Studies of Multi-Heme Cytochromes from *Geobacter sulfurreducens*

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Microbial reduction of U(VI) to U(IV) is a promising strategy for the in situ remediation of uranium, and thus is a primary focus of the NABIR program. The *Geobacteraceae* family predominates in the reduction of uranium in subsurface environments. Current studies are focusing on the model organism, *Geobacter sulfurreducens*, which is capable of using Fe(III) as its terminal electron acceptor. Intensive functional genomics and physiological studies are in progress in Prof. Derek Lovley's laboratory, and the complete genome sequence of this organism is available from TIGR. The *G. sulfurreducens* genome contains a large number (>100) of cytochromes *c*; they function in the metal-reduction pathways. We are studying two types of cytochromes that are required for the reduction of Fe(III). These are the three-heme cytochromes (c_7 family), and the membrane-bound 89 kDa cytochrome FerB, which contains 12 hemes. The c_7 three-heme cytochromes are related to the well-studied c_3 four-heme cytochromes found in sulfur-reducing bacteria. The c_7 cytochromes appear to be characteristic of a family of bacteria that can reduce Fe(III). We are also developing heterologous expression systems for the production of multi-heme cytochromes *c*.

The c_7 cytochrome (PpcA) was originally purified from the periplasm of *G. sulfurreducens*; it was shown to reduce Fe(III), U(VI), and Cr(VI). We found, using BLAST searches, that the bacterial genome codes for four other homologs of the c_7 protein and, in addition, for three other proteins that are polymers of the c_7 domains. Two of the polymers have four repeats, and one has nine repeats; they contain 12 and 27 hemes, respectively. At present it is not clear under what conditions the c_7 homologs are expressed and how they are used by *G. sulfurreducens*.

We have previously expressed cytochrome PpcA in *E. coli*; and purified it, crystallized it, and determined its structure by x-ray diffraction using 1.45 Å data. The core of this molecule is formed by the three hemes, surrounded by the 71 amino acid residues of the protein. We have located the chromate binding site. The packing of the molecules in the crystal lattice can serve as a model for the structures of the poly-c_7 cytochromes. Comparing the sequences of the c_7 domains in the polymers with the c_7 sequences suggests that the polymers are a new type of cytochrome, with different heme coordination for one of the hemes in each domain. Structural studies of these closely related molecules will help elucidate the features that are responsible for their functions.