Identification and Characterization of Microbial Proteins Important for Extracellular Electron Transfer Reactions

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Microbial proteins and protein complexes involved in extracellular electron transfer reactions play a critical role in the biogeochemical cycling of metals. These proteins usually form electron transfer pathways that link the extracellular metal redox reactions and metabolic activities inside microbial cells. Our research goal is to establish a molecular-level understanding of the roles of the key proteins of these electron transfer pathways in Fe(II/III) redox transformations in the environment.

Originally discovered in the dissimilatory metal-reducing bacterium *Shewanella oneidensis* MR-1 (MR-1), key components of the Mtr (i.e., metal-reducing) pathway exist in all characterized strains of metal-reducing *Shewanella*. A survey of sequenced microbial genomes has identified homologues of the Mtr pathway in other dissimilatory Fe(III)-reducing bacteria, including *Aeromonas hydrophila*, *Ferrimonas balearica* and *Rhodoferax ferrireducens* and in the Fe(II)-oxidizing bacteria *Dechloromonas aromatica* RCB (RCB), *Gallionella capsiferriformans* ES-2 (ES-2) and *Sideroxydans lithotrophicus* ES-1 (ES-1). The putative Mtr pathways identified from the Fe(II)-oxidizing bacteria are named as Mto (i.e., metal-oxidizing) pathways to distinguish them from those found in the Fe(III)-reducing bacteria. The apparent widespread distribution of Mtr/Mto pathways in both Fe(III)-reducing and Fe(II)-oxidizing bacteria suggests a bi-directional electron transfer role, and emphasizes the importance of this type of extracellular electron transfer pathway in microbial redox transformation of Fe.

The *mtoABCD* and *cymA* genes of the Mto pathways identified from the Fe(II)-oxidizing bacteria ES-1, ES-2 and RCB were cloned for heterologous expression and subsequent characterization. MtoA is believed to be inserted into MtoB and mediates electron transfer across the outer membrane between extracellular Fe(II) and MtoD in the periplasm. Consistent with this prediction, cloned *mtoA* of ES-1 partially complemented an MR-1 mutant without MtrA with regard to ferrihydrite reduction. Purified MtoA of ES-1 was a decaheme *c*-type cytochrome and oxidized soluble Fe(II). Oxidation of Fe(II) by MtoA was pH- and Fe(II)-complexing ligand-dependent. Thermodynamic modeling shows that redox reaction rates for the different Fe(II)-complexes correlated with their respective estimated reaction-free energies. Thus, characterization results are consistent with the prediction that MtoA of ES-1 may be involved in Fe(II) oxidation by ES-1.

In the Mtr pathway of MR-1, MtrABC transfer electrons from the periplasm, across the outer membrane to the surfaces of extracellular Fe(III)-containing minerals. To characterize their interfacial electron transfer rate to solid phase Fe(III) oxides, MR-1 MtrABC were incorporated into proteoliposomes. Immuo-gold localization confirmed that MtrC was exposed at the surfaces of proteoliposomes, which is consistent with the results of proteolytic digestion. Under the conditions tested, MR-1 MtrABC in proteoliposomes facilitated electron transfer between reduced methyl viologen inside proteoliposomes, as the electron donor, to goethite (α-FeOOH) and lepidocrocite (γ-FeOOH) external to the proteoliposomes in the absence of any electron shuttle, such as flavins, at rates of $1288 \pm 39\ s^{-1}$ and $8756 \pm 505\ s^{-1}$, respectively. These results demonstrate the intrinsic ability of MR-1 MtrABC to transfer electrons to solid phase Fe(III) oxides at physiologically relevant rates.

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