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 Prof. Derek Lovley's laboratory, and the complete genome sequence of this organism is available from TIGR. We are studying cytochromes from the c_7 family that are required for the reduction of Fe(III).

Previously, we determined the three-dimensional structure of three-heme cytochrome c_7 PpcA (coded by ORF01023) characterized by Lloyd et al., and we identified in the *G. sulfurreducens* genome ORFs for several of its homologs. Four of the ORFs are the same size as PpcA; three other ORFs are polymers of c_7-type domains, two of which consist of four domains and one of nine domains.

We have now cloned, expressed, purified, and crystallized all four three-heme homologs of PpcA, coded by ORFs 601, 603, 2938, and 1734 that have 77, 62, 65, and 57% identity respectively with PpcA. We have determined the structure of two of them, ORF00603 and ORF01734. Although these proteins are highly homologous to each other, their structures differ: that is, their surface characteristics and the arrangement of the hemes are different. We found that their thermal stabilities are also different, and it is expected that they will have different reduction properties as well. Lovley's group is working on the elucidation of the physiological functions of the above homologs, which will make it possible to determine the functional correlates of the observed structural differences.

We further developed methods to express cytochromes *c* with up to 12 hemes in *E. coli* and to purify them. We cloned and expressed individual domains, two contiguous domains, and recently the complete four-domain protein coded by ORF03300. We purified and crystallized domains C and D, the two-domain protein CD, and the complete four-domain protein. We have determined the x-ray structures of domain C and the two-domain protein CD. These c_7-type domains that form the polymers represent a new family of cytochromes *c* that has not been previously described. Based on the three-dimensional structures of individual domains, they differ from PpcA. While two of the hemes are bis-histidine coordinated, as found in cytochromes c_7 and c_3, the third one is coordinated by a histidine and a methionine that are expected to make its redox potential more positive than those of the other two. Indeed, the midpoint reduction potential of domain C is –105mV, 50mV more positive than that of PpcA, as determined by our collaborator Prof. Carlos Salgueiro (Universidade Nova de Lisboa). The structure of the two-domain protein shows that the domains form a chain, which is as we have predicted based on the packing of the molecules in the crystals formed by PpcA. The arrangement of all four domains in the complete molecule coded by ORF03300 will be of special interest, as we recently collected diffraction data on the complete protein.