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Nanowires, Capacitors, and Other Novel Outer-Surface Components Involved in Electron Transfer to Fe(III) Oxides in Geobacter Species

Lovley, Derek R.
University of Massachusetts

RESULTS TO DATE: In the past year studies have primarily focused on elucidating the role of pili in electron transport to Fe(III) oxide in Geobacter sulfurreducens. As summarized in last year?s report, it was previously found that pili are specifically expressed during growth on Fe(III) oxide and that Fe(III) oxide reduction is inhibited if the gene for the structural pilin protein is deleted. However, it was also found that a pilin-deficient mutant of G. sulfurreducens could attached to Fe(III) oxide as well as wild type.

These results suggested that the pili might have a more direct role in electron transfer to Fe(III) oxides. In order to evaluate this, pili and other proteins exposed on the outer surface of G. sulfurreducens were sheared from the surface. These proteins were then examined with an atomic force microscope (AFM) fitted with a conducting tip in order to simultaneously profile topography and tip-to-substrate conductivity. When a voltage was applied to the tip there was a strong current response through the pilus filaments to the underlying graphite substrate, consistent with pili being highly conductive . In contrast, the non-pilin proteins that had also been sheared from the outer cell surface had no detectable conductivity and in instances in which the non-pilin proteins covered the pili filaments, they insulated the pili from the conductive tip. Repeated scans on the same region of the pilus filaments while applying a sweep-bias voltage demonstrated the linear, ohmic correspondence between current and voltage applied. When similar studies were carried out with pili from Shewanella oneidensis, no conductance was detected. This is consistent with the concept that Shewanella species produce electron shuttles to transfer electrons onto Fe(III) oxides. Pili from the non-metal reducer, Pseudomonas aeruginosa, were also not conductive.

The finding that the pili of G. sulfurreducens are highly conductive suggests that G. sulfurreducens requires pili in order to reduce Fe(III) oxides because pili are the electrical connection between the cell and the surface of the Fe(III) oxides. This contrasts with the nearly universal concept that outer-membrane cytochromes are the proteins which transfer electrons to Fe(III) oxide in Fe(III) reducer. However, the concept of pili serving as the terminal reductase for Fe(III) oxides is in accordance with the conservation of pilins in the Geobacteraceae and the lack of conservation of c-type cytochromes. Furthermore, it seems unlikely that outer-membrane cytochromes, which have to be at least partially embedded in the membrane, would have sufficient exposure on the outer cell surface to permit efficient electrical contact between the cytochrome and Fe(III) oxide, especially in soils in which Fe(III) oxides exist as heterogeneously dispersed coatings on clays and other particulate matter. In contrast, conductive pili extend the electron transfer capabilities well beyond the cell surface. The pilus apparatus is anchored in the periplasm and outer membrane of gram-negative cells, thus offering the possibility of pili accepting electrons from periplasmic and/or outer membrane electron transfer proteins. These intermediary electron transfer proteins need not be the same in all organisms, consistent with the differences in cytochrome content and/or composition in different Geobacteraceae. The likely function of the pili is to complete the circuit between these various intermediary electron carriers and the Fe(III) oxide.

In addition to serving as a conduit for electron transfer to Fe(III) oxides, pili could conceivably be involved in other electron transfer reactions. For example, pili of individual Geobacter cells are often intertwined, raising the possibility of cell-to-cell electron transfer via pili. These biologically produced nanowires might be useful in nanoelectronic applications with the possibility of genetically modifying pilin structure and/or composition to generate nanowires with different functionalities.

A manuscript summarizing these pilin studies has been accepted for publication in Nature.

Studies outlined in detail in last year?s report on: 1) the function of the most abundant protein in the outer membrane of G. sulfurreducens; 2) the role of a secretion system in secreting a multi-copper outer-membrane protein required for Fe(III) oxide reduction; 3) the role of outer-membrane c-type cytochromes in U(VI) reduction; and 4) the discovery of an outer-membrane cytochrome specifically required for Fe(III) oxide reduction were completed and manuscripts for each of these studies have been submitted for publication.

DELIVERABLES: 1. Afkar, E. A., G. Ruggera, and D. R. Lovley. 2005. A novel Geobacteraceae-specific outer membrane protein, OmpJ, is essential for electron transport to Fe (III) and Mn (IV) oxides in Geobacter sulfurreducens.(submitted). 2. Lovley, D. R., D. E. Holmes, and K. P. Nevin. 2004. Dissimilatory Fe(III) and Mn(IV) reduction. Adv. Microb. Physiol. 49:219-286. 3. Mehta, T., S. E. Childers, R. Glaven, T. Mester, and D. R. Lovley. 2005. A novel type II secretion system required for the secretion of a novel multi-copper protein specifically required for the reduction of insoluble, but not soluble Fe(III) in Geobacter sulfurreducens.(submitted). 4. Mehta, T., M. V. Coppi, S. E. Childers, and D. R. Lovley. 2005. Outer membrane cytochromes required for Fe(III) and Mn(IV) oxide reduction in Geobacter sulfurreducens.(submitted). 5. Reguera, G., K. D. McCarthy, T. Mehta, J. Nicoll, M. T. Tuominen, and D. R. Lovley. 2005. Extracellular electron transfer via microbial nanowires. Nature. (in press). 6. Shelobolina, E. S., S. A. Sullivan, M. V. Coppi, C. Leang, L. DiDonato, J. E. Butler, and D. R. Lovley. 2005. Evaluation of the role of c-type cytochromes in U(VI) reduction by Geobacter sulfurreducens.(submitted).