Genetic evidence that multiple cytochrome nanowires are necessary for Fe(III) oxide reduction in *Geobacter sulfurreducens*

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Abstract

Geobacter sulfurreducens is a dissimilatory metal-reducing microorganism capable of utilizing insoluble acceptors via extracellular electron transfer. While a large number of multiheme *c*-type cytochromes expressed by *G. sulfurreducens* are implicated in linking its cytoplasmic respiratory chain to materials beyond its outer membrane, whether these proteins have specific roles in reduction or recognition of particular metals is unknown. Recently, structures of three extracellular conductive *c*-type cytochrome filaments, often referred to as nanowires, were reported. Comprised of either OmcS, OmcE, or OmcZ, these nanowires are long polymers of protein subunits with a core of closely spaced hemes, with no similarity in sequence, fold, glycosylation, subunit size, or diameter. We utilized a markerless deletion approach to construct single, double, and triple-deletion strains in an isogenic background to investigate possible roles of OmcS, OmcE, and OmcZ. When soluble Fe(III) or the organic acceptor fumarate were electron acceptors, no defects were observed in any mutant. When freshly precipitated Fe(III) oxide was tested as an electron acceptor, mutants lacking *omcE* were strongly affected, reducing Fe(III) approximately half as fast. No other single mutant (Δ*omcS* or Δ*omcZ*) showed a defect. Double mutants containing only *omcE* (Δ*omcSZ*) also showed a defect, suggesting other proteins could be required in addition to OmcE. The double mutant containing only *omcZ* (Δ*omcSE*) also showed a partial defect, while double mutants containing only *omcS* (Δ *omcEZ*) were completely unable to reduce Fe(III) oxide. The triple (Δ*omcESZ*) mutant was also unable to reduce Fe(III) oxides. Taken together, this indicates that genes for two separate nanowires are necessary to completely reduce this form of Fe(III) oxide. This is the first evidence that *omcZ*, which has only been implicated in electron transfer to electrodes, could also be needed for metal reduction. With the recent discovery of two completely unrelated mutltiheme cytochrome nanowires in thermophilic Archaea, different conductive filaments with different substrate specificities may have repeatedly evolved to facilitate extracellular respiration.

Keywords

nanowires, metal reduction, extracellular electron transfer, iron cycling, anaerobic metabolism, environmental geochemsitry

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Conflicts of interest

The authors have declared that no competing interests exist.