Studies of Multi-heme Cytochromes from *Geobacter sulfurreducens*

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The *Geobacteraceae* family predominates in the reduction of uranium in subsurface environments. We are focusing on the model organism, *Geobacter sulfurreducens;* its genome contains a large number (>100) of cytochromes *c* that function in the metal reduction pathways. Intensive functional genomics and physiological studies are in progress in Derek Lovley's laboratory (Univ. of Massachusetts), and the complete genome sequence of this organism has been determined by Methe et al., 2003. We are studying cytochromes from the c_7 family that are required for the reduction of Fe(III).

We have determined the three-dimensional structures of five 3-heme cytochromes c_7 coded by the *G. sulfurreducens* genome. Although these proteins are highly homologous to each other, their structures differ, specifically in their surface characteristics and the arrangement of the hemes. We found that their thermal stabilities are different, and they have different reduction potentials. Laurie DiDonato, of Derek Lovley's group, found that the physiological functions of the above homologs are also different, which might make it possible to determine the functional correlates of the observed structural differences.

PpcA, the most abundant cytochrome c_7 in the periplasm, differs from the other four; it was only possible to crystallize it in the presence of deoxycholate. In the structure of PpcA, there is a binding cavity for the deoxycholate; the structures of the other four homologs did not reveal any such binding sites. This binding site in PpcA might be occupied in vivo by a quinone molecule that plays a role in the function of PpcA.

Since the PpcA and its homologs differ by several residues, it is difficult to pinpoint the residue or residues responsible for their different properties. To understand the roles of the individual residues in PpcA, we have made 25 site-specific mutants and determined the structures of 10 of them. The site-specific mutants were designed to probe the role of residues that are close to the hemes, or to influence the surface charge of the molecule. The change in redox potential of the mutants of the conserved residue F15 buried between the hemes was determined by our collaborator, Carlos Salgueiro (Universidade Nova de Lisboa). Jon Lloyd (Univ. of Manchester) is collaborating with us to study the reduction of metals such as U(VI) and Np(V) by the site-specific mutants of cytochrome c_7 PpcA and its homologs.

The *G. sulfurreducens* genome also codes for two proteins (GSU0592 and GSU1996, also called ORF0901 and ORF3300, respectively), which consist of four c_7-type domains. Previously, we have determined the structures of domain C and domains CD coded by gene GSU1996. We now have crystallized both full-length proteins and collected diffraction data for both.