Mechanisms for the Reduction of Actinides and Tc(VII) in *Geobacter sulfurreducens*

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Uranium and technetium are the primary radioactive metals contaminating subsurface environments at Department of Energy (DOE) sites. Dissimilatory Fe(III)-reducing microorganisms can control the mobility of these contaminants through the enzymatic reduction of highly soluble U(VI) and Tc(VII) to insoluble tetravalent forms that will precipitate from groundwater and be immobilized in the subsurface. The objective of the proposed research is to characterize the mechanisms of U(VI) and Tc(VII) reduction in the dissimilatory Fe(III)-reducing bacterium *Geobacter sulfurreducens*. In a previous two-year NABIR-funded study, we purified and characterized a periplasmic cytochrome c_7 that reduced a range of electron acceptors in vitro, including U(VI), Fe(III), and humics. Confirmation that the protein was required for the reduction of these electron acceptors in vivo was obtained using a deletion mutant lacking the structural gene for the cytochrome. The mechanism of enzymatic Tc(VII) reduction was also characterized in whole cells. The oxidation of hydrogen was coupled to the reduction of Tc(VII) leading to the precipitation of Tc(IV) in the periplasm. The involvement of a periplasmic Ni/Fe hydrogenase was implicated by CO profiling.

The aims of this new study are to use the tools of biochemistry and molecular biology to confirm the identity of the genes encoding the relevant U(VI) and Tc(VII) reductases in *G. sulfurreducens*, and to elucidate the detailed mechanisms of U(VI) and Tc(VII) reduction by the corresponding enzymes. Furthermore, we propose to explore the range of other metals and radionuclides reduced by *Geobacter sulfurreducens*—including Np(V), Pu(IV) and Hg(II)—and identify the roles of the U(VI) and Tc(VII) reductases in the reduction of these other priority pollutants.

The specific hypotheses that will guide our research are:

(1) Cytochrome c_7 functions as the U(VI) reductase of *G. sulfurreducens* in vivo, and is also capable of reducing and modifying the solubility of other actinide species [including Np(V) and complexed Pu(IV)] and toxic metals [including Co(III) and Hg(II)] via a mechanism that is distinct to that catalyzing the transfer of electrons to insoluble Fe(III) oxides.

(2) Key amino-acid residues can be mutated to identify regions of cytochrome c_7 that are required for the reduction of metals and radionuclides.

(3) A periplasmic Ni/Fe hydrogenase is the Tc(VII) reductase of *G. sulfurreducens*. This enzyme is also able to reduce a range of other electron acceptors, including U(VI) directly when hydrogen is supplied as the electron donor.

(4) Additional genes may be required for U(VI) reduction in *G. sulfurreducens*, and can be identified by transposon mutagenesis.

This project is in collaboration with co-PIs Dr. Francis Livens and Dr. Iain May of the Radiochemistry Centre, Manchester, and we will also continue successful collaborations with experts in protein crystallography (Dr. Marianne Schiffer of the Argonne National Laboratory) and the molecular biology of *Geobacter* (Dr. Derek Lovley of the University of Massachusetts).