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Abstract

This thesis concerns the biological process of iron reduction mediated by microbially produced extracellular redox-active organic molecules. Two different iron reducing bacteria, *Shewanella oneidensis* strain MR-1 and *Pseudomonas chlororaphis* strain PLC1391, were studied. *S. oneidensis* can grow by reducing ferric iron [Fe(III)] as a terminal electron acceptor in anaerobic respiration (i.e., dissimilatory iron reduction), whereas *P. chlororaphis* is a plant root isolate that cannot respire iron but can reduce it under certain conditions. Previous studies had suggested that *S. oneidensis* produces extracellular electron shuttles as a strategy for transferring electrons to an external electron acceptor (e.g., poorly crystalline iron (hydr)oxides, $Fe(OH)_3$), however this had not been shown. To investigate this, a new method was developed to measure iron reduction at a distance using $Fe(OH)_3$-coated porous glass beads. Because 91% of the iron in the beads is not directly accessible to cells, reduction in excess of 9% indicates that iron is being reduced by a process that involves a soluble mediator. Given this assay, it was shown that Fe(III) reduction at a distance is an active process that requires anaerobic conditions and coincides with biofilm formation on the beads. Although the structure of the mediator is unknown at this time, it presumably functions either as an electron shuttle or as an iron chelator. The possibility that *S. oneidensis* excretes a soluble quinone for this purpose (derived from the menaquinone biosynthetic pathway) was ruled out, but it was shown that these quinones are present in culture fluids and can be used by cells to make

menaquinone. Regardless of the nature of the mediator, it appears to act locally within the biofilm-bead environment for *S. oneidensis*. In the case of *P. chlororaphis*, this work shows that redox active secondary metabolites (e.g., phenazine antibiotics) can promote microbial mineral reduction. *P. chlororaphis* can reductively dissolve poorly crystalline iron and manganese oxides whereas a mutant in one of the phenazine biosynthetic genes (*phzB*) cannot; the addition of purified phenazine-1-carboxamide (PCN) rescues the mutant strain. The small amount of PCN produced relative to the large amount of Fe(III) reduced in cultures of *P. chlororaphis* implies that PCN is recycled multiple times; moreover, poorly crystalline iron (hydr)oxide can be reduced abiotically by reduced PCN. This suggests that PCN functions as an electron shuttle rather than an iron chelator, consistent with the observation that dissolved Fe(III) is undetectable in culture fluids. Multiple phenazines and the glycopeptidic antibiotic, bleomycin, can also stimulate mineral reduction by *S. oneidensis* MR-1. Because diverse bacterial strains that cannot grow on iron can reduce phenazines, and thermodynamic calculations suggest that phenazines have lower redox potentials than poorly crystalline iron (hydr)oxides in a range of relevant environmental pH (5 to 9), it seems likely that natural products like phenazines promote microbial mineral reduction in the environment. Whether cycling of microbially produced extracellular redox-active organic molecules serves a physiological function remains to be determined.

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