DOE/BES Geosciences Research Program, Summaries of Geosciences Research
Final Technical Report

Grantee: Idaho State University
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Title: Redox Interaction of Cytochromes and Bacteria with Oxide Surfaces: Probing Redox-Linked Conformation Change

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Project Description:

The biochemistry of bacterial proteins involved in redox transformations of metals and minerals is, without dispute, an important area of research. Nevertheless, most studies on bacterial metal transformation have focused not on biochemistry but on genetics and genomics. The objective of this research is to better understand the role of conformation change in electron transfer from cytochromes to minerals, a process that underpins respiratory metal reduction by bacteria in nature and in bioremediation strategies, including reductive immobilization of radioactive contaminants. Our DOE-funded work is specifically focused on answering long-standing questions about the biochemical behavior of these very interesting proteins, and our findings thus far have already made impacts in the fields of environmental microbiology and biogeochemistry. Among the key findings from the project are 1) Successful large-scale production of biomass for protein isolation; 2) Purification of several c-type cytochromes for biochemical study; 3) Characterization of these proteins using spectrophotometric and electrochemical techniques; 4) Examination of protein conformational change and redox activity towards metal oxides using a small mass cytochrome c from *Acidiphilium cryptum;* 5) Proteomic characterization of *A. cryptum* biofilms; 6) Training of 2 undergraduate research assistants; 7) Publications and several meeting presentations.

Results:

Purification of several mono- and polyheme cytochrome c from *A. cryptum* and *G. sulfurreducens*. These include the 10.1 kDa monoheme periplasmic cytochrome c and 42kDa monoheme outer membrane cytochrome c from A. cryptum, and OmcB and OmcS from *G. sulfurreduc*ens.

Spectrophotometric analyses of the redox properties of purified cytochromes. We have demonstrated interesting redox properties, in that for A. cryptum, electron transfer to a soluble Fe(III) chelate is preferred at low pH (3.0), and reactions do not

proceed at pH 7.0. This is consistent with the acidic environments (periplasm pH 6.0, outside pH 2-3) that the proteins occupy.

Training of undergraduate researchers (Andy Fielding, Sean Clark), and support of a Research Associate (Mike Swenson).

Sabbatical leave travel for Magnuson to the University of Wyoming, to work with collaborators Carrick Eggleston and Patricia Colberg. Magnuson gave a seminar for the UW Physiology and Zoology Department in Fall 2007, and performed experiments on c-type cytochromes using the newly-acquired Optical Waveguide Lightmode Spectrometer (OWLS).

Studies with OWLS and with Quartz Crystal Microbalance. We examined two cytochromes, PpcA and OmcB (from Geobacter sulfurreducens) for their comparative adsorption properties to glass waveguide surfaces. Essentially, OWLS and QCM help to determine how quickly and how strongly proteins adsorb to surfaces, and how the degree of protein hydration changes during this process. PpcA, a small periplasmic cytochromes, was found to be compact, and showed slower adsorption kinetics than OmcB (Cell surface cytochrome), which appears to be more 'floppy' and adsorbs and desorbs faster from surfaces (Fig. 1). This suggests that the protein is tailored for quick electron discharge to a mineral surface, followed by rapid desorption and 'reloading' with more electrons.

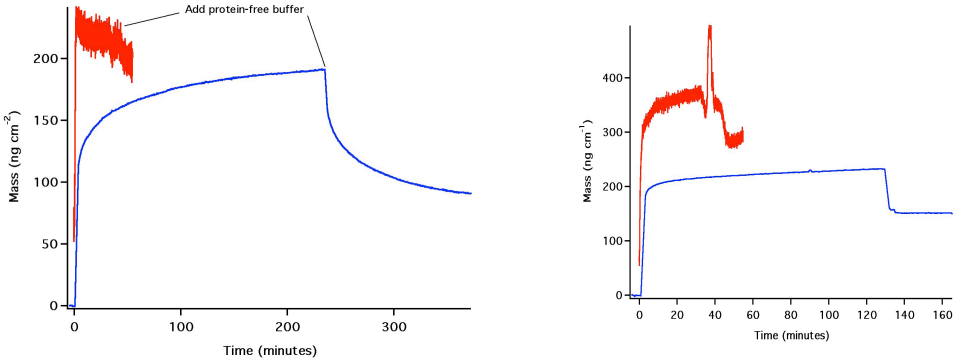

Figure 1. OWLS (Blue trace) and QCM (Red trace) data for PpcA (left) and OmcB (right). Not rapid adsorption and desorption kinetics of OmcB compared to PpcA.

During Sabbatical Leave in 2007-2008, Magnuson performed experiments using the innovative OWLS platform at the University of Wyoming, working with collaborators Eggleston and Colberg. OWLS essentially measures the amount of material (proteins, polysaccharides, or any other biomolecule of interest) that adsorbs to a test surface under a given set of conditions (pH, ionic strength). Two proteins were examined, as well as several synthetic metal oxide surfaces. Our findings were quite interesting, showing for the first time that cytochromes c from metal-respiring bacteria have a surface interaction property in addition to a catalytic electron transfer ability. PpcA, a periplasmic cytochrome from G. sulfurreducens, showed more desorption from an Al-oxide surface, and subsequent calculations showed that this protein is more contact in structure with less complexed water. The protein OmcB (G. sulfurreducens OM cytochrome c) shows surface specificity as well (Figure 2). We then examined mineral dependent surface adsorption with ApcA (from *Acidiphilium cryptum*) for their comparative adsorption properties to glass waveguide surfaces coated with either

silicon dioxide, titanium oxide, or iron oxide. Our results were quite intriguing, in that we did in fact observe surface-dependent behaviors. This technique has shown that mineral-specific adsorption of cytochromes c occurs, and that when coupled with quartz crystal microbalance analysis, corroborates protein homology models predicting structure of these cytochromes.

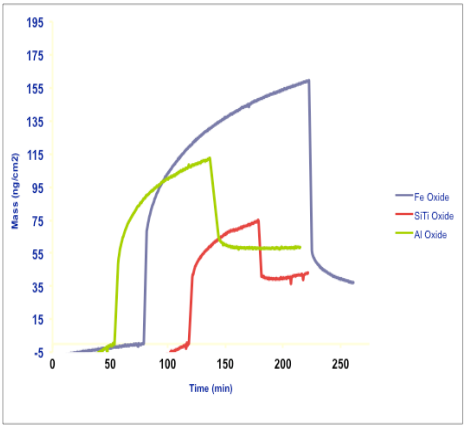

Figure 2. Results of OWLS experiments where OmcB was tested on several different metal oxide surfaces. The protein preparation was exactly the same in all experiments. From this it can be determined that OmcB shows a higher affinity for Fe-oxide, as well as a greater overall desorption. This result is expected for a protein that is evolved to interact reversibly with its physiologic electron acceptor.

Fluorescence spectroscopy. We have continued characterization of the cytochromes c purified from *Geobacter* and *Acidiphilium*, and are now using Fluorescence Spectroscopy to examine conformational change and redox state. Studies on ApcA (10.1 kDa cytochromes c from *A. cryptum*) show a distinct shift in the Tyrosine adsorption band, suggesting that conformation is different depending on whether the protein is oxidized or reduced. Studies are ongoing with the other representative cytochromes.

Homology Modeling using PHYRE. Using genome sequence data for *Acidiphilium* and *Geobacter*, we have constructed homology models of cytochromes c using PHYRE. These models are extremely useful in understanding protein structure, and thus far correlate with other laboratory observations. For example, ApcA (*A. cryptum*) is a relative compact protein with a well-defined heme binding site, and easily identifiable His and Met heme coordinating residues.

Examination of redox protein complexes that reside in membrane vesicles and the extracellular biofilm matrix. We have moved from studies of single proteins to examination of redox protein complexes. Experimental evidence suggests that *Acidiphilium cryptum* (one of our model organisms) forms outer membrane vesicles when grown under biofilm-forming conditions. These vesicles are known to contain c-type cytochromes, and represent a viable means of extracellular electron transport to mineral surfaces.

Structural determination of ApcA and ApcB, two periplasmic cytochromes that play roles in both Fe(III) and Cr(VI) reduction. We have continued our collaboration with Dr. John Cort of the Pacific Northwest National Laboratory on NMR structure determination of the periplasmic cytochromes ApcA and ApcB from *A. cryptum*. The

structures of ApcA in both oxidized and reduced states have been solved, and experiments are underway to obtain NMR spectral data for ApcB.

Voltammetry Studies. We have conducted electrochemical experiments on purified cytochromes and conformed that these proteins are electrophysiologically adapted for electron transport. ApcA, a mono-heme cytochrome, shows a midpoint potential of +200mV, within the range required for respiratory electron transfer to occur to Fe(III) at acidic pH (E=+700mV at pH 2). Moreover, these experiments have determined that ApcA can transfer electrons to soluble Fe(III) compounds at low pH. Taken together, the results suggest that ApcA is an electron transfer mediator to Fe(III) under Fe(III)-respiring growth conditions.

Deliverables:

Publications:

Kahre, N., D.M. Lovelace, C.M. Eggleston, M.W. Swenson, and T.S. Magnuson. 2006. Redox-linked conformation change and electron transfer between monoheme c-type cytochromes and oxides. Geochim. Cosmochim. Acta 70: 4332-4342.

Cummings, D.E., and T.S. Magnuson. 2007. Microbial Fe(III) reduction: ecological and physiological considerations. In Manual of Environmental Microbiology, 3rd Edition. American Society for Microbiology Press.

Magnuson, T.S., M.W. Swenson, L.A. Deobald, A.J. Paszczynski, and D.E. Cummings. 2010. Physiology and proteogenomics of Cr(VI) reduction in *Acidiphilium cryptum* JF-5. Biometals 23: 1129-38.

Magnuson, T.S. 2011. Commentary: How the xap locus put electrical 'zap' in *Geobacter sulfurreducens* biofilms. J. Bacteriol. 193(5): 1021-2.

Cort, J.R., M.W. Swenson, and T.S. Magnuson. 2011. 1H, 13C, and 15N backbone, side-chain, and heme chemical shift assignments for oxidized and reduced forms of the monoheme c-type cytochrome ApcA isolated from the acidophilic metal-reducing bacterium *Acidiphilium cryptum*. Biomol. NMR Assign. 5(1):89-92.

Invited Seminars and Meeting Presentations:

Magnuson, T.S., M.W. Swenson, R. Brown, T. Salazar. 2010. Proteomics of extracellular electron transport structures in an acidophilic bacterium. International Society for Microbial Ecology, Seattle, WA.

Sycheva, L.V., C.M. Eggleston, P.J.S. Colberg, T.S. Magnuson, and L. Shi. 2010. Redox-linked conformation change observed for adsorbed metal-reducing bacterial cytochromes. Goldschmidt 2010, Knoxville, TN.

T. S. Magnuson, M. Swenson, R. Brown, T. Salazar, B. Shakya. 2010. Biochemistry and proteomics of biofilm redox proteins in an acidophilic iron-respiring bacterium. 110th General Meeting, American Society for Microbiology, San Diego CA.

Thorne, J., and T.S. Magnuson. 2009. Synthesis of a knockout gene fragment for ApcA of *Acidiphilium cryptum*. 109th General Meeting, American Society for Microbiology, Philadelphia PA.

Cummings, D.E., D. Kerk, D. Sims, P. Richardson, B. Briggs, M. Swenson, and T.S. Magnuson. 2008. The *Acidiphilium cryptum* genome reveals capacities for metal transformation and mineral colonization. UCLA-Lake Arrowhead Conference on Microbial Genomics, Lake Arrowhead, CA.

M.W. Swenson, T.S. Magnuson, P.J.S. Colberg, and C.M. Eggleston. 2008. Functional Characterization of c-type Cytochromes from Iron-Respiring Bacteria. 18th V.M. Goldschmidt Conference, Vancouver, BC.

Swenson, M.W., P.J.S. Colberg, C.M. Eggleston, and T.S. Magnuson. 2008. Purification and Functional Characterization of Two Periplasmic C-Type Cytochromes from *Acidiphilium cryptum*. American Society for Microbiology General Meeting, Boston, MA.

Swenson, M.W., and T.S. Magnuson. 2009. Identification of Excreted and Surface Associated Proteins in Acidiphilium cryptum JF-5. 109th General Meeting, American Society for Microbiology, Philadelphia PA.

Magnuson, T.S., and M.W. Swenson. 2010. Collaborative Research Between ISU and the PNNL. Pacific Northwest National Laboratory, Richland WA.

Magnuson, T.S. et al. 2008. Microbial mineral transformation: A preponderance of possible permutations. Department of Geosciences, Oregon State University, Corvallis OR.

Magnuson, T.S. et al. 2008. Discovering the power of microbes for remediation and energy. Department of Microbiology, Molecular Biology, and Biochemistry, University of Idaho, Moscow ID.

Magnuson, T.S. et al. 2007. Harnessing mineral-transforming microbes for remediation and energy. Department of Zoology and Physiology, University of Wyoming, Laramie WY.

Magnuson, T.S., and M.W. Swenson. 2007. Redox and solution behavior of c-type cytochromes from mineral transforming bacteria. 17th Goldschmidt Symposium, Cologne Germany (Magnuson also served a a session chair).

Gresham, T.G., B. Briggs, M. Swenson, M. Day, L. Yang, M. A. Thomas, P. P. Sheridan, D. Sims, P. Richardson, D. Kerk, D. E. Cummings, and T. S. Magnuson. 2007. Genome Sequencing and Annotation of the Acidophilic Metal-reducing Bacterium Acidiphilium cryptum JF-5. Department of Energy-Joint Genome Institute Users Meeting, Walnut Creek, CA.

Magnuson, T.S. et al. 2006. Acidophiles and Metals: Implications for Bioremediation. American Chemical Society, San Francisco CA.

Magnuson, T.S. et al. 2006. Biochemical and genomic inquiries into the metal-reducing acidophile Acidiphilium cryptum JF-5. INRA Environmental Biofilms Symposium, Bozeman MT.

Tyler, T.L., D.E. Cummings, and T.S. Magnuson. 2006. Chemiluminescence-based detection of a novel chromate reductase on polyacrylamide gels. 106th General Meeting, American Society for Microbiology, Orlando, FL.

Magnuson, T.S., D.E. Cummings, T.L. Tyler, M.E. Swenson. 2006. Emerging mechanisms of electron transport in iron reducing bacteria. INRA Environmental and Subsurface Science Symposium, Big Sky, MT.