Journal of Industrial Microbiology, (1995) 14, 159–163

Bacterial reduction of hexavalent chromium

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(Received 28 March 1994; accepted 10 August 1994)

Key words: Chromium reduction; Enzymatic; Aerobic; Anaerobic; Metals; Redox potential

SUMMARY

Cr(VI)-reducing bacteria are widespread and Cr(VI) reduction occurs under both aerobic and anaerobic conditions. Under aerobic conditions, both NADH and endogenous cell reserves may serve as the electron donor for Cr(VI) reduction. Under anaerobic conditions, electron transport systems containing cytochromes appear to be involved in Cr(VI) reduction. High cell densities are necessary to obtain a significant rate of Cr(VI) reduction. Cr(VI) reduction by bacteria may be inhibited by Cr(VI), oxygen, heavy metals, and phenolic compounds. The optimum pH and temperature observed for Cr(VI) reduction generally coincide with the optimal growth conditions of cells. The optimum redox potential for Cr(VI) reduction has not yet been established.

INTRODUCTION

Chromium has been designated as a priority pollutant by the US EPA due to its carcinogenicity and mutagenicity [3,21]. It is one of the most widely used metals in industry, resulting in large quantities being discharged into the environment. Chromium is present in industrial wastes primarily in the hexavalent form as chromate and dichromate. Major sources of chromium include the metal finishing industry, petroleum refining, leather tanning, iron and steel industries, inorganic chemicals production, textile manufacturing and pulp producing. In addition, chromium compounds are added to cooling water to inhibit corrosion and are contained in some preservatives and fire-retardant chemicals used in wood preservatives [20]. The chromium discharges from these industrial processes was estimated at approximately 10 000 lbs per day by the US EPA [28].

In contrast to most metals, chromium is usually soluble under oxidizing conditions, and only limited removal can be achieved by conventional precipitation methods [20]. Biological transformation of Cr(VI) to Cr(III) has only recently been recognized. Trivalent chromium is less soluble in most water systems, and great potential exists for the ultimate removal of chromium by biological processes. In such processes, Cr(VI) may serve as an electron acceptor for the oxidation of organic compounds. Microbial cells are known to be able to develop resistances to virtually all toxic heavy metals. Bacterial resistance to chromate may be plasmid-borne or due to chromosomal mutations as reviewed elsewhere [2]. The dissimilatory reduction of Cr(VI) and other metals has also recently been reviewed [16]. This paper reviews the environmental aspects of Cr(VI) reduction by bacteria with an emphasis on the effect of several environmental factors.

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Chromium-reducing bacteria

Many bacteria can reduce chromium. Table 1 summarizes bacteria which reduce Cr(VI) to Cr(III). They belong to a variety of genera most of which are facultative anaerobes and widespread in nature. Earlier studies assumed that Cr(VI) was reduced to Cr(III) by observing medium color change from yellow to white [5,13,29]. Recently, Cr(V) was detected as an intermediate in *Pseudomonas ambigua*, indicating that the reduction of Cr(VI) to Cr(III) is at least a two-step reaction [27]. Direct evidence has only recently been provided to show that Cr(VI) was completely and quantitatively transformed to Cr(III) by *Escherichia coli* [23] and *Agrobacterium radiobacter* [15].

Although chromium-reducing bacteria are widespread, high cell densities are generally required for significant Cr(VI) reduction to occur. Cell protein of *D. vulgaris* ATCC 29579 at 1.1 mg ml^{-1} buffer was needed to completely reduce 470 μM Cr(VI) in 100 min [17]. The rate of Cr(VI) reduction increased with increasing cell density as observed with *E. coli* ATCC 33456 [25], *Pseudomonas fluorescens* LB 300 [31], *Bacillus* sp. [31], *A. radiobacter* EPS-916 [15], and *Enterobacter cloacae* HO1 [29]. However, the specific rate of Cr(VI) reduction (normalized by cell mass) did not necessarily follow the same pattern. The specific rate of Cr(VI) reduction by *E. coli* was higher at relatively lower cell densities with a maximum of 86 mg Cr h^{-1} g^{-1} dry weight observed at a cell density of 3×10^8 cells ml^{-1} [25].

Electron donors for Cr(VI) reduction

Chromium-reducing bacteria may utilize a variety of organic compounds as electron donors for chromium reduction (Table 1). However, the majority of the known electron donors are natural aliphatic compounds, mainly low-molecular-weight

TABLE 1

Microbial populations that transform Cr(VI) to Cr(III)

Organism	Substrate/redox condition	Reference
Achromobacter eurydice	acetate, glucose/anaerobic	[4]
Aeromonas dechromatica	galactose, mannose, melibiose, sucrose, fructose, lactose, cellobiose, arabinose, mannitol, dulcitol, sorbitol, glycerol/anaerobic	[12]
Agrobacterium radiobacter	glucose, fructose, maltose, lactose, mannitol, glycerol/aerobic	[15]
Bacillus cereus	acetate, glucose/anaerobic	[4]
Bacillus sp.	glucose/aerobic	[31]
Bacillus subtilis	acetate, glucose/anaerobic	[4]
Desulfovibrio vulgaris	hydrogen/anaerobic	[17]
Enterobacter cloacae	acetate, glycerol, glucose/anaerobic	[19]
Escherichia coli	acetate/anaerobic	[11]
Escherichia coli ATCC 33456	glucose, acetate, propionate/aerobic and anaerobic	[23]
Micrococcus roseus	acetate, glucose/anaerobic	[4]
Pseudomonas aeruginosa	acetate, glucose/anaerobic	[4]
Pseudomonas dechromaticans	peptone, glucose/anaerobic	[22]
Pseudomonas chromatophila	ribose, fructose, fumarate, lactate, acetate, succinate, butyrate, glycerol, ethylene glycol/anaerobic	[13]
Pseudomonas ambigua G-1	nutrient broth/aerobic	[5]
Pseudomonas fluorescens LB 300	glucose/aerobic	[1]
Pseudomonas putida PRS 2000		[7]

carbohydrates, amino acids and fatty acids. Hydrogen may serve as the electron donor in *Desulfovibrio vulgaris* [17]. Enhanced Cr(VI) reduction was observed in several ATCC cultures including *P. fluorescens* 27663, 31483, 17573, *E. coli.* 15489, *Ent. cloacae* 529, 29893, and 35930 in growth media containing yeast extract or nutrient broth but not in minimal salts medium containing a sole carbon source [31]. Yeast extract or nutrient broth alone may reduce Cr(VI) presumably by organic compounds with sulfhydryl groups, especially in the absence of oxygen [31].

Although Cr(VI) reduction was observed during the growth phase, cell growth is not necessarily required for Cr(VI) reduction. Resting cells of *P. fluorescens* [1] and *E. coli* [25] reduced Cr(VI) at the same rate as in the growth media. Cr(VI) reduction was also noted with resting cells of *D. vulgaris* [17] and *A. radiobacter* [15].

Influence of Cr(VI) concentration on Cr(VI) reduction

The rate of Cr(VI) reduction depends upon the concentration of Cr(VI). The time required for complete reduction increased progressively as the initial concentration of Cr(VI) increased in the cultures of *Ent. cloacae* [29], *E. coli* [25], *P. fluorescens* [31], and *Bacillus* sp. [31]. However, the rate of Cr(VI) reduction may not be inhibited by high levels of Cr(VI) during the early phase of reduction. The initial specific rate of Cr(VI) reduction by *E. coli* as normalized by cell dry weight, was higher at a higher initial Cr(VI) concentration [25]. However, the opposite trend was observed with *Ent. cloacae* cultures in which lower initial rates of Cr(VI) reduction were obtained with higher initial Cr(VI) concentrations [9,10].

Influence of dissolved oxygen on Cr(VI) reduction

Bacterial reduction of Cr(VI) may occur both aerobically and anaerobically (Table 1). As illustrated in Fig. 1, the aerobic activity of Cr(VI) reduction is generally associated with soluble proteins with NADH as an electron donor either for requirement or for enhanced activity [5,8,23,31]. In the

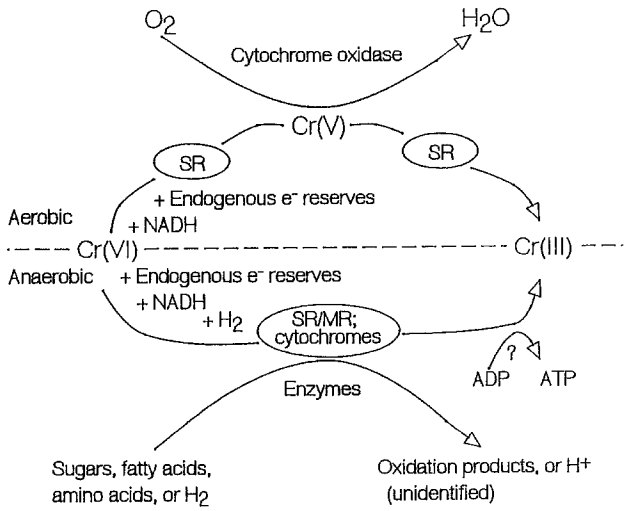

Fig. 1. Mechanisms of Cr(VI) reduction in bacteria. Under aerobic conditions, organisms may reduce Cr(VI) through action of a soluble reductase (SR) using NADH or endogenous electron reserves as an electron donor. Organisms may also reduce Cr(VI) under anaerobic conditions via the mediation of either a soluble reductase (SR), a membrane-bound reductase (MR), or both reductases (SR + MR) with possibility of involving cytochrome contents of *b*, *c* and *d*.

absence of added electron donors, chromium-reducing organisms may utilize endogenous reserves for the reduction of Cr(VI) through the activity of soluble reductase [8,23,31]. However, the physiological functions of the electron flow to Cr(VI) through the soluble reductase have not been thoroughly examined. Under anaerobic conditions, Cr(VI) serves as a terminal electron acceptor through the respiratory chains of *Ent. cloacae* [30], *E. coli* [23], and *D. vulgaris* [17]. Recent studies have also implicated the membrane-bound respiratory chain in the transfer of reducing equivalents to Cr(VI) through cytochrome *c* in *Ent. cloacae* [29] and cytochromes *b* and *d* in *E. coli* [23]. In the absence of oxygen, the soluble reductase activity may mediate electron transport to Cr(VI) as observed in *E. coli* [23] and *D. vulgaris* [17] in which the cytochrome c_3 in the soluble protein fraction of *D. vulgaris* was needed for Cr(VI) reduction. Although several carbohydrates, amino acids, fatty acids, and hydrogen, as well as NADH and endogenous reserves may be used as the electron donor, their oxidation products during anaerobic Cr(VI) reduction have not been identified. In addition, there is still no evidence to show that electron transport to Cr(VI) may yield energy to support anaerobic growth of Cr(VI)-reducing strains.

Although chromium-reducing bacteria are widespread, only two species, *A. radiobacter* EPS-916 [15] and *E. coli* ATCC 33456 [23], were reported to reduce Cr(VI) in liquid media both aerobically and anaerobically. However, these organisms reduced Cr(VI) better under anaerobic conditions than under aerobic conditions. *A. radiobacter* EPS-916 actively reduced 0.05 mM chromate while growing aerobically but it reduced up to 0.15 mM chromate under anaerobic conditions [15]. A further study on Cr(VI) reduction by *E. coli* ATCC 33456 revealed that Cr(VI) reduction was repressed by dissolved oxygen and an apparent uncompetitive inhibition behavior of oxygen was observed. The maximum specific Cr(VI) reduction rate, k, decreased from 0.5 mmol g^{-1} cell h^{-1} under anaerobic conditions to 0.27 mmol g^{-1} cell h^{-1} in the presence of oxygen [23]. From a thermodynamic point of view, oxygen is the preferred final electron acceptor as compared to Cr(VI) since higher energy levels will be obtained by cells through the electron transport systems involving cytochrome (Fig. 2). This may explain why lower rates of Cr(VI) reduction were always noted in the presence of oxygen.

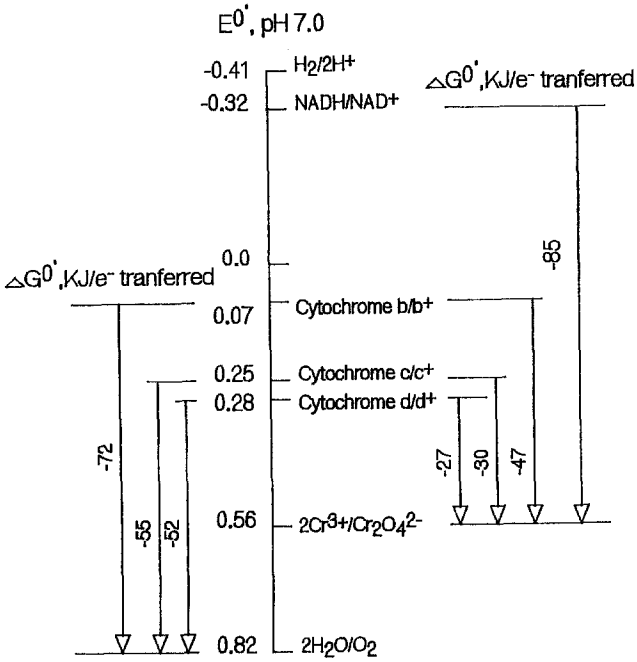

Fig. 2. Thermodynamic profile of bacterial respiration with oxygen or Cr(VI) as possible electron acceptors. The $\Delta G^{0'}$ was calculated based on the listed standard electron potential as shown in references [6] and [14].

Effect of other electron acceptors

Inhibition of sulfate and nitrate on Cr(VI) reduction has not been reported for aerobic cultures. Sulfate up to 1 mM and nitrate at 200 μM had no effect on chromate reduction both with whole cells and with cell-free supernatant fluid of *P. putida* [8]. Sulfate at 10 mM and nitrate at 16 mM did not affect Cr(VI) reduction by whole cell and cell-free extract of *Bacillus* sp. [31]. In anaerobic cultures, the rate of Cr(VI) reduction by *E. coli* was not affected by up to 83 mM sulfate and 129 mM nitrate [25]. Sulfate concentrations as high as 50 mM did not inhibit Cr(VI) reduction in *D. vulgaris* [17]. However, Cr(VI) reduction activity of *Ent. cloacae* HO1 under anaerobic conditions was reduced to 68% in the presence of 25 μM $ZnSO_4$ and was reduced to 84% by 5 mM $NaNO_3$ [10].

Optimum pH and temperature for Cr(VI) reduction

The optimal pH and temperature for Cr(VI) reduction generally coincide with the optimal growth conditions of cells. Cr(VI) reduction by *Ent. cloacae* was observed at pH 6.0–8.5 and at temperatures of 20–40 °C with pH and temperature optima ranging from 7.0–7.8 and 30–37 °C, respectively [10]. Cr(VI) reduction in *E. coli* was evaluated within a pH range of 3 to 8 and a temperature range of 10 to 45 °C and the observed maximum initial specific rate of Cr(VI) reduction occurred at pH 7 and a temperature of about 36 °C. The rate data were found to well fit the Arrhenius equation [25]. For another Cr(VI)-reducing species, *Bacillus* sp., the optimal conditions for Cr(VI) reduction took place at pH 7 and 30 °C [31].

Redox potential and Cr(VI) reduction

The best range of redox potential for Cr(VI) reduction has not yet been well established. Using washed, resting cells of *A. radiobacter* pregrown under different carbon and energy sources, the rate of Cr(VI) reduction was shown to be greater in cell suspensions at −240 mV than with −198 mV [15]. When Cr(VI) was introduced to *E. coli* cultures with a redox potential greater than −140 mV, no reduction of Cr(VI) was observed within the first hour [4]. However, in *B. subtilis* cultures, even with a high initial redox potential value of about +250 mV, Cr(VI) reduction occurred after one hour of incubation as the redox potential decreased [4]. Cr(VI) reduction by *Bacillus* sp. occurred over a wide range of redox potential. Cr(VI) reduction began almost immediately at the beginning with a redox potential of about +250 mV and continued at a constant rate throughout the incubation despite a rapid drop

of redox potential to a low of −500 mV after 48 h when the growth phase was evident [31].

Low redox potential generated by microbial activity was shown not to affect the reduction of Cr(VI) as demonstrated by using *E. coli* ATCC 33456 [25]. *E. coli* ATCC 33456 was inoculated to a very low cell concentration (10^4 cells ml^{-1}) and incubated anaerobically with glucose as the sole carbon source and Cr(VI) in the form of CrO_4^{2-} as a potential electron acceptor. The anaerobic culture of *E. coli* ATCC 33456 quickly lowered the redox potential to below −400 mV despite a very low initial cell density. However, no significant Cr(VI) reduction was noted even under such a low redox potential. Cr(VI) reduction, on the other hand, occurred in the aerobic culture with a high initial cell density (10^{10} cells ml^{-1}). Cr(VI) reduction continued even after the redox potential of the aerobic culture increased to above +150 mV from a low of about −500 mV after 6 h incubation. In addition, a rapid decrease of the redox potential in the aerobic culture, from above +200 mV to below −500 mV after 6 h incubation, did not seem to significantly enhance Cr(VI) reduction as compared to Cr(VI) reduction in the positive redox potential range.

Inhibition of Cr(VI) reduction by metals

Cr(VI)-reducing organisms are susceptible to several heavy metals. Cr(VI) reduction by *Ent. cloacae* was completely inhibited by 0.5 mM Zn^{2+} or was reduced to 70% by 0.5 mM Cu^{2+} [18]. Cr(VI) reduction by *E. coli* was reduced to 80 and 84% of control by 0.8 mM Zn^{2+} and 3 mM Cu^{2+}, respectively, while up to 0.2 mM Cd^{2+} and 0.1 mM Pb^{2+} did not exhibit inhibition [25]. Strong inhibition of Cr(VI) reduction by Hg^{2+} and Ag^{2+} in *P. putida* was characterized as noncompetitive with K_I of 20 μM observed for both Hg^{2+} and Ag^{+} [8]. However, at a concentration of 100 μM, a range of 11 metals including Zn^{2+} and Cu^{2+} had no effect on Cr(VI) reduction by *D. vulgaris* [17]. The reduced form of Cr(VI), Cr^{3+}, did not inhibit Cr(VI) reduction by *P. putida* at 200 μM [17].

Inhibition of Cr(VI) reduction by phenolic compounds

In addition to heavy metals, phenolic compounds have also been found as co-contaminants in chromium-containing wastes and chromium-polluted sites. Inhibition of Cr(VI) reduction by phenolic compounds was observed with a few Cr(VI)-reducing organisms. Phenol and *p*-cresol at 5 mM each and 2-chlorophenol at 2 mM severely inhibited both Cr(VI) reduction and cell growth of *P. fluorescens* LB 3000 [31]. The toxic effect of three phenolic compounds on Cr(VI) reduction by *E. coli* ATCC 33456 was recently assessed by examining the inital specific Cr(VI) reduction rate [23]. Anaerobic cultures of *E. coli* ATCC 33456 were more susceptible to phenolic compounds than the aerobic cultures, with concentrations causing 50% reduction in Cr(VI) reduction activity being 9, 12, and 56 mM for *p*-cresol, 2-chlorophenol, and phenol, respectively. While in aerobic cultures, the corresponding concentrations which caused 50% decrease in Cr(VI) reduction rate were 15, 20, and 82 mM, respectively.

Environmental application of bacterial Cr(VI) reduction

Recent work has revealed the potential of using Cr(VI)-reducing microorganisms for detoxifying Cr(VI)-contaminated environments or for the treatment of Cr(VI)-containing wastes even if the mechanisms of Cr(VI) reduction have not been fully understood. The finding that biological reduction of Cr(VI) occurs near a neutral pH and over a moderate temperature range implies that no costly chemical reagents and extensive energy inputs are required. However, the need for added carbon sources and inhibition of heavy metals, the most likely co-contaminants, may render biological treatment of industrial effluents less efficient [18]. Another challenge to be overcome before the application of biological treatment for Cr(VI) is Cr(VI) toxicity which may lead to cell inactivation and loss of Cr(VI) reduction capacity [24,33]. Recently, an innovative two-stage bioreactor was evaluated for the treatment of Cr(VI) by separating cell growth from the Cr(VI) contacting phase to avoid Cr(VI) toxicity [26]. Results indicated that Cr(VI) was completely removed under appropriate operating conditions and that higher Cr(VI) removal rates were obtained with higher Cr(VI) loading rates.

For industrial effluents which do not contain organic compounds for Cr(VI) reduction, joint treatment of organic pollutants and Cr(VI) should be considered. Recently, the potential of simultaneous detoxification of Cr(VI) and organic compounds was demonstrated by using defined co-cultures which utilize a range of toxic aromatic compounds including phenol, 2-chlorophenol, toluene, and benzene as carbon sources for Cr(VI) reduction [32]. However, further studies are needed to characterize bacterial interactions in the co-culture. Such an understanding is essential for the ultimate disposal of Cr(VI) and organic pollutants by biological processes.

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