Electron exchange between ferredoxin, cytochrome, and hematite

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ABSTRACT: The use of solid ferric hydr(oxides) as respiratory electron acceptors by dissimilatory iron-reducing bacteria imposes constraints on the properties of molecules involved in the process. Several metalloproteins change redox properties when sorbed on electrode surfaces. Sorption-induced folding and orientation changes may be involved in electron transport to mineral surfaces. Here, we present initial results for the interaction of ferredoxin (an iron sulfur protein) and c-type cytochrome (a heme-based protein) with hematite surfaces. The results imply that the redox properties of ferredoxin depend on its sorbed concentration, the presence of other adsorbates (impurities as well as cytochrome), and pH. This is consistent with observations of the properties of these biomolecules on non-mineral electrodes.

1 INTRODUCTION

It has been known for well over a decade that a number of species of Bacteria and Archaea can couple the reduction of Fe³⁺ and other metals to the oxidation of organic matter. Indeed, this respiratory strategy may have been among the first to evolve (e.g. Lovley 2002). These organisms have been the subject of growing interest because of their novel electron transport pathways and their bioremediation potential. In order to understand and optimize the usefulness of these organisms, it is necessary to understand the pathways by which they transfer electrons to Fe³⁺ and other metals. Electron transfer to a solid mineral presents biochemical challenges that differ from those encountered in the use of gasses (or dissolved gasses) as respiratory electron acceptors. Some species of dissimilatory iron reducing bacteria (DIRB) apparently require direct contact with ferric minerals in order to accomplish iron reduction (e.g. Nevin & Lovley 2000), while others appear to have the ability to synthesize soluble “electron shuttle” molecules (although this is controversial; e.g. Newman & Kolter, 2000; Hernandez & Newman, 2001).

Among the molecules implicated in the outer-membrane electron transport chain used in Fe³⁺ reduction are quinones (e.g., menaquinone), c-type cytochromes, and possibly melanin (Myers & Myers 1993, 1997; Newman & Kolter 2000; Turick et al. 2002). Several DIRB species can reduce ferric minerals when provided with electron shuttle molecules such as anthraquinone-2,6-disulfonate (AQDS) and humic acids (e.g. Lovley et al. 1996; Zachara et al. 1998). Here, we report initial steps taken toward understanding whether another molecule, iron-sulfur protein (ferredoxin), could act as an electron shuttle between cytochromes (associated with the outer membrane of DIRB) and an iron oxide surface. Wirtz et al. (2000) have shown that ferredoxin can act as an electron shuttle between indium-doped tin oxide (ITO) electrodes and cytochrome P450.

The [2Fe-2S] iron sulfur protein (ferredoxin) of the well-studied and ubiquitous cytochrome bc₁ complex (e.g. Crofts & Berry 1998) has recently been found to be part of the ability of some DIRB to reduce iron (Kaufmann & Lovley 2001), along with cytochromes and quinones.

Our goal is to investigate a similar system to that of Wirtz et al. (2002), substituting hematite for the ITO and focusing on the ability of ferredoxin to shuttle electrons between cytochrome (which would be associated with DIRB outer membrane) and the hematite surface (which would likely be separated from the outer membrane by a lipopolysaccharide...
layer). This is a preliminary report presenting some AFM results for c-type horse heart cytochrome sorbed to hematite, and cyclic voltammetry results for ferredoxin, and ferredoxin together with cytochrome, on hematite. AFM of ferredoxin, voltammetry of cytochrome on hematite, and corresponding adsorption studies the subject of ongoing work.

2 METHODS

Natural n-type hematite with a charge carrier density of about 2.0 x 10\(^3\) atom% due to Sn and Ti impurities was used (see Eggleston et al. 2003). Horse heart cytochrome c was purchased from Sigma and dissolved in 1 M ammonium phosphate at a concentration of 0.3 mg L\(^{-1}\). Atomic force microscope (AFM) imaging was accomplished both by evaporating a droplet of cytochrome solution onto a hematite sample, and by in-situ imaging in a diluted (0.03 mg L\(^{-1}\)) solution. Spinach ferredoxin was purchased from Sigma in a liquid (35% solution) 0.15M Trizma and NaCl buffer, pH 7.5. Hematite crystals were immersed in in MOPS buffer at pH 7, and the ferredoxin solution injected as received (MOPS buffer was used in similar cyclic voltammetry experiments by Wirtz et al. 2002) to give a solution concentration of 0.5 mg L\(^{-1}\). Subsequent additions of ferredoxin solution gave concentrations of 0.88 and 1.1 mg L\(^{-1}\). The solution pH was checked after each change of aqueous conditions, including acidifications with HCl. AFM was conducted using a Digital Instruments Nanoscope IIIa controller running a Multimode AFM in contact mode both in air and in aqueous solution. Cyclic voltammetry was conducted with several different voltage ranges, in deaerated solution, at 25 mV s\(^{-1}\).

3 RESULTS

3.1 Cytochrome imaging

Preliminary AFM imaging of c-type cytochromes deposited on hematite in different ways are presented in Figure 1. In general, the results show that c-type cytochromes adhere strongly to hematite surfaces, to the extent that AC AFM imaging modes are not necessary in order to obtain images of cytochromes, even in aqueous solution. These results imply that the forces of interaction between the cytochromes and the surface are probably greater than the forces that hold the protein in a particular conformation, so that the molecules are likely denatured upon sorption.

Figure 1. Top: (15x15 \(\mu\)m) Aggregated 10-20 nm particles of cytochromes (lower left) deposited on a clean hematite surface (upper right); Middle: 5x5 \(\mu\)m area cleared of cytochrome by a high-force scan (~200 nN); steps and terraces are readily apparent; Bottom: 5x5 \(\mu\)m area after re-sorption of cytochrome aggregates from the surrounding solution. The scan field near center was created by scanning at high force, followed by subsequent imaging on about 2 nN net force.
3.2 Cyclic voltammetry, ferredoxin

Cyclic voltammetry of hematite electrodes in ferredoxin-free control buffer as well as ferredoxin-containing solutions are presented in Fig. 2. In the initial control, cyclic voltammogram shows broad hysteresis, as expected for the capacitance of a semiconductor. Scans started at the positive potential, scanned toward the negative endpoint, and then returned to the positive end potential. The general increase in current toward negative potential is most likely caused by incipient iron reduction at the hematite surface, followed by its oxidation on the return to positive potential, similar to the observations of Balko & Clarkson (2001). With the addition of ferredoxin, a broad new oxidation peak appears in the vicinity of 0 to –0.1 mV (there is a very broad reduction peak at about –0.1 mV range, but it is obscured by the space-charge capacitance changes caused by pH changes; both peaks are more evident in the series of 5 scans presented in Fig. 3).

Acidification was undertaken in order to investigate whether creation of a positively charged surface would attract the negatively charged redox functional region of the ferredoxin molecule (Wirtz et al. 2002). There are only slight increases in current in response to lowered pH, however (Fig. 2). Addition of another aliquot of ferredoxin (to 1.1 mg L⁻¹) resulted in a dramatic change in the cyclic voltammogram, with the oxidation peak shifting to about 0.2 mV (without ferredoxin, voltammograms to higher positive potentials showed no oxidation peak). This implies that either an increased surface coverage of ferredoxin (perhaps together with other lipids and proteins from the impure ferredoxin solution) results in significant changes in the redox properties of the ferredoxin molecule.

Returning to neutral pH and lower ferredoxin concentrations with a new hematite sample, we investigated the possibility that sorption or ordering/orientation of ferredoxin on the hematite surface is slow. Figure 3 shows increased redox current associated with ferredoxin on each of five successive scans over a three minute period, implying slow sorption or ordering/orientation kinetics. Addition of cytochrome c (0.3 mg L⁻¹ solution) to the solution investigated in Figure 3 results in a drastically altered voltammogram (Fig. 4) in which, although the oxidation peak has not shifted greatly, the reduction peak occurs at about –0.3V. However, it is not known whether this peak is due to ferredoxin or cytochrome. In any case, the redox properties of the mixed impurity, ferredoxin, and cytochrome layer on the hematite surface clearly change in response to sorption of different substances to the surface, implying environment-dependent molecular properties. These observations are similar, for example, to observed denaturing of cytochrome upon sorption to a metal electrode, but stabilization of the cytochrome by coadsorbing it with a lipid, bipyridine, or phosphate layer (Boussaad et al. 1998).
CONCLUSION

Our results show that cytochrome c adheres strongly to the hematite surface, allowing relatively easy non-AC AFM imaging in situ. The redox properties of ferredoxin appear to depend on surface coverage as well as the presence of other biomolecules. The results are consistent with the idea that the surface environment can affect the redox properties of sorbed biomolecules through stabilization of particular conformations or molecular orientations. Future work will concentrate on the role of ferredoxin as an electron shuttle between membrane-bound cytochrome and a ferric oxide surface.

REFERENCES