Engineering bacteria for superior Chromate bioremediation

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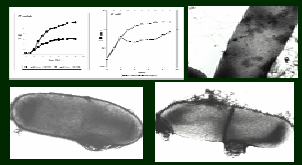
Introduction

Chromate [(Cr(VI)] is a major heavy metal contaminant at the DOE waste sites (1). Being soluble, it can leach into drinking water supplies and being a carcinogen thereby poses a serious health hazard. Its reduction to Cr(III) by bacteria represents a promising avenue for its bioremediation because Cr(III) is insoluble and nontoxic. Most bacteria possess the capacity to reduce chromate; however, their effectiveness is compromised by the fact that chromate is toxic to them as well (Fig. 1) (2), and thus an important way to improve bacterial chromate bioremediation efficiency is to reduce Cr(VI) toxicity to them.

Fig. 1. Chromate inhibits growth of bacteria and generates surface and other abnormalities.



Results and discussion

Fig. 2. A major reason for chromate toxicity. Cr(VI) enters the cell by "fooling" the sulfate transport system. Inside, it is attacked vicariously by several flavoproteins with essential metabolic roles. An example is lipoyl dehydrogenase (LpDH) which is involved in energy metabolism. These enzymes reduce Cr(VI) by one-electron transfer, which generates the highly reactive Cr(V) radical. Redox cycling of Cr(V) produces reactive oxygen species (ROS) that damage the cell (2-5).

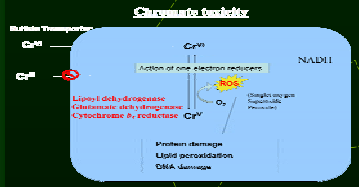


Fig. 3. Obligatory four-electron reducers. A mammalian enzyme, called DT-diaphorase, catalyzes obligatory two-electron reduction of its substrates (6). We reasoned that a similar enzyme in bacteria, in dimeric form, would reduce chromate to Cr(III) in one step, leaving only one electron to generate ROS. Finding such enzymes in bacteria and managing chromate flow primarily through them would provide a "safe" pathway for chromate reduction.

By using *in silico* approaches and classical biochemical methods combined with those of modern molecular biology, we found several such enzymes in bacteria, cloned the relevant genes and overproduced them in electrophoretically pure form (4,7,8). Several lines of evidence, including rapid scan spectrophotometric, electron spin, and ROS generation measurements confirmed that these enzymes do indeed bring about largely a one-step chromate reduction (5,9,10). These enzymes have a broad substrate specificity (4,5,8,9,10).

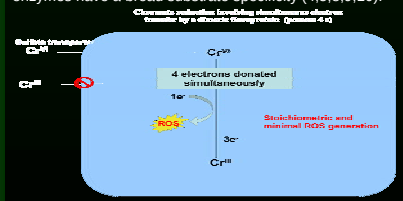


Table 1. ROS generation by two-electron reducers and their ability to outcompete one-electron chromate reducers. While LpDH transfers some 70% of the available electrons to ROS, YieF, for example, transfers only 25%. Further, when both LpDH and YieF are simultaneously present in an in vitro reaction mixture, still only 25% electrons go to ROS, indicating that the presence of YieF preempts chromate reduction by LpDH.

NADPH ELECTRONS TRANSFERRED TO CHROMATE & H_2O_2 (μM; t = ∞)

Enzyme	NAD(P)H e⁻ consumed	e⁻ used in chromate reduction	e⁻ used in H_2O_2 formation
YieF/LpDH	70	53 (74%)	18 (26%) (74%)
ChrR/LpDH	75	35 (48%)	41 (48%)
NfsA/LpDH	80	30 (37%)	50 (63%)

Fig. 4. In vivo confirmation. A mutant in one such enzyme exhibits increased chromate toxicity, while a strain overproducing the same enzyme shows decreased toxicity.

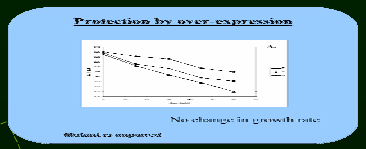


Fig. 5. Strategy for decreasing chromate toxicity to the remediating bacteria. This strategy is to improve the affinity and other characteristics of the two-electron reducing "safe" enzymes so that they can essentially circumvent chromate channeling through the dangerous one-electron reducing pathways.

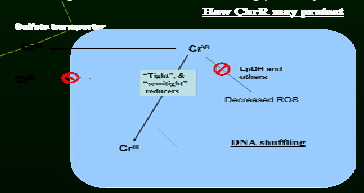


Figure 6. Experimental setup for enzyme evolution through DNA shuffling.

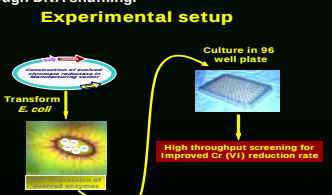


Table 2. Improved enzymes. After two rounds of shuffling, we have achieved 40-fold increase in Vmax for chromate reduction when assayed in crude extracts. Kinetics of evolved purified proteins are shown in this Table. A 10-fold improvement in Vmax (product Y3) and four-fold improvement (product Y1) can be seen. Further improvements are underway but already Y3 should prove more useful for high chromate situations and Y1 for a wide range of these concentrations as shown by it's lowest Km (12).

Strain	Vmax (nmol Cr (VI) mg protein^{-1} min^{-1})	Km (μM)	Kcat (S^{-1})	Kcat/Km ($S^{-1}M^{-1}$)
YieF	294	380	20	$5X10^4$
Y1	1,111	60.8	74	$1.3X10^5$
Y2	1,588	304	39	$1.3X10^5$
Y3	3,339	270	222	$8.2X10^5$

Figure 7. All the chromate reductases we have examined can also reduce U(VI), thus raising the possibility of remediating two of the DOE contaminants through the activity of the same enzyme (11); this activity too is being improved.

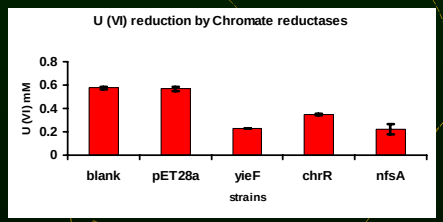
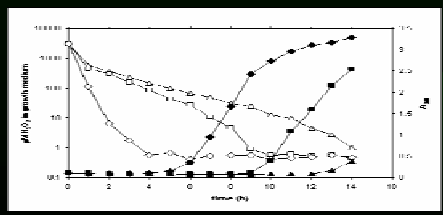
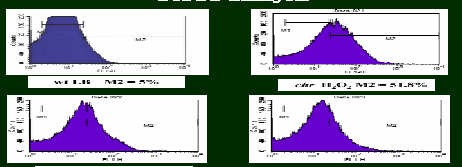


Fig. 8-10. We have suggested that chromate reduction is unlikely to be the biological role of the above enzymes (5,7,9). This does not argue against the potential of these enzyme for bioremediation; however, it is of interest to discern what such a role might be, to better gauge possible consequences of manipulating the activity and expression of these enzyme. Studies with the wild type mutant and overproducing strains show that this role is protection against the oxidative stress primarily of H_2O_2. Growth pattern (Fig. 8), FACS analysis of cellular H_2O_2 concentration (Fig. 9) and protein carbonylation measurements (Fig. 10) all support this conclusion (10).





Conclusions. Through these studies we have established for the first time that:

1. Single electron reduction of chromate is indeed a major reason for chromate toxicity.
2. Two electron reducers exist that can more safely reduce chromate.
3. Their efficiency can be increased so that they can conceivably essentially prevent chromate channeling into the unsafe pathways.
4. The soluble chromate reductases can also reduce U(VI) raising the exciting possibility of constructing combinatorial bacteria engineered to efficiently remediate more than one heavy metals of the DOE sites.

References:

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