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Chromate reduction by *Rhodobacter sphaeroides*

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Rhodobacter sphaeroides **grew in the presence of up to 43 μM chromate and reduced hexavalent chromium to the trivalent form under both aerobic and anaerobic conditions. Reduced chromium remained in the external medium. Reductase activity was present in cells of *Rb. sphaeroides* independent of whether chromate was present or not in the growth medium. The reducing activity was found in the cytoplasmic cell fraction and was dependent on NADH. The chromate-reducing enzyme was purified by anion exchange, hydroxyapatite and hydrophobic interaction chromatography, and gel filtration. The molecular weight of the enzyme was 42 kDa as determined by gel filtration. The optimum of the reaction is at pH 7.0 and 30°C. The enzyme activity showed a hyperbolic dependence on the concentrations of both substrates, NADH and chromate, with a maximum velocity at 0.15 mM NADH. A K_m of 15±1.3 μM CrO_4^{2-} and a V_{max} of 420±50 μmol min^{-1} mg $protein^{-1}$ was determined for the enzyme isolated from anaerobically grown cells and 29±6.4 μM CrO_4^{2-} and 100±9.6 μmol CrO_4^{2-} min^{-1} mg $protein^{-1}$ for the one from aerobically grown ones.** *Journal of Industrial Microbiology & Biotechnology* (2000) **25,** 198–203.

Keywords: *Rhodobacter sphaeroides*; chromate reduction; cytoplasmic localization; enzyme purification

Introduction

Chromium is present in the environment as either Cr(III) or Cr(VI). Chromate (Cr(VI)) is highly soluble; in bacteria, it is transported rapidly across the cell membranes via the sulfate pathway and reduced in the cytoplasm to trivalent Cr(III). Trivalent chromium, which interacts with proteins and nucleic acids, however, is far less soluble than hexavalent chromate and does not pass through biological membranes [25].

The high solubility of hexavalent chromium makes it difficult to remove the metal from waste water and leachates by conventional precipitation methods. During recent years, many bacteria were discovered which reduce Cr(VI) to Cr(III). *Pseudomonas putida* reduces chromate aerobically [14], while for *Escherichia coli* [28], *P. fluorescens* [5], and *Bacillus* sp. [7], chromate reduction was shown under both aerobic and anaerobic culture conditions. Furthermore, many indigenous soil bacteria reduce hexavalent chromium under aerobic conditions [3]. Cr(VI) reduction seems to be ubiquitous in soil bacteria similar to the reduction of chlorate [9]. Recently, Tebo and Obraztsova [32] discovered a sulfate-reducing bacterium that grows on hexavalent chromium, using it even as sole electron acceptor. With this variety of organisms, the removal of chromium by biological processes [34] may become possible at neutral pH, without chemical additives and without formation of toxic byproducts. The enzyme-reducing chromate has been isolated from several organisms. The reducing activity in *Pseudomonas* and *Aeromonas* strains grown under anaerobic conditions is located in membrane preparations [23]. In contrast, *P. putida* reduces hexavalent chromate aerobically [14] and here, the activity was found in the soluble protein fraction. Chromate reduction was also detected in *Rhodobacter sphaeroides* [21], with the chromate-reducing activity located in the soluble fraction.

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Many members of the Rhodospirillaceae show a high resistance towards toxic heavy metal oxides and oxyanions [19]. For *Rb. sphaeroides*, resistance to selenite, tellurite, and a number of rare earth oxides as well as chromate was demonstrated. These bacteria grow phototrophically under anaerobic conditions as well as both aerobically and anaerobically in the dark [13,26]. They reduce a wide range of oxyanions and often deposit the elemental form within the cells [16,20]. We examined the ability of *Rb. sphaeroides* to reduce hexavalent chromate, and characterized the reduction reaction and the enzyme involved.

Materials and methods

Bacterial strains and culture conditions

Rb. sphaeroides 158 (ATCC 17023) was grown at 30°C anaerobically in the light (14 W m^{-2}) in 100 ml screw-capped bottles using the medium of Sistrom [30] containing 2.4 g l^{-1} succinic acid as carbon source and 1 g l^{-1} casein hydrolysate. The bottles were flushed with N_2 for 2 min before inoculation [22]. For aerobic cultures, 250 ml Erlenmeyer flasks filled with 100 ml of medium were incubated at 30°C in the dark on a rotary shaker (200 rpm).

Rb. capsulatus (ATCC 11166), *Rs. rubrum* S1 (ATCC 11170), *Rc. tenuis* (ATCC 25093), and *Rb. blasticus* (ATCC 33485) were grown similarly under the same conditions.

Experimental conditions

Sterile K_2CrO_4 was added in various concentrations as indicated. Controls were done with sterile medium and heat-killed cells.

Resting cells were produced by washing bacteria from the mid-exponential growth phase with salt solution (Sistrom medium lacking the C-source and casein hydrolysate) and suspending in the same solution to 1.3×10^8 cells ml^{-1} (measured as turbidity calibrated by microscopic cell counting) [24]. Permeabilized cells were produced with 2% (v/v) Triton X-100 after Campos *et al.* [7] and suspended in 20 mM Tris–HCl buffer pH 7.0 to 1.3×10^8 cells ml^{-1} for 2 h.

Cell extracts were obtained from washed cells from mid-exponential phase cultures, suspended in 50 mM Tris–HCl pH 8.0 (containing 50 μg ml^{-1} DNAse, 0.1 mM phenylmethylsulfonyl fluoride, 4 mM iodoacetamide, and 2 mM EDTA) at 50-fold concentration and disrupted in a French pressure cell. The homogenate was centrifuged at 5000×*g* for 15 min to remove cell debris and intact cells, then at 20,000×*g* for 20 min to obtain the membrane fraction. The supernatant fluid was then centrifuged at 150,000×*g* for 1 h to separate the chromatophores from the cytoplasm [15]. Sphaeroplasts produced after the method of Sabaty *et al.* [27] were used to obtain cytoplasm and periplasm separately.

For the purification of chromate reductase, the soluble fraction was fractionated on a DE-52 column (2.2×5.5 cm^2, Whatman Ltd., Maidstone, Great Britain) equilibrated with 20 mM Tris–HCl pH 7.0. The proteins were eluted with a 0.1–0.6 M step gradient of NaCl 20 mM, Tris–HCl pH 7.0, and fractions of 1.5 ml tested for chromate reduction. The active fractions were pooled and applied to a hydroxyapatite column (high resolution, Fluka, Switzerland) equilibrated with 10 mM Na-phosphate buffer pH 7.0. The proteins were eluted with a 10–200 mM step gradient of Na-phosphate buffer at pH 7.0 and the active fractions concentrated by ultrafiltration. Ammonium sulfate was added to a final concentration of 1 M for separation on a butyl-sepharose (Pharmacia, Sweden) column. Proteins were eluted in a 1.0–0 M step gradient of ammonium sulfate in 20 mM Tris–HCl pH 7.0.

The molecular weight of the enzyme was estimated on a FPLC Sephadex G-75 column (Pharmacia) with 50 mM Tris–HCl pH 7.0 containing 0.15 M NaCl as elution buffer. Alcohol dehydrogenase (150 kDa), bovine serum albumin (66 kDa), carbonic anhydrase (29 kDa), and cytochrome *c* (12 kDa) served as molecular weight standards.

Analytical methods

The growth of the bacteria was monitored by the optical density at 660 nm. Hexavalent chromium was determined spectrophotometrically at 540 nm in the supernatant of the cultures after reaction with *S*-diphenylcarbazide in acid solution [33]. Chromate reduction was assayed by following the decrease of hexavalent chromium in the medium. The assay mixture contained the sample, NADH (0.15 mM), Tris–HCl (pH 7.0, 20 mM), and chromate. One unit of chromate reductase is defined as the amount of enzyme which catalyzed the reduction of 1 μmol of chromate per minute at 30°C.

Total chromium in the supernatant of cultures was measured with inductively coupled plasma atomic emission spectroscopy (ICP-AES). To reoxidize reduced chromium, samples were adjusted to pH 8 with 6 M NaOH and another 0.25 ml 6 M NaOH was added per milliliter of sample. The mixture was boiled for 2 min, cooled, and 0.5 ml 1 M NaHClO per milliliter was added. After stirring the solution for 30 s, it was boiled again for 1 min and after cooling it, the sample was assayed for hexavalent chromium [10]. Protein concentration was determined according to Bradford [6], with bovine serum albumin as a standard.

Denaturing sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) was done according to Laemmli [17] and the proteins were silver-stained (modified after Blum *et al.* [4]).

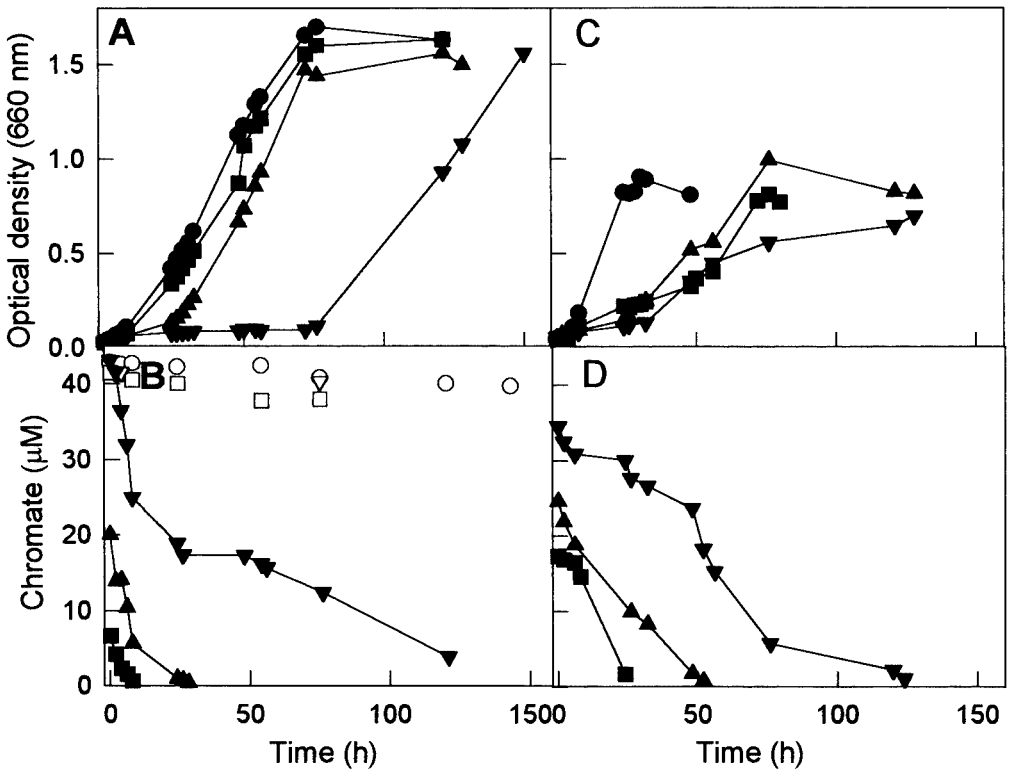

Figure 1 Growth and chromate reduction by *Rb. sphaeroides* in the presence of chromate added to the medium at the beginning of incubation. (A and B) Cells grown anaerobically in the light. (C and D) Cells grown aerobically in the dark. (A and C) Growth. (B and D) Chromate concentration in medium. (○) Sterile medium and (□) autoclaved bacteria with 40 μM CrO_4^{3-}; (▽) total chromium; (●) no chromate; (■, C) 7 μM K_2CrO_4, (D) 17 μM K_2CrO_4; (▲, C) 20 μM K_2CrO_4, (D) 25 μM K_2CrO_4; (▼, C) 43 μM K_2CrO_4, (D) 35 μM K_2CrO_4.

Gel electrophoresis of non-denatured proteins was done with a neutral buffer system. The gels were stacked at pH 7.0 and the proteins separated at pH 8.0 [12] in the presence of 1 mg^{-1} ml lithium dodecyl sulfate, an agent less denaturing than SDS [29]. Gel electrophoresis was done with 30 mA for 90 min at 4°C. The lanes of each sample were cut in small slices which were extracted for 1 h at 4°C in Eppendorf tubes containing 200 μl 20 mM Tris–HCl pH 7.0. The supernatant was assayed for chromate reductase. Active samples were again separated by SDS gel electrophoresis to control the purity of the enzyme and for determination the subunit of molecular weight.

Results

Reduction of chromate by whole cells of Rb. sphaeroides

Rb. sphaeroides grew in the presence of chromate in concentrations of up to 43 μM chromate both anaerobically in the light as well as aerobically in the dark (Figure 1). At this chromate concentration, the lag phase lasted 3 days, but after 150 h, cultures had reached the same density as cells grown in the absence of chromate. Reduction of chromate occurred under both anaerobic and aerobic conditions (Figure 1B and D), though under anaerobic conditions the initial reaction rate was significantly higher than in aerobiosis. Incubation with 20 μM chromate resulted in a reduction rate of 1.6 μmol CrO_4^{2-} h^{-1} for anaerobic and 0.146 μmol CrO_4^{2-} h^{-1} for aerobic cells during the first 6 h after inoculation. The total amount of chromium remained constant throughout the experiments (Figure 1B). Of the six oxidation states of chromium, only two — Cr(III) and Cr(VI) — are associated with microbial activity [8]; the difference between the total amount of chromium and its hexavalent species likely consists of trivalent chromium. By reoxidising the samples, hexavalent chromium was fully recovered. When cells were separated by centrifugation, lysed with alkali, and oxidized, no free chromate was detected; thus, neither Cr(III) nor Cr(VI) had been trapped within the cells. In sterile medium and in medium containing autoclaved bacteria, the amount of hexavalent chromium remained constant (Figure 1B). Thus, reduction of hexavalent chromium to the trivalent form was clearly a biological reaction.

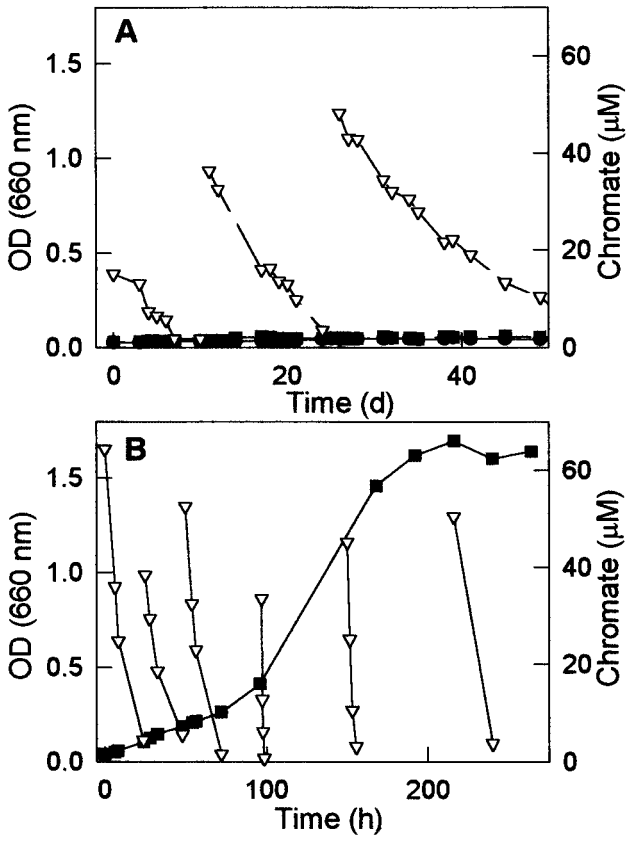

Figure 2 Growth and chromate reduction of *Rb. sphaeroides* with repeated addition of chromate. (A) Anaerobic dark, (B) anaerobic light. (●) Optical density at 660 nm in the absence of chromate; (■) optical density at 660 nm in the presence of chromate; (▽) chromate concentration in medium; repeated addition of CrO_4^{3-} from 15 to 50 μM in (A) and between 35 and 64 μM in (B).

Table 1 Cellular localization of chromate reductase activity in anaerobically grown cells (mean of three experiments)

Fraction	Activity (%)
Periplasm	14±11
Cytoplasm	76±6
Outer membranes	27±9
Chromatophores	8±6

Experiments with other phototrophic bacteria show that likewise, *Rb. capsulatus*, *Rs. rubrum*, *Rc. tenuis*, and *Rb. blasticus* reduced hexavalent chromium with similar rates (data not shown). All these strains reduced 20 μM chromate completely during 25 h of anaerobic incubation in the light.

With *Rb. sphaeroides*, chromate did not act as alternative electron acceptor in anaerobic dark respiration. No growth was observed over 50 days in the dark in the absence of oxygen. However, hexavalent chromium in the medium was still completely reduced, even after repeated addition of chromate (Figure 2A). Under these conditions, the reduction rate was slower than in the light. The rate remained constant after repeated addition of chromate, suggesting that the reduction activity is constitutive. When such cells were placed in the light, they immediately started growing and reached the same optical density after 7 days as cells grown in the light from the beginning. Chromate reduction proceeds under phototrophic conditions in all phases of growth (Figure 2B). Repeated addition of different amounts of chromate, however, did not increase resistance towards the metal.

The reducing rate of resting cells accounted for 80% of cells growing on Sistrom medium; thus, an external carbon source was not needed to reduce chromate (data not shown). Cells permeabilized by Triton X-100 retained only 20% of the activity of control cells, indicating that the rate of reduction was not limited by the transport of hexavalent chromium into the cells, but the process was rather inhibited by a damaged membrane. Interestingly, the addition of 30 μg of cytochrome *c* from *Saccharomyces cerevisiae* to 100 μg protein from the cytoplasm resulted in a 30% stimulation of the chromate reduction rate (data not shown).

Isolation and characterization of chromate reductase from Rb. sphaeroides

Anaerobically grown mid-exponential cells were fractionated into the outer membrane, the periplasm, the cytoplasm, and the

Table 2 Purification scheme of chromate reductase from anaerobically grown cells

Fractionation step	Volume (ml)	Total protein (mg)	Total activity (U)	Specific activity (U mg^{-1})	Yield (%)	Purification (fold)
Cytoplasm	16.3	55.4	93,000	1.68	100	(1)
DE-52	30.0	4.8	91,200	19.0	98	11.3
Hydroxyapatite	7.5	0.5	12,900	26	13.8	15.7
Butyl-sepharose	7.2	0.1	9,200	90	10	53.8
Gel filtration	2.0	0.04	2,730	68	3	40.6

In each purification step, the active fractions from the previous one were concentrated and applied to the next chromatography step. Gel filtration was done with the active fractions from hydroxyapatite chromatography.

chromatophores. The main activity was found in the cytoplasm, indicating that the reductase is a soluble protein (Table 1).

Using ion exchange, hydroxyapatite, and hydrophobic interaction chromatography or gel filtration as the final step, a 40–50-fold purification of the enzyme was obtained (Table 2). The purification process is illustrated by SDS-PAGE (Figure 3A). For anaerobic cells, the effect of all the purification steps from crude extract to the proteins separated by LiDS gel electrophoresis is shown; for aerobic cells, only the results of the last steps of purification are presented. The number of bands decreased with purification. A second LiDS gel electrophoresis resulted in only two bands, both showing chromate reduction activity (Figure 3A, lanes g, h; Figure 3B, lanes d, e). With gel filtration, molecular masses of 35 and 42 kDa, respectively, were determined for the two fractions containing reducing activity. Similar molecular masses were also observed on SDS gels (Figure 3A, lane f). Enzyme activity followed Michaelis–Menten kinetics concerning NADH with a maximum activity at 0.15 mM NADH (Figure 4A). NADPH, instead of NADH, at the same concentration lowered the activity by 45%. The optimal pH of the enzyme was pH 7.0 and the optimal temperature was 30°C (data not shown). For chromate, a K_m of 15±1.3 μM CrO_4^{2-} and a V_{max} of 420±50 μmol CrO_4^{2-} min^{-1} mg^{-1} protein were obtained (Figure 4B). Chromate reductase from aerobically grown cells using the same purification scheme yielded a 40-fold purification. LiDS electrophoresis resulted in the same molecular masses for the active proteins of 35 and 42 kDa (Figure 3B, lanes c and d). Chromate reductase from aerobic cells resulted, however, in slightly different kinetic parameters compared to the enzyme from anaerobic cells which had a K_m of 29±6.4 μM CrO_4^{2-} and a V_{max} of 100±9.6 μmol CrO_4^{2-} min^{-1} mg^{-1} protein (data not shown).

Discussion

Cells of *Rb. sphaeroides* reduced chromate under anaerobic and aerobic conditions. Reoxidation of the product formed indicates that chromate is reduced to its trivalent form and excreted into the medium. Whether there is a specific export mechanism as in other organisms [1,2] remains to be tested.

Rb. sphaeroides starts growing only when a certain amount of the chromate added has been reduced. Thus, the cells have to partially detoxify their environment before growth is initiated. Interestingly during exponential growth, the growth rate is independent of whether chromate is present in the medium and the reduction rate parallels the cell density. To avoid toxic effects, small amounts of chromate were given repeatedly. The reduction rate remained constant after each chromate pulse and no product

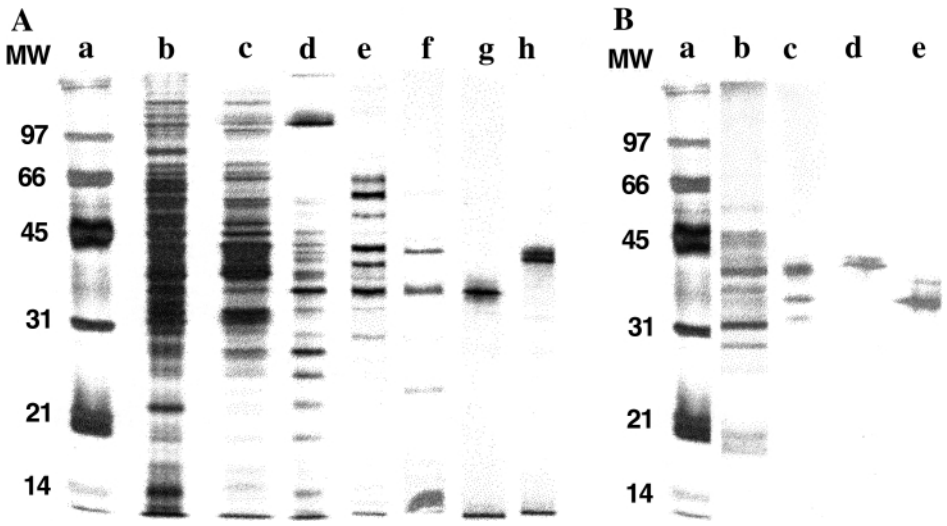

Figure 3 (A and B) SDS gel electrophoresis of the fractions containing chromate reduction activity at various stages of purification (silver staining). (A) From anaerobic cells: (a) molecular weight standards in kiloDalton, (b) crude cytoplasm, (c) active fractions after ion exchange chromatography, (d) active fractions after hydroxyapatite chromatography, (e) active fractions after hydrophobic interaction chromatography (butyl-sepharose), (f) active fractions after gel filtration, (g, h) active bands from LiDS gel electrophoresis. (B) From aerobic cells: (a) molecular weight standards in kiloDalton, (b) active fractions after hydroxyapatite chromatography, (c) active fractions after hydrophobic interaction chromatography, (d, e) active bands from LiDS gel electrophoresis.

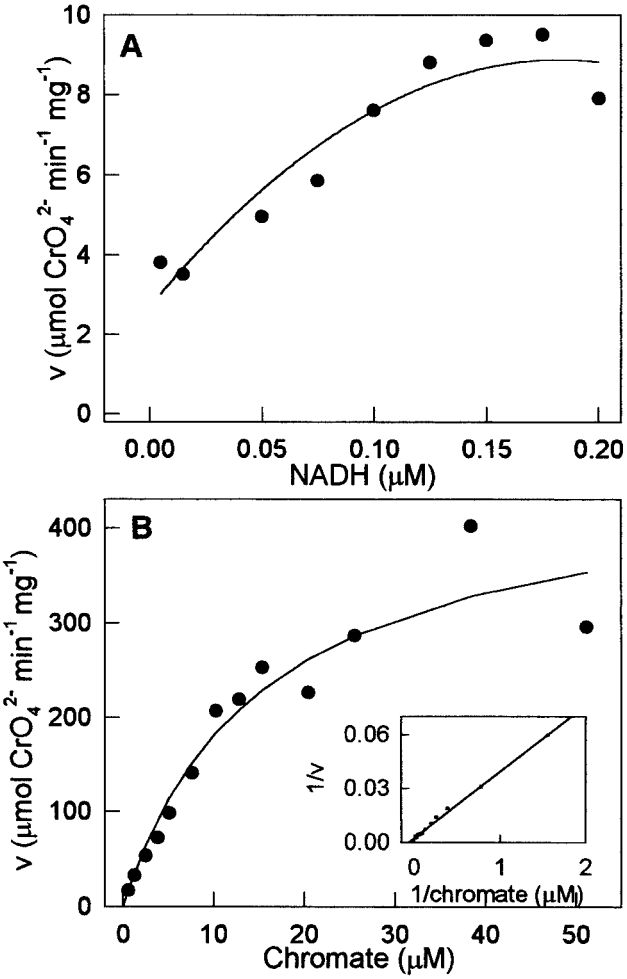

Figure 4 Dependence of chromate reductase from anaerobically grown cells on (A) NADH concentration and (B) chromate concentration. (A) Enzyme preparations after ion exchange chromatography (Figure 3A, lane c) were incubated with varying concentrations of NADH and 2.6 μM chromate. (B) Enzyme preparations after hydroxyapatite chromatography (Figure 3A, lane d) were incubated with varying concentrations of chromate and 0.15 mM NADH. Insert: Hill's plot.

inhibition by trivalent chromium was observed (Figure 2). This is rather important in view of a possible practical use of these bacteria for bioremediation. A continuous reduction system has been presented for *Enterobacter cloacae* in a bioreactor running with a constant chromate feed rate [11]. Furthermore, it must be noted that as in other organisms [5,18], cell growth is not a prerequisite for efficient reduction, as resting cells still reduce chromate with an activity of 80% that of growing ones. The effect of permeabilization suggests that chromate reduction is coupled to metabolic processes requiring an energised membrane [18], similar to *En. cloacae* HO1 [16]. Obviously, *Rb. sphaeroides* needs an intact and functional membrane for the process. This is furthermore strengthened by the molecular properties of the chromate reductase. The protein precipitates in 80% ammonium sulfate and the elution properties in anion exchange and hydrophobic interaction chromatography suggest that it contains hydrophobic surfaces. Although the enzyme is present in the cytoplasmic fraction as in *E. coli* [28] or a *Bacillus* sp. [7], it might have been attached to the cell membrane. Chromate-reducing activity is measured independent of whether chromate was present during growth, as in *P. fluorescens* strain LB300 [5], and is not increased by successive addition of chromate. This indicates that chromate reductase is not induced by chromate. Chromate reductase may be either constitutively present or the activity of an unspecific reductase with a different physiological role. However, the addition of structural isomorphic ions like sulfate, manganate, and phosphate had only a negligible effect on the enzyme activity (data not shown). Reduction in cell-free systems from *Rb. sphaeroides* requires NADH as in *Bacillus* sp. [7], *P. putida* [14], and *P. ambigua* [31], NADPH being less efficient.

It was hoped that the amino acid sequence of the enzyme could offer more information; however, the N-terminal sequence (20 amino acids were sequenced) of the 35 kDa band of the purified chromate reductase had no significant homology to genes available from databanks (data not shown). Both gel filtration and gel electrophoresis indicate two distinct active proteins for the active chromate reductase of *Rb. sphaeroides*, with molecular masses of 42 and 35 kDa. The 35 kDa protein might be a part of the 42 kDa part due to proteolytic cleavage. This was also found for the enzyme from aerobically cultivated cells, suggesting that it is the same protein under anaerobic and aerobic conditions. The reducing activity of the active fractions from hydroxapatite chromatography (Figure 3A, lane d; Figure 4B, lane b) from both aerobically and anaerobically grown cells follows Michaelis–Menten kinetics. In both cases, the temperature optimum of 30°C and the optimal pH of 7.0 were found, identical to the optimal growth conditions. However, the enzyme from aerobically grown cells had a four times lower V_{max} and a two times higher K_m than the enzyme of anaerobic cells (both after the purification with a hydroxyapatite column).

Conclusions

Rb. sphaeroides is highly resistant towards Cr(VI) and has a constitutive chromate reductase. This might be a good basis for its use in bioremediation. Experiments with real liquid waste should allow us to decide whether these bacteria can be used to detoxify chromate pollution in a large-scale process.

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References

1 Alvarez AH, R Moreno-Sanchez and C Cervantes. 1999. Chromate efflux by means of the ChrA chromate resistance protein from *Pseudomonas aeruginosa*. *J Bacteriol* 181: 7398–7400.
2 Anton A, C Grosse, J Reissmann, T Pribyl and DH Nies. 1999. CzcD is a heavy metal ion transporter involved in regulation of heavy metal resistance in *Ralstonia* sp. strain CH34. *J Bacteriol* 181: 6876–6881.
3 Bader JL, G Gonzalez, PC Goodell, AS Ali and SD Pillai. 1999.

Aerobic reduction of hexavalent chromium in soil by indigenous microorganisms. *Biorem J* 3: 201–211.
4 Blum H, H Beier and HJ Gross. 1987. Improved silver staining of plant proteins, RNA and DNA in polyacrylamide gels. *Electrophoresis* 8: 93–99.
5 Bopp LH and HL Ehrlich. 1988. Chromate resistance and reduction in *Pseudomonas fluorescens* strain LB300. *Arch Microbiol* 150: 426–431.
6 Bradford MB. 1976. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein–dye binding. *Anal Biochem* 26: 7688–7693.
7 Campos J, M Martinez-Pacheco and C Cervantes. 1995. Hexavalent chromium reduction by a chromate-resistant *Bacillus* sp. strain. *Antonie van Leeuwenhoek* 68: 203–208.
8 Cervantes C and S Silver. 1992. Plasmid chromate resistance and chromate reduction. *Plasmid* 27: 65–71.
9 Coates JD, U Michaelidou, RA Bruce, SM O'Connor, JN Crespi and LA Achenbach. 1999. Ubiquity and diversity of dissimilatory (per)chlorate-reducing bacteria. *Appl Environ Microbiol* 65: 5234–5241.
10 Fischer H. 1990. Praktikum in allgemeiner Chemie. Versuchsanleitungen 1. Teil, Universität Zürich, pp. 157–159.
11 Fujie K, HY Hu, X Huang, Y Tanaka, K Urano and H Ohtake. 1996. Optimal operation of bioreactor system developed for the treatment of chromate wastewater using *Enterobacter cloacae* HO-1. *Water Sci Technol* 33: 173–182.
12 Hames BD. 1994. One-dimensional polyacrylamide gel electrophoresis. In: Hames BD and D Rickwood (Eds.), Gel Electrophoresis of Proteins. A Practical Approach. IRL, Oxford, pp. 1–147.
13 Imhoff JF. 1995. Taxonomy and physiology of phototrophic purple bacteria and green sulfur bacteria. In: Blankenship RE, MT Madigan and CE Bauer (Eds.), Anoxygenic Photosynthetic Bacteria. Kluwer Academic Publ., Dordrecht, pp. 1–15.
14 Ishibashi Y, C Cervantes and S Silver. 1990. Chromium reduction in *Pseudomonas putida*. *Appl Environ Microbiol* 56: 2268–2270.
15 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: 4734–4740.
16 Komori K, P Wang, K Toda, H Ohtake. 1989. Factors affecting chromate reduction in *Enterobacter cloacae* strain HO1. *Appl Microbiol Biotechnol* 31: 567–570.
17 Laemmli UK. 1970. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature* 227: 680–685.
18 Llovera S, R Bonet, MD Simon-Pujol and F Congregado. 1993. Chromate reduction by resting cells of *Agrobacterium radiobacter* EPS-916. *Appl Environ Microbiol* 59: 3516–3518.
19 Moore MD 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.
20 Moore MD and S Kaplan. 1994. Members of the family *Rhodospirillaceae* reduce heavy metal oxyanions to maintain redox poise during photosynthetic growth. *Am Soc Microbiol News* 60: 17–23.
21 Nepple BB and R Bachofen. 1997a. Influence of hexavalent chromium on the growth and protein composition of *Rhodobacter sphaeroides*. In: Abstracts of the Euroconference. Microbial Response to Stress: What's New and How Can It Be Applied? Sesimbra, Portugal, p. 99.
22 Nepple BB and R Bachofen. 1997b. Induction of stress proteins in the phototrophic bacterium *Rhodobacter sphaeroides*. *FEMS Microbiol Lett* 153: 173–180.
23 Ohtake H and S Silver. 1994. Bacterial detoxification of toxic chromate. In: GR Chaudhry (Ed.), Biological Degradation and Bioremediation of Toxic Chemicals. Chapman & Hall, London, pp. 403–415.
24 Ohtake H, E Fujii and K Toda. 1990a. A survey of effective electron donors for reduction of toxic hexavalent chromium by *Enterobacter cloacae* (strain HO1). *J Gen Appl Microbiol* 36: 203–208.
25 Ohtake H, E Fujii and K Toda. 1990b. Bacterial reduction of hexavalent chromium: kinetic aspects of chromate reduction by *Enterobacter cloacae* HO1. *Biocatalysis* 4: 227–235.
26 Ormerod JG. 1992. Physiology of photosynthetic prokaryotes. In: Mann NH and NG Carr (Eds.), Photosynthetic Prokaryotes, Plenum Press, New York, pp. 93–120.
27 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 conditions. *Arch Microbiol* 162: 335–343.
28 Shen H and YT Wang. 1993. Characterization of enzymatic reduction of hexavalent chromium by *Escherichia coli* ATCC 33456. *Appl Environ Microbiol* 59: 3771–3777.
29 Sinclair JF, JF Healey, R McAllister, HL Bonkowsky and P Sinclair. 1981. Improved retention of heme with increased resolution of microsomal proteins in polyacrylamide gel electrophoresis. *Anal Biochem* 114: 316–321.
30 Sistrom WR. 1977. Transfer of chromosomal genes mediated by plasmid R68:45 in *Rhodospeudomonas sphaeroides*. *J Bacteriol* 131: 526–532.
31 Suzuki T, N Miyata, H Horitsu, K Kawai, K Takamizawa, Y Tai and M Okazaki. 1992. NAD(P)H-dependent chromium(VI) reductase of *Pseudomonas ambigua* G-1: a Cr(V) intermediate is formed during the reduction of Cr(VI) to Cr(III). *J Bacteriol* 174: 5340–5345.
32 Tebo BM and AY Obraztsova. 1998. Sulfate-reducing bacterium grows with Cr(VI), U(VI), Mn(IV), and Fe(III) as electron acceptors. *FEMS Microbiol Lett* 162: 193–198.
33 Urone PF. 1955. Stability of colorimetric reagent for chromium, *S*-diphenylcarbazides, in various solvents. *Anal Chem* 27: 1354–1355.
34 Wang YT and H Shen. 1995. Bacterial reduction of hexavalent chromium. *J Ind Microbiol* 14: 159–163.