified from Pratt and Myers (1993). For most experiments, 0·13–0·15 mg CM were used. However, for initial experiments, various amounts (0·09–0·52 mg) of subcellular fractions were used to confirm that the rates were a function of the amount of enzyme added (i.e. substrate was not limiting). Subcellular fractions of MR-1 were incubated in glass tubes in a total volume of 2·5 ml containing 28 mmol l^{-1} potassium phosphate (pH 7·5) and 10 mmol l^{-1} sodium formate (pH 7·5) under O_2-free conditions in an anaerobic chamber (Pratt and Myers 1993) at room temperature (approximately 23 °C). Samples were pre-incubated for 5 min prior to the addition of Na_2CrO_4 to give a final concentration of 20 μmol l^{-1}. Of the Cr(VI) species, chromate dominates in water at a pH of 6–8 (Calder 1988) and is the form of Cr(VI) expected for environments typically inhabited by *S. putrefaciens*. The reduction of Cr(VI) was stopped by the addition of 127·5 μl 2 mol l^{-1} Na_2CO_3 (2·5 ml l^{-1} reaction volume). The concentration of remaining Cr(VI) was measured colorimetrically by 1,5-diphenyl-carbazide (DPC) in a sulphuric acid solution (pH 2) (Sandell 1959) after removal of interfering reducing material by a charcoal/aluminium oxide mixture (Ryberg 1986; Pratt and Myers 1993; Myers and Myers 1998a). Specifically, 400 mg charcoal/Al_2O_3 (1/1, w/w) were added to adsorb potentially interfering reducing material; after filtration through 0·2 μm syringe filters, 1·0 ml filtrate was mixed with 1·0 ml 1% DPC/2 mol l^{-1} H_2SO_4 (1/1, v/v). After 15 min, the absorbance at 540 nm was read against a blank in which Na_2CrO_4 was omitted; Cr(VI) concentrations were determined from a standard curve with Na_2CrO_4 as the standard. In some experiments, other potential electron donors substituted for formate as indicated.

The effects of electron transport inhibitors and other compounds on Cr(VI) reductase activity were examined. Stock solutions of these compounds were prepared just before use as follows: 10 mmol l^{-1} *p*-chloromercuriphenylsulphonate (pCMPS), 0·1 mol l^{-1} sodium azide and 0·1 mol l^{-1} potassium cyanide (KCN) stocks were in distilled water; 5 mmol l^{-1} 2-heptyl-4-hydroxyquinolone-*N*-oxide (HQNO) and 25 mmol l^{-1} antimycin A were in 95% ethanol; 10 mmol l^{-1} diphenyliodonium chloride (DPIC) was dissolved in 10% dimethylsulphoxide (DMSO); 2·5 mmol l^{-1} flavin mononucleotide (FMN) was dissolved in water. The final concentrations used are listed in the Results.

Electron Paramagnetic Resonance (EPR) Spectroscopy

Cr(V), a d^1 paramagnetic species, has a distinct EPR spectrum at conventional X-band frequency that consists of a sharp line at $g = 1{\cdot}98$ (Stearns and Wetterhahn 1994; Molyneux and Davies 1995). Relative changes in the levels of Cr(V) with time can be estimated by comparison of changes in the signal intensity (Stearns and Wetterhahn 1994). Cr(VI) reduction experiments were established as described above

except that initial Cr(VI) levels were 400 μmol l^{-1} to provide an adequate signal for detection by EPR, and the amount of CM was increased to 1·5 mg. Aliquots (0·3 ml) of the Cr(VI) reduction assays were taken at periodic intervals and frozen in quartz tubes by immersion in liquid nitrogen (77 K). The samples were stored at 77 K for up to 1 week until analysed by EPR; it has been demonstrated that Cr(V) is stable for at least several months at 77 K. The samples in the quartz tubes were placed in a finger Dewar with liquid nitrogen and the EPR spectra were recorded using a Varian Century Series Spectrometer, which includes a Gauss meter for magnetic field calibration and a frequency counter. Instrument settings were as follows: 1·0 G modulation amplitude, 4×10^4 receiver gain, 0·128 s time constant, 9·078 GHz microwave frequency, sweep width = 400 G, field set = 3300 G, modulation frequency = 100 kHz, scan time = 2 min; all samples were analysed at two levels of microwave power, 16 and 34 dB. Representative scans were repeated to verify reproducibility.

RESULTS

Localization of Cr(VI) reductase activity

Cells of MR-1 were fractionated into purified membrane fractions (CM, IM and OM) and a soluble fraction (periplasm plus cytoplasm) by an EDTA-lysozyme-Brij protocol as described and used by Myers and Myers (1992b, 1993a,b, 1997a,c, 1998b). These subcellular fractions were comparable with the analogous fractions from previous experiments on the basis of cytochrome content and buoyant density (Table 1), as well as NADH oxidase activity (Fig. 1). The SDS-PAGE profiles (Fig. 2) were comparable with those from previous experiments (Myers and Myers 1992b, 1993a, 1997ac). Together, these data indicated a prominent separation of the various subcellular fractions of MR-1.

To assay for Cr(VI) reductase activity *in vitro*, formate

Table 1 Characteristics of membrane fractions isolated from cells grown anaerobically with fumarate*

Subcellular fraction†	Buoyant density (g cm^{-3})‡	Specific cytochrome content§
CM	1·079 (± 0·005)	0·674 (± 0·045)
IM	1·194 (± 0·003)	1·87 (± 0·221)
OM	1·230 (± 0·005)	2·24 (± 0·042)
Soluble	—	1·44 (± 0·163)

* All values represent the mean (± S.D.) for three different preparations.
† CM, cytoplasmic membrane; IM, intermediate membrane; OM, outer membrane; soluble, periplasm plus cytoplasm.
‡ Determined by isopycnic ultracentrifugation in sucrose gradients.
§ Expressed as the difference between the absorbances at the peak and trough of the Soret region from reduced-minus-oxidized difference spectra per mg of protein (Myers and Myers 1992b).

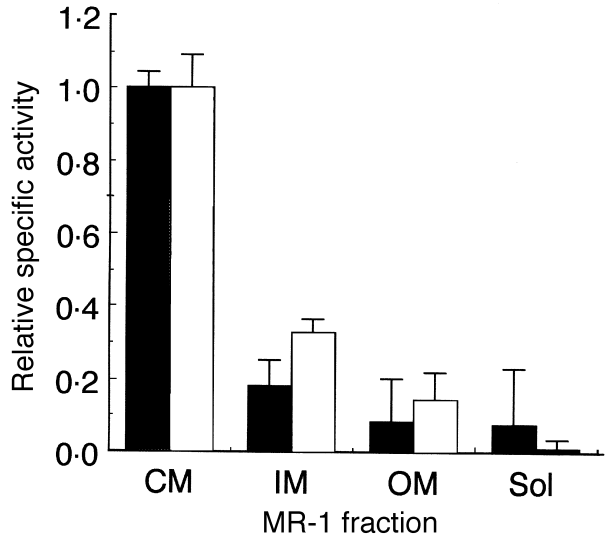

Fig. 1 Distribution of Cr(VI) reductase and NADH oxidase activities among subcellular fractions prepared from anaerobically grown MR-1 cells. Specific activities are expressed relative to that observed with CM, which was arbitrarily set to 1·0. Results represent the mean ± SD from duplicate assays of each of three independent subcellular fractionations. (■), Cr(VI) reductase; (□), NADH oxidase

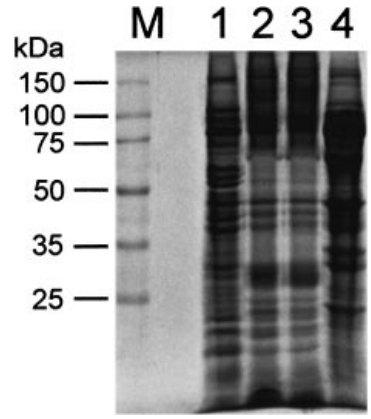

Fig. 2 SDS-PAGE profiles of subcellular fractions prepared from MR-1 cells grown anaerobically with fumarate. The gel was stained for protein with ProBlue stain. Lanes 1–4 were loaded with 50 μg protein of each of the following fractions: CM, cytoplasmic membrane (1); IM, intermediate membrane (2); OM, outer membrane (3); and soluble, periplasm plus cytoplasm (4). The sizes of the molecular mass markers (M) in kDa are indicated at left

was used as the electron donor because it is a recognized physiological electron donor in this bacterium (Lovley *et al.* 1989; Myers and Nealson 1990a). Formate-dependent Cr(VI) reductase activity was readily detected in the CM of cells grown under anaerobic conditions, with values ranging from 2·3 to 3·8 nmol min^{-1} mg^{-1} protein in CM fractions from three independent subcellular fractionations. Relative to the CM, the other subcellular fractions of MR-1 had much lower specific Cr(VI) reductase activities (Fig. 1). The relative distribution of Cr(VI) reductase among the subcellular fractions approximated to that of NADH oxidase (Fig. 1), a CM-associated marker (Osborn *et al.* 1972; Scott *et al.* 1976; Nishimura *et al.* 1986; Myers and Myers 1992b), further supporting its localization to the CM.

As formate was used as the electron donor for Cr(VI) reductase assay, the level of formate dehydrogenase (FDH) in these subcellular fractions could potentially limit the relative Cr(VI) reduction rates. However, this was not the case as the FDH levels (Myers and Myers 1993a) were at least 100-fold that of Cr(VI) reductase, indicating that the donor FDH activity was not a limiting factor in detecting or quantifying the Cr(VI) reductase activity.

Characterization of Cr(VI) reductase

As Cr(VI) reductase activity was found primarily in the CM, the remaining studies were conducted using CM. Essentially, no Cr(VI) reductase activity was seen when the CM was heat-denatured prior to use, or when formate was omitted (Fig. 3). The addition of ferric chloride did not change the rate of Cr(VI) reduction catalysed by the CM (Fig. 3).

Alternative electron donors were tested for their ability to support Cr(VI) reduction. Relative to formate, neither L-lactate nor NADPH supported Cr(VI) reduction, whereas NADH supported even higher rates than formate (Fig. 4).

The effects of various electron transport inhibitors on formate-dependent Cr(VI) reductase activity were assessed. pCMPS inhibited Cr(VI) reductase by 87% (Table 2), which is much greater than its approximately 40% inhibition of formate dehydrogenase (Myers and Myers 1993a). These data suggested that an active sulphhydryl centre (e.g. iron-sulphur protein) (Kröger and Innerhofer 1976) was part of the formate-dependent Cr(VI) reductase. Azide inhibited Cr(VI) reductase by 51% (Table 2) but had little effect on formate dehydrogenase (Myers and Myers 1993a). Cyanide markedly inhibited Cr(VI) reductase (Table 2) but this could be the result of an ability to prominently inhibit the formate dehydrogenase of MR-1 (Myers and Myers 1993a). Both HQNO and antimycin A markedly inhibited Cr(VI) reductase activity (Table 2) but have essentially no effect on formate dehydrogenase (Myers and Myers 1993a). These data suggest the involvement of *b*- and *c*-type cytochromes (Singer 1979; de Vries *et al.* 1982) in the formate-dependent reduction of Cr(VI). The CM of MR-1 contains both *b*- and *c*-type cytochromes (Myers and Myers 1992b). As HQNO also acts as inhibitor of certain quinone sites (Cox *et al.* 1970; Newton

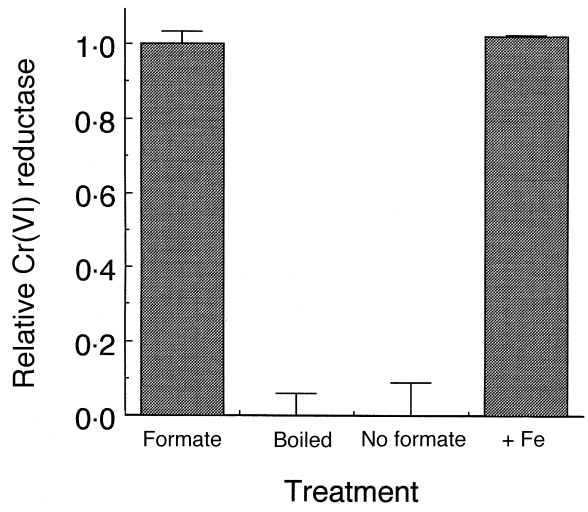

Fig. 3 Effect of various treatments on the *in vitro* Cr(VI) reductase activity of the CM. Formate, CM + 10 mmol l^{-1} formate; boiled, CM boiled for 10 min before use +10 mmol l^{-1} formate; no formate, CM only; +Fe, CM + 10 mmol l^{-1} formate + 13 μmol l^{-1} $FeCl_3$. Results represent the mean ± SD from duplicate assays of each of three independently prepared cytoplasmic membrane fractions

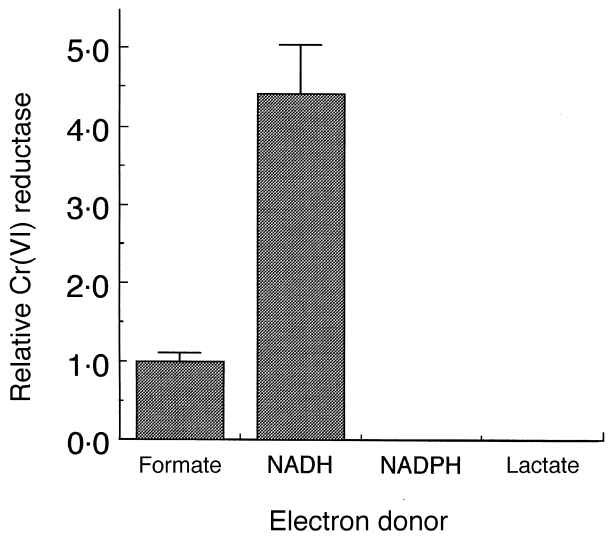

Fig. 4 Effect of alternate electron donors on the relative *in vitro* Cr(VI) reductase activity of the CM. The final concentration of each electron donor was 2·0 mmol l^{-1}. Activities are expressed relative to that observed with formate, which was arbitrarily set to 1·0. Results represent the mean ± SD from duplicate assays of each of two independently prepared cytoplasmic membrane fractions

Table 2 Effect of metabolic inhibitors on the *in vitro* anaerobic reduction of Cr(VI) by the cytoplasmic membrane of MR-1

Inhibitor*	Relative Cr(VI) reductase activity†
None	1·000 ± 0·063
0·5 mmol l^{-1} pCMPS	0·128 ± 0·181
1·0 mmol l^{-1} azide	0·490 ± 0·204
0·2 mmol l^{-1} KCN	0·346 ± 0·238
None (ethanol vehicle)	1·000 ± 0·105
0·01 mmol l^{-1} HQNO	0·335 ± 0·109
0·1 mmol l^{-1} antimycin A	0·180 ± 0·209

* pCMPS, *p*-Chloromercuriphenylsulphonate; KCN, potassium cyanide; HQNO, 2-heptyl-4-hydroxyquinolone-*N*-oxide.
† Activity seen relative to that with no inhibitor. Results represent the mean ± S.D. from duplicate assays of each of three independently prepared cytoplasmic membrane fractions.

et al. 1971), it is possible that quinones are part of the electron transport chain linking formate oxidation to Cr(VI) reduction.

DPIC, an inhibitor of FMN-containing flavoproteins (O'Donnell *et al.* 1994), completely inhibited Cr(VI) reductase relative to the control with DMSO, the solvent for DPIC (Fig. 5). The addition of 10 μmol l^{-1} FMN stimulated Cr(VI) reductase approximately 20-fold (Fig. 5), consistent with the ability of FMN to mediate electron transfer between a wide variety of chemicals and functional groups (Massey and Hemmerich 1980). Stimulation by FMN was blocked by DPIC (Fig. 5). The stimulation by FMN was not the result of a direct interaction between FMN and Cr(VI) because FMN had no effect on Cr(VI) reduction when L-lactate was the electron donor.

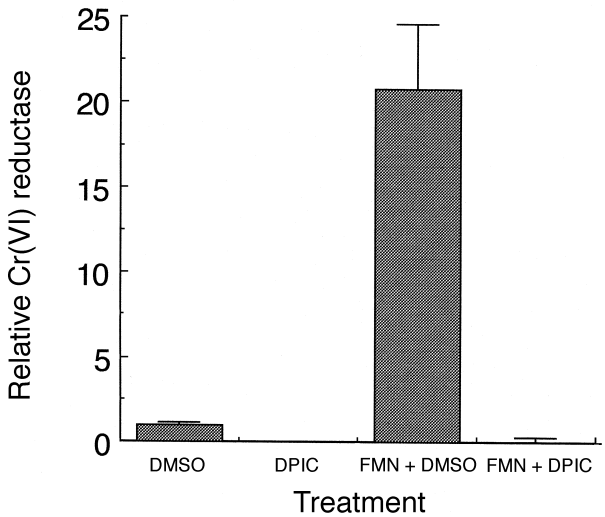

Fig. 5 Effect of a flavoprotein inhibitor (DPIC, 0·1 mmol l^{-1}) and FMN (10 μmol l^{-1}) on the relative *in vitro* formate-dependent Cr(VI) reductase activity of the CM. Activities are expressed relative to that observed with an equal volume of 10% DMSO, the solvent for DPIC. Results represent the mean ± SD from duplicate assays

EPR spectra clearly showed a production of Cr(V) ($g = 1{\cdot}98$) during reduction of Cr(VI) by the CM (Fig. 6). The Cr(V) signal was absent when either Cr(VI) or formate or CM was excluded (not shown), implying that the Cr(V) signal is due to the reduction of Cr(VI) by formate-dependent electron transport components in the CM. The prominent Cr(V) signal was detected at the earliest time point, and this signal persisted over a 2 h time course at the end of which, Cr(VI) reduction was not complete (Fig. 7).

DISCUSSION

The reduction of Cr(VI) has been reported for a number of bacterial genera, including *Pseudomonas* (Bopp and Ehrlich 1988; Gopalan and Veeramani 1994; Bhide *et al.* 1996; Turick and Apel 1996), *Bacillus* (Campos *et al.* 1995), *Enterobacter* (Wang *et al.* 1989; Rege *et al.* 1997), *Thiobacillus* (Sisti *et al.* 1996) and sulphate-reducing bacteria (Fude *et al.* 1994). In some cases, the reduction of Cr(VI) is indirectly mediated by the production of reducing agents as metabolites; for example, *Thiobacillus ferrooxidans* generates sulphite and thiosulphate which can reduce Cr(VI) at pH 1·7 (Sisti *et al.* 1996), and sulphate-reducing bacteria reduce Cr(VI) indirectly via the production of sulphide (Fude *et al.* 1994). In other cases, the bacteria utilize sugars (e.g. molasses (Bhide *et al.* 1996), glucose (Campos *et al.* 1995) or sucrose (Rege *et al.* 1997)) as carbon and energy sources to mediate Cr(VI) reduction. Cells of *Enterobacter* can also use acetate or glycerol to support Cr(VI) reduction (Wang *et al.* 1989), whereas *Pseudomonas fluorescens* can metabolize citrate (DeLeo and Ehrlich 1994). As far as is known, MR-1 represents the first case of a bacterium that can use formate to mediate Cr(VI) reduction via a CM-associated electron transport chain.

While the localization of the Cr(VI) reductase activity in some strains has not been determined, the activities in *Bacillus* strain QC1-2 (Campos *et al.* 1995) and *Pseudomonas putida* (Ishibashi *et al.* 1990) are associated with the cytosolic and soluble fractions, respectively. In contrast, the Cr(VI) reductase activities in *Enterobacter* and *Ps. fluorescens* appear to be membrane-associated (Bopp and Ehrlich 1988; Wang *et al.* 1990). The Cr(VI) reductase activity in MR-1 is clearly localized to the CM, in comparison with the periplasmic localization of its fumarate reductase (Myers and Myers 1992a) and the OM localization of its ferric reductase (Myers and Myers 1993a), suggesting that the Cr(VI) reductase activity is distinct from that of fumarate and iron reductases.

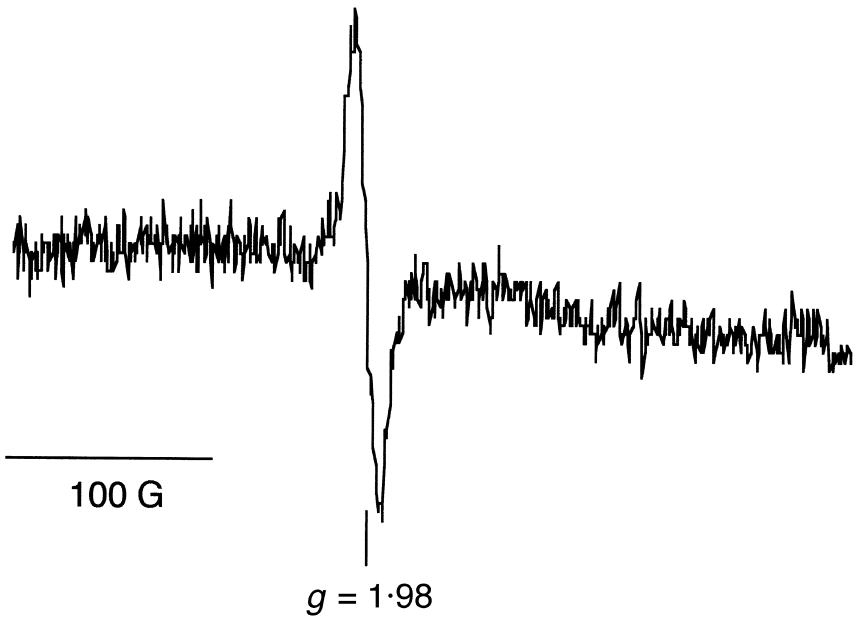

Fig. 6 EPR spectrum of Cr(V) generated by CM under anaerobic conditions. EPR microwave power was 16 dB. All other EPR instrument settings were as described in the methods. The spectra were collected under stringent conditions (1 G modulation amplitude); the signal intensity becomes much greater at higher modulation amplitudes

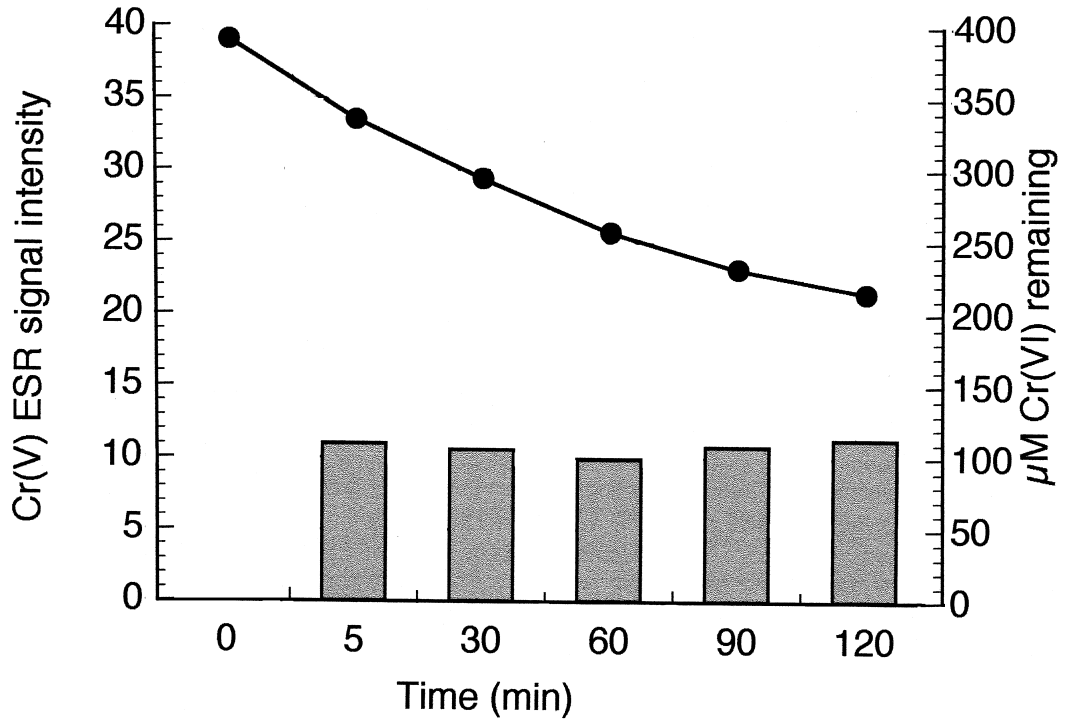

Fig. 7 Time course of Cr(V) signal intensity at 16 dB (vertical bars) relative to the level of Cr(VI) remaining at each time point (●). The Cr(V) signal intensity represents the $I\Delta H^2$ of the first derivative spectrum (I = peak-to-trough intensity; ΔH = line width between peak minimum and maximum) (Stearns and Wetterhahn 1994). The pattern of Cr(V) signal intensity was not changed significantly by using I to represent signal intensity

As the TMAO reductase (TorA) from MR-1 is very similar in sequence to the periplasmic TorA of *Shewanella massilia* (Czjzek *et al*. 1998), it seems likely that the Cr(VI) reductase activity of MR-1 is also distinct from that of its TMAO reductase. While MR-1 can also reductively dechlorinate tetrachloromethane (Petrovskis *et al*. 1994), this activity in *S. putrefaciens* 200 is associated with both the membrane and periplasmic fractions (Picardal *et al*. 1993), suggesting that it is distinct from the CM-associated Cr(VI) reductase reported here.

As Fe(II) can reduce Cr(VI) (Myers and Myers 1998a), it would be possible for Cr(VI) reduction to be driven indirectly by the reduction of Fe(III) to Fe(II). However, the fact that iron did not stimulate Cr(VI) reduction by the CM implies that Cr(VI) reductase activity was not the result of iron reduction. However, as the Fe(III) reductase of MR-1 is localized in its outer membrane (Myers and Myers 1993a), it is possible that intact cells could mediate Cr(VI) reduction in two ways: (i) directly by the CM-associated Cr(VI) reductase described here, and (ii) indirectly due to chemical reduction by Fe(II) generated by the Fe(III) reductase in the outer membrane. The latter implies that in environments where both metals are present, Fe(II) should not accumulate until Cr(VI) reduction is complete. This is similar to the reported interaction of Fe(II) and MnO_2 (Myers and Nealson 1988b).

As these cells were grown with fumarate as the electron acceptor, it is apparent that Cr(VI) is not necessary during growth for the expression of Cr(VI) reductase activity. In addition, as cell fractionation procedures were conducted in the presence of O_2, it is apparent that Cr(VI) reductase activity is not irreversibly inhibited by exposure to O_2. As there are no known 3-electron donors in biological systems, the reduction of Cr(VI) to Cr(III) cannot occur as a single step. The Cr(V) EPR data (Fig. 6) indicate that at least some of the Cr(VI) reduction must therefore proceed through an initial single electron transfer. As the Cr(V) signal did not continually increase throughout the time course (Fig. 7), it is possible that Cr(V) is not the terminal product and that at least some of the Cr(V) was subsequently being reduced to Cr(III), the next stable oxidation state. As Cr(IV) is difficult to detect, it is not known whether this intermediate was formed as part of the reduction process.

During these experiments, the CM of MR-1 could reduce initial levels of 20 μmol l^{-1} Cr(VI) to undetectable levels, suggesting that it could serve to reduce soluble Cr(VI) in contaminated systems completely. In preliminary experiments, cells of MR-1 in LM medium (Myers and Myers 1994) supplemented with fumarate and either formate or lactate could similarly reduce sequential additions of 20 μmol l^{-1} Cr(VI) to undetectable levels under anaerobic conditions. It is currently unknown whether MR-1 cells can generate energy by using Cr(VI) as a terminal electron acceptor for anaerobic respiration. The localization of Cr(VI) reductase activity to the CM, and the inhibition by various electron transport inhibitors, suggests that energy generation may be possible. However, assessment of growth linked to Cr(VI) reduction is difficult to determine in batch culture because $\geqslant 100\,\mu$mol l^{-1} CrO_4^{2-} is toxic to cells under these conditions.

In summary, Cr(VI) reductase activity was found to be associated with the CM of anaerobically grown MR-1 cells, and either formate or NADH could serve as electron donors. Based on its localization and other properties, the Cr(VI) reductase was distinct from the fumarate and Fe(III) reductases in this bacterium, and was not irreversibly inhibited by exposure to O_2. Inhibitor studies suggest the involvement of a multi-component electron transport chain that could include cytochromes, quinones, flavoproteins, and proteins with iron-sulphur centres. Additional studies on the Cr(VI) reductase activity in MR-1 will be useful in assessing its potential for the bioremediation of Cr(VI)-contaminated sites or industrial effluents.

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