Biomolecular Strategy to Decrease Chromate Toxicity to Remediating Bacteria

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Cr(VI) (chromate) is a widespread, toxic, and soluble environmental pollutant. Bacteria can reduce chromate to insoluble and less toxic Cr(III); thus, chromate-bioremediation-utilizing bacteria are of interest. Chromate, however, is toxic to the remediating bacteria; application of genetic and protein engineering approaches can ameliorate this problem and also improve chromate bioremediation in other respects [1]. Towards this end, we have conducted an extensive study of bacterial chromate reductases [2–6]. A major reason for chromate toxicity is the generation of reactive oxygen species (ROS) during its reduction. Using enzyme purification and in silico approaches, we cloned several bacterial genes with presumptive capacity to encode chromate reductase activity and have examined several characteristics using pure enzymes. The propensity to generate ROS during chromate reduction was examined using rapid scan kinetics, electron spin resonance measurements, as well as H_2O_2 generation. In this respect, these enzymes, all of which are flavoproteins and coexist in bacterial cells, fall into three categories. The first is exemplified by the protein YieF, which belongs to a protein family known for its obligatory two-electron reduction of electrophiles. The YieF dimer did not generate the flavin semiquinone form of the enzyme, nor (likely) also Cr(V) during chromate reduction, and transferred only 25% of the reductant (NADH) electrons to H_2O_2. Thus, YieF tightly maintained its obligatory two-electron transfer characteristic even when catalyzing a three-electron-requiring Cr(VI) to Cr(III) reduction ("tight chromate reductases"). The second category is exemplified by the proteins ChrR and NfsA, which belong to a similar family of proteins. However, flavin semiquinone forms of NfsA and Cr(V) were generated during chromate reduction, and over 25% of the NADH electrons were consumed in generating ROS, indicating these enzymes maintain their obligatory electron transfer characteristic only semi-tightly during chromate reduction ("semi-tight chromate reductases"). ChrR was shown to protect *P. putida* against chromate toxicity, and YieF is likely to be even more effective in this respect. The third category is exemplified by lipoyl dehydrogenase. It was previously shown that large and persistent amounts of Cr(V) are generated during chromate reduction by this enzyme, and we found that 60% of the NADH electrons were directed to ROS generation during chromate reduction catalyzed by this enzyme ("single electron chromate reducers"), which was more than generated by either of the above two enzyme categories. Thus, our strategy to minimize chromate toxicity is to generate bacteria in which the flow of chromate is enhanced through the reductive pathways represented by the tight and semi-tight chromate reductases, so as to preempt its reduction by the single electron reducers of the cell.

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