Display and retraction of outer membrane cytochromes by *Shewanella oneidensis* in response to electron acceptor availability

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Morphological and compositional analyses of the surface of *Shewanella oneidensis* strain MR-1 were conducted and compared under conditions of electron acceptor limitation and electron acceptor repletion. Atomic force microscopy (AFM) reveals that cells display nanoscale domains that protrude through the outer membrane when availability of electron acceptor is limited. These protrusions disappear within seconds after relieving electron acceptor limitation using a variety of dissolved electron acceptors. Mutants of MR-1 that do not produce outer membrane porin-forming protein (gspD) associated with the type II secreton (T2S) machinery do not produce protrusions under either electron acceptor limited or excess conditions. Surface enhanced Raman spectroscopy (SERS) clearly reveals that heme containing proteins are selectively displayed on surface of wild type MR-1 cells in response to acceptor limitation. SERS spectra for heme is absent from surfaces of cells under conditions of electron acceptor excess. The gspD mutant does not display surface heme under either electron acceptor limited or excess conditions. Correlation of data from AFM and SERS supports our hypothesis that heme containing cytochromes, possibly those implicated in solid phase iron and manganese oxide reduction, exist in complexation with proteins of the T2S to form a functional protein complex that extends and retracts through the outer membrane in direct response to electron acceptor availability. The results of our studies provide support for a physiologically based model describing distribution and mobility of redox-reactive heme proteins on microbial cell surfaces.

Role of proteins in silicification

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Recent experimental work on microbial silicification has shown that different microorganisms are capable of being silicified with different degrees of fidelity. This species-specific pattern is not surprising considering that the actual mechanisms of silicification rely, in part, on the microorganisms providing reactive surface ligands that initially adsorb polymeric/colloidal silica from solution. At present, the silica immobilisation stage appears to occur via two distinct mechanisms: (1) hydrogen bonding between hydroxyl ions in the silica polymer with hydroxy functional groups and (2) metal cation-hydroxide bridging between the silica and negatively-charged functional groups. We are currently investigating a third mechanism, namely the reaction between silica and positively-charged amine groups.

Using the biofilm-forming bacterium *Sulfuri-hydrogenibium azorense*, we show that when grown as H_2-oxidisers, the cells respond to increasing silica concentrations by producing excess protein. Importantly, potentiometric titrations show abundant amine functional groups on the organic surfaces, while transmission electron microscopy reveals silica colloids aggregated within the surrounding biofilm, but not associated with the wall.Preliminary protein-denaturing gel electrophoresis study of *Anabaena* sp. 7120, grown in polymerising silica solutions, indicate the induction of specific membrane proteins not normally found in non-silica growth conditions. The protein component of the plasma membrane changed at various silica concentrations, despite polymerisation experiments indicating that silica was not bound to the cell surface. This suggests that *Anabaena* possesses signal-transduction pathways appropriate to a biomineralization response.