Studies of multi-heme cytochromes from *Geobacter sulfurreducens*

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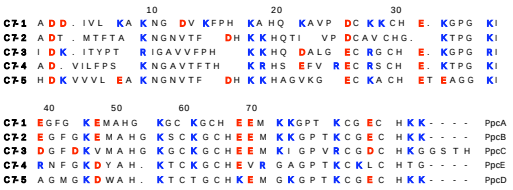
Introduction

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 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 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).

Previously, we expressed in *E. coli* (Londer et al., 2002) and determined the three-dimensional structure at 1.45 Å resolution (Pokkuluri et al., 2004a) of the three-heme cytochrome c_7 (PpcA, coded by ORF01023) characterized by Lloyd et al., 2003. Further we identified in the *G. sulfurreducens* genome ORFs for several of its homologs (Pokkuluri et al., 2004a). 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, that contain 12 and 27 hemes respectively.

Small *c7* cytochromes, PpcA homologs

We cloned, expressed, purified, crystallized and determined the structures by X-ray diffraction all four three-heme homologs of PpcA, coded by ORFs 601, 603, 2938, 1734; their crystallographic refinement is in progress. Though these proteins have highly homologous sequences and three-dimensional structures their surface characteristics differ from each other. We also found that they have different thermal stabilities and different reduction potentials. Laurie DiDonato in Prof. Lovley's group determined that the physiological function of the above homologs is also different; disabling the individual genes coding for them results in different iron reduction rates.



Aligned sequences of the homologs illustrated the different distribution of charged residues (acidic residues Asp and Glu shown in red and basic residues Lys and Arg shown in blue). Insertions and deletions of residues also result in different arrangements in space of the side chains causing variation in surface electrostatic potential.

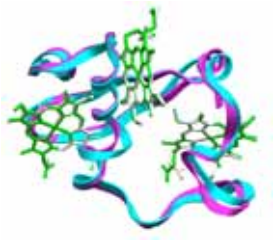
Summary of data

	PpcA	PpcB	PpcC	PpcE	PpcD
	c_7	c_7-2	c_7-3	c_7-4	c_7-5
No.of residues	71	71	75	70	72
Calculated pI	9.2	9.0	8.8	9.5	9.0
Eapp (mV)*	-155	-151	-152	-129	-150
% of CD signal † at 90° compared to 25°	81	72	74	36	69
X-ray data resolution , Å	1.45	1.35	2.25	1.60	1.34
Current R-factor %	18.2	15.2	27.4	19.3	16.4

*Redox potential determined by Prof. Carlos Salgueiro (Universidade Nova de Lisboa)
† Stability, measured as change in circular dichroism with temperature at the heme absorption band

The structure of PpcA, the c_7 which is most abundant in the periplasm, differs most significantly from the structures of the other homologs. In PpcA, heme I and heme IV are further apart, the Fe to Fe distance is 20.8 Å compared with an average of 18.3 ± 0.2 Å in the other four structures. PpcA has a pocket where a guest molecule is located in its structure, not observed in the other homolog structures. This pocket might be occupied by a quinone molecule *in vivo.*

Overlap of PpcA and PpcD structures
PpcA: C_ in blue, hemes & deoxycholic acid in green
PpcD: C_ in magenta, hemes in gray



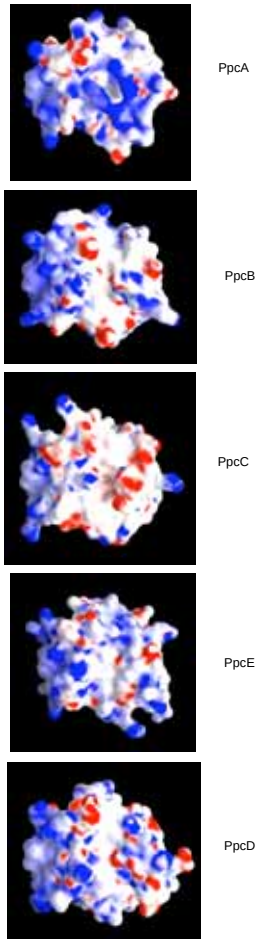
Overlap of PpcB and PpcE structures
PpcB: C_ in blue, hemes in green
PpcE: C_ in magenta, hemes in gray

Discussion

The distribution of charged residues results in different electrostatic potential on the surfaces of the molecules. This suggests that each cytochrome c_7 (PpcA homolog) might interact with different proteins in the electron transfer chain required for iron reduction.

The challenge will be to identify the interacting molecules and to determine which residues are responsible for the differences in properties of the five c_7 proteins so mutants can be made to convert one into the other.

Electrostatic potential on the molecular surfaces calculated by the program GRASP are shown (negative in red; positive in blue); the molecules are in the same relative orientation. PpcC, PpcD and PpcE were overlapped on PpcB using all the hemes; PpcA was overlapped using heme III and heme IV only. For this presentation the guest molecule, deoxycholate was removed from the calculation, resulting in a cavity surrounded by positive potential.

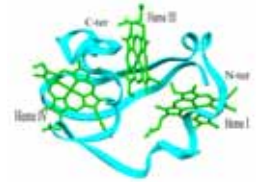


c_7-type four domain polymer

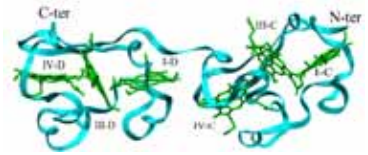
We developed methods to express cytochromes *c* with up to 12 hemes in *E. coli*. We purified and crystallized domains C, the two domain protein – domains CD, and the complete four domain protein coded by ORF03300.

We have determined the x-ray structures of domain C (Pokkuluri et al., 2004b) and domains CD. These *c*7-type domains that form the polymers represent a new family of cytochromes *c* that has not been previously described. 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 which is expected to make its redox potential more positive than those of the other two. Indeed, the midpoint reduction potential (Eapp) of domain C is -105mV, 50mV more positive than that of PpcA.

The structure of the two domain protein (CD) shows that the domains could form a chain as we have predicted based on the packing of the molecules in the crystals formed by PpcA.



The structure of the domain C (R-factor 19.5% for 1.7 Å resolution data) with C_ in blue and hemes in green.



The preliminary structure of the CD two-domain unit, CD. Hemes of neighboring domains are close: the Fe atoms of heme IV-C and heme I-D are 14.9 Å apart.

Crystals of the four domain molecule

References

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Acknowledgments

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