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Reduction of U(VI) and toxic metals by Desulfovibrio *cytochrome* c_3
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The central objective of our proposed research was twofold: 1) to investigate the structure-function relationship of *Desulfovibrio desulfuricans* (now *Desulfovibrio alaskensis* G20) cytochrome c_3 with uranium and 2) to elucidate the mechanism for uranium reduction in vitro and in vivo. Physiological analysis of a mutant of *D. desulfuricans* with a mutation of the gene encoding the type 1 tetraheme cytochrome c_3 had demonstrated that uranium reduction was negatively impacted while sulfate reduction was not if lactate were the electron donor. This was thought to be due to the presence of a branched pathway of electron flow from lactate leading to sulfate reduction. Our experimental plan was to elucidate the structural and mechanistic details of uranium reduction involving cytochrome c_3.We proposed

1. to elucidate the structure-function relationship of tetraheme cytochrome c_3 interaction with uranium;
2. to determine the mechanism by which uranium is reduced *in vitro*; and
3. to determine the array of positively charged metals that can be reduced by this cytochrome.

Our collaborative efforts have resulted in

1) perfecting the purification of wild-type cytochrome c_3 in our laboratory,
2) crystal structure determination, and
3) analysis of key amino acid residues in the cytochrome c_3 by effects on U(VI) reduction.

Pattarkine, M.V., J.J. Tanner, C.A. Bottoms, Y.-H. Lee, and J.D. Wall. 2006. *Desulfovibrio desulfuricans* G20 tetraheme cytochrome structure at 1.5 Å and cytochrome interaction with metal complexes. J. Mol. Biol. 358(5):1314-1327.

The structure of the type I tetraheme cytochrome c3 from *Desulfovibrio desulfuricans* G20 was determined to 1.5A ° by X-ray crystallography. In addition to the oxidized form, the structure of the molybdate-bound form of the protein was determined from oxidized crystals soaked in sodium molybdate. Only small structural shifts were obtained with metal binding, consistent with the remarkable structural stability of this protein. In vitro experiments with pure cytochrome showed that molybdate could oxidize the reduced cytochrome, although not as rapidly as U(VI) present as uranyl acetate. Alterations in the overall conformation and thermostability of the metal-oxidized protein were investigated by circular dichroism studies. Again, only small changes in protein structure were documented. The location of the molybdate ion near heme IV in the crystal structure suggested heme IV as the site of electron exit from the reduced cytochrome and implicated Lys14 and Lys56 in binding. Analysis of structurally conserved water molecules in type I cytochrome c3 crystal structures identified interactions predicted to

be important for protein stability and possibly for intramolecular electron transfer among heme molecules.

Miller, S.M. 2005. Examination of specific amino acid residues of *Desulfovibrio desulfuricans* cytochrome c_3 in electron transfer. Master of Science thesis presented to the Graduate School of the University of Missouri-Columbia.

Sulfate-reducing bacteria (SRB) are strictly anaerobic microorganisms present throughout the environment. These microorganisms are able to utilize a variety of electron donors and couple the oxidation of those compounds to the reduction of sulfate, with sulfate as the terminal electron acceptor and hydrogen sulfide as an end product of respiration. The generation of hydrogen sulfide is problematic because of its corrosive effects on metals and concrete. On the positive side, there exists the potential to utilize the metabolic properties of the bacteria for the bioremediation of toxic metals such as uranium. Many of the SRB are capable of altering the redox state of uranium from soluble U(VI) to the insoluble mineral uraninite U(IV) that is less biologically available. Of particular interest in this investigation is the involvement of the predominant c-type tetraheme cytochrome, cytochrome c_3, implicated as a metal reductase in SRB metabolic processes. To explore this possibility, a number of mutant cytochrome c_3 proteins were generated and electron transfer capabilities to metals and metal complexes were examined. UV spectroscopy was used to observe the redox properties of wild-type and mutant cytochromes with the addition of uranium and molybdate. Oxidation and reduction was observed to be similar to non-mutant, for the mutations F19A, C45A, K66A, K72A, and M80K. However, the K14A mutant was not oxidized when molybdate was added to the reduced protein. This lysine residue may represent a critical point of interaction between the cytochrome and the metal.