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Particle size effect and the mechanism of hematite reduction by the outer membrane cytochrome OmcA of *Shewanella oneidensis* MR-1

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Abstract

The cycling of iron at the Earth's near surface is profoundly influenced by dissimilatory metal reducing microorganisms, and many studies have focused on unraveling electron transfer mechanisms between these bacteria and Fe(III)-(oxyhydr) oxides. However, these efforts have been complicated by the fact that these minerals often occur in the micro- to nanosize regime, and in relevant natural environments as well as in the laboratory are subject to aggregation. The nature of the physical interface between the cellular envelope, the outer-membrane cytochromes responsible for facilitating the interfacial electron transfer step, and these complex mineral particulates is thus difficult to probe. Previous studies using whole cells have reported reduction rates that do not correlate with particle size. In the present study we isolate the interaction between the decaheme outer-membrane cytochrome OmcA of *Shewanella oneidensis* and nanoparticulate hematite, examining the reduction rate as a function of particle size and reaction products through detailed characterization of the electron balance and the structure and valence of iron at particle surfaces. By comparison with abiotic reduction via the smaller molecule ascorbic acid, we show that the reduction rate is systematically controlled by the sterically accessible interfacial contact area between OmcA and hematite in particle aggregates; rates increase once pore throat sizes in aggregates become as large as OmcA. Simultaneous measure of OmcA oxidation against Fe(II) release shows a ratio of 1:10, consistent with a cascade OmcA oxidation mechanism heme by heme. X-ray absorption spectroscopies reveal incipient magnetite on the reacted surfaces of the hematite nanoparticles after reaction. The collective findings establish the importance of accessibility of physical contact between the terminal reductases and iron oxide surfaces, and through apparent consistency of observations help reconcile behavior reported at the larger more complex scale of whole cell studies.

Keywords: Hematite; Nanoparticle; Size effect; Microbial iron reduction; Aggregation electron transfer; Cytochrome

1. INTRODUCTION

In the natural environment, reduction of sparingly soluble Fe(III) oxide minerals to the highly soluble $Fe(II)_{(aq)}$ form by dissimilatory metal-reducing bacteria (DMRB) is a prevalent redox process that controls iron cycling in the

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subsurface, and can influence the fate and transport of redox-active metals, as well as organic and inorganic contaminants (e.g., Lovley et al., 1996; Islam et al., 2004). To access extracellular oxide mineral electron acceptors, it has been proposed that c-type cytochromes work in concert to comprise a long-range electron transport network across the periplasm and outer membrane of the cell envelope, terminating at decaheme cytochromes whose function is to facilitate the interfacial electron transfer (ET) step (Shi et al., 2009; Clarke et al., 2011; Liang et al., 2016). Along with MtrC, OmcA is one of these terminal reductases. It is found on the outer membrane and in the extracellular polymeric substances of *Shewanella oneidensis* MR-1, an extensively studied DMRB for its role in environmental biogeochemistry and its considerable potential influence on the remediation of contaminated environments (Fredrickson et al., 2008; Cao et al., 2011).

The 85 kDa OmcA shows broad redox potentials ranging from −320 to −20 mV versus SHE (Standard Hydrogen Electrode) (Shi et al., 2006). A variety of laboratory measurements have demonstrated that its 10 heme groups are involved in ET to metal acceptors, along with those in MtrC (Shi et al., 2006; Xiong et al., 2006; Wang et al., 2008; Ross et al., 2009; Johs et al., 2010). Determination of the molecular structure of MtrF, an MtrC homolog, and more recently the structure determination of OmcA itself (Edwards et al., 2014), suggest that these proteins transfer electrons directly to the surface of metal oxides via one or more solvent-exposed hemes (Clarke et al., 2011; Shi et al., 2012a) within a conserved staggered cross heme wire motif. For example, with regards to the widespread Fe(III)-oxide mineral hematite (α-Fe_2O_3), experimental and computational studies have shown that purified OmcA is able to both specifically bind to hematite surfaces and transfer electrons to it (Xiong et al., 2006; Kerisit et al., 2007; Eggleston et al., 2008; Lower et al., 2008; Wigginton et al., 2009; Johs et al., 2010).

Despite recent discoveries relating to the structural, electrochemical, and spectroscopic characteristics for these key outer-membrane cytochromes, resolving their function at a detailed level requires close examination of how properties of the electron accepting metal oxide itself can affect the interfacial ET step. At the whole cell level, in experiments with *S. oneidensis*, metal oxide reduction rates were shown to depend on characteristics of the metal oxide such as particle size, surface area, surface structure, solubility, mineral structure, impurity, and crystallinity (Roden and Zachara, 1996; Neal et al., 2003; Bonneville et al., 2009; Bose et al., 2009; Ekstrom et al., 2010). For small particle sizes, such as for many of the metal oxides in nature (Hochella et al., 2008), additional characteristics such as extent of particle aggregation and size distribution become important, in principle, because they can impact the steric accessibility of solid surface area to cytochromes, which themselves are of nanometer size and situated in a geometrically constrained configuration on the outer membranes of cells.

In spite of its importance for understanding the interfacial ET rate, unraveling the impacts of nanoparticle properties on DMRB activity has been difficult. Attempts using whole cell studies have left the issue unresolved. Roden (2003) suggested that initial rates of microbial Fe(III) oxide reduction are linearly correlated to BET surface area and the reactive surface site density of the solid phase. However, Bose et al. (2009) reported that the microbial reduction rates for 11, 12, 30, 43, and 99 nm hematite nanoparticles are not proportional to particle size, and postulated that metal oxide particles of different sizes may attach to DMRBs in different modes. Yan et al. (2008) suggested that the reduction rates should be directly correlated to the bacteria-hematite contact area, not to the total oxide surface area. Studies to date thus appear to be in conflict, possibly because of the complexities of understanding the manifold of interactions between whole cells and very small particles overall (Roden, 2003; Yan et al., 2008; Bose et al., 2009; Bosch et al., 2010). Novel experiments are needed that minimize the number of modes of physical and chemical interaction between the particles and terminal reductases to understand mechanisms of ET between DMRB and metal oxides in the environment.

The present study investigates the particle size effect directly, making use of purified OmcA as the electron donor and hematite particles with well-defined sizes as electron acceptors. We report reduction kinetics of hematite particles with mean primary particle sizes of 15, 30, 55, and 173 nm, by OmcA, and we compare to the size-dependence of the hematite reduction kinetics by ascorbic acid, a reducing agent analog for biogeochemical organic compounds in nature (Echigo et al., 2012). Characteristics of the hematite particles before and after reaction with OmcA were investigated using a variety of methods, including in situ micro-X-ray diffraction (μ-XRD), transmission electron microscopy (TEM), and Fe $L_{2,3}$-edge X-ray absorption (XA)/X-ray magnetic circular dichroism (XMCD) spectroscopy. The observed size effect is resolved and discussed in terms of separate factors such as primary particle size itself, the molecular size of the reductant, and particle aggregation. This study is the first investigation of the effect of particle size on ET kinetics between hematite and an important c-type cytochrome in the transmembrane electron transfer network of a primary DMRB.

2. MATERIALS AND METHODS

All glassware and plastic bottles for wet chemical experiments were soaked in 1% HNO_3 overnight and then rinsed several times with distilled and deionized water before use. All chemicals, plastic syringes, tubes, vials, pipette tips, and syringe filters were deoxygenated for at least 24 h inside the glovebox prior to use. All spectroscopic kinetic measurements as well as batch reactions (including OmcA and ascorbic acid reduction experiments) were conducted in the glovebox. Degassed and deionized water (DDW) was stored in an anoxic chamber for preparation of all solutions and suspensions. All chemicals were reagent grade or better. All experiments were performed in triplicate. All reduction experiments were performed at least in triplicate in a high-purity dry N_2 glovebox (<1 ppm O_2).