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Electron Transfer by *Desulfobulbus propionicus* to Fe(III) and Graphite Electrodes

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*Desulfobulbus propionicus* was able to grow with Fe(III), the humic acids analog anthraquinone-2,6-disulfonate (AQDS), or a graphite electrode as an electron acceptor. These results provide an explanation for the enrichment of *Desulfobulbaceae* species on the surface of electrodes harvesting electricity from anaerobic marine sediments and further expand the diversity of microorganisms known to have the ability to use both sulfate and Fe(III) as an electron acceptor.

Electrical energy can be harvested from marine sediments when a graphite electrode emplaced in marine sediment (anode) is connected by an electrical circuit to another electrode (cathode) in overlying aerobic water (22). Microbial activity in the sediments is required for current production (2, 8, 25, 28), because both electrodes and Fe(III) oxides represent insoluble, extracellular electron acceptors.

**Dissimilatory Fe(III) reduction.** The representative *Desulfo- bulbaceae* species, *D. propionicus* (DSMZ 2032), was obtained from DSMZ (German Collection of Microorganisms and Cell Cultures; Braunschweig, Germany) and was grown in a slightly modified version of NB Basal medium (14), which contained the following (per liter): 0.42 g of KH$_2$PO$_4$, 0.22 g of K$_2$HPO$_4$, 0.2 g of NH$_4$Cl, 0.38 g of KCl, 0.36 g of NaCl, 0.75 g of CaCl$_2$·2H$_2$O, 0.10 g of MgCl$_2$·6H$_2$O, 1.8 g of NaHCO$_3$, and 0.5 g of Na$_2$CO$_3$, as well as 1 μM Na$_2$SeO$_4$ and trace minerals and vitamins. This medium differed from that typically used to culture *D. propionicus* (DSMZ medium 194) in that it contained selenium and fivefold more calcium. Sulfate other than that present in the trace metal solution (300 μM) was omitted to prevent extensive sulfide production with subsequent abiotic reduction of Fe(III). Strict anaerobic techniques were used throughout, and cultures were incubated at 30°C in the dark. Organic acids (21), cell numbers (19), Fe(II), and total iron (19, 20) were monitored as previously described. Growth with various electron donors and acceptors was considered positive only after six consecutive transfers.

When *D. propionicus* was grown on pyruvate alone, 6.67 ± 0.33 mM (n = 3) pyruvate was fermented to 4.22 ± 0.51 mM acetate and 2.23 ± 0.35 mM propionate according to the following reaction: 3CH$_3$COCOO$^-$ + 3H$_2$O→2CH$_3$COO$^-$ + CH$_3$CH$_2$COO$^-$ + 2HCO$_3^-$ + 2H$^+$.

These results are similar to those of previous studies that have shown that acetate and propionate are formed in a 2:1 ratio when *Desulfobulbus propionicus* is grown on pyruvate in the absence of an electron acceptor (20).

Pyruvate consumption by *D. propionicus* differed significantly from fermentation when a soluble form of iron was provided as an electron acceptor. For example, when pyruvate (mean ± standard deviation, 19.20 ± 0.68 mM; n = 3) was provided as the electron donor with Fe(III)-citrate (50 mM) as the electron acceptor, Fe(III) was reduced and 18.65 ± 0.36 mM acetate was formed, accompanied by cell growth (Fig. 1a). The stoichiometry of pyruvate consumption and Fe(III) reduction was consistent with the following reaction: CH$_3$COCOO$^-$ + 2Fe$^{3+}$ + 2H$_2$O→CH$_3$COO$^-$ + HCO$_3^-$ + 2Fe$^{2+}$ + 3H$^+$. *D. propionicus* was able to grow with several other soluble...
Electron acceptors, including Fe(III)-NTA (5 mM), Fe(III)-pyrophosphate (10 mM), and anthraquinone-2,6-disulfonate (AQDS; 5 mM), with pyruvate as the electron donor. Propionate, lactate, and hydrogen also served as electron donors for growth on all forms of soluble Fe(III) evaluated as well as AQDS. The mechanism(s) for Fe(III) reduction appeared to be independent of the mechanism(s) for sulfate reduction, because *D. propionicus* continued to reduce Fe(III)-citrate in the presence of 1 to 10 mM molybdate, an inhibitor of sulfate reduction.

There was a mixture of pyruvate fermentation and Fe(III) reduction when poorly crystalline Fe(III)-oxide (100 mM) (17) was provided as the electron acceptor with pyruvate (7.15 mM) as the electron donor. In the presence of Fe(III)-oxide, electron transfer to a graphite electrode. In order to evaluate the ability of *D. propionicus* to transfer electrons to an electrode, the inoculum was grown fermentatively on lactate (10 mM). The cells were pelleted via centrifugation, washed, and then resuspended in 20 ml of fresh anoxic medium lacking electron donor or acceptor. Ten milliliters of this cell suspension was inoculated into the anaerobic anodic chamber (250 ml of medium) of a two-chambered electrode system, constructed as previously described (2, 3). The electrodes were (in centimeters) 2.34 by 7.02 by 1.17 cm (cathode) and 4.72 by 7.02 by 1.17 cm (anode). The anode was poised with a potentiostat (AMEL instruments, Milan, Italy) at a constant potential to support growth. *D. propionicus* could also be continually cultured with hydrogen as the electron donor and poorly crystalline Fe(III)-oxide as the electron acceptor when acetate (0.1 mM) was provided as a carbon source (Fig. 1b).

**Electron transfer to a graphite electrode.** In order to evaluate the ability of *D. propionicus* to transfer electrons to an electrode, the inoculum was grown fermentatively on lactate (10 mM). The cells were pelleted via centrifugation, washed, and then resuspended in 20 ml of fresh anoxic medium lacking electron donor or acceptor. Ten milliliters of this cell suspension was inoculated into the anaerobic anodic chamber (250 ml of medium) of a two-chambered electrode system, constructed as previously described (2, 3). The electrodes were (in centimeters) 2.34 by 7.02 by 1.17 cm (cathode) and 4.72 by 7.02 by 1.17 cm (anode). The anode was poised with a potentiostat (AMEL instruments, Milan, Italy) at a constant potential to support growth. *D. propionicus* could also be continually cultured with hydrogen as the electron donor and poorly crystalline Fe(III)-oxide as the electron acceptor when acetate (0.1 mM) was provided as a carbon source (Fig. 1b).

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**D. propionicus** was grown in the electrode chamber with 2.42 mM pyruvate provided as the electron donor with a poised electrode as the sole electron acceptor, 0.33 mM acetate, and 0.29 mM propionate was formed. Thus, 26.4% of the pyruvate metabolized was associated with oxidation of pyruvate to acetate coupled to electron transfer to the electrode.

When spent medium from a lactate-grown culture was replaced with fresh medium containing more lactate (1 mM), current production immediately resumed (Fig. 3). This suggested that, as was previously seen with *Geobacter sulfurreducens* (3) and *Rhodoferax ferrireducens* (4), cells of *D. propionicus* attached to the electrode, rather than planktonic cells, were primarily responsible for the current production.

When S0 (20 g/liter) was added as a potential electron donor for electron transfer to the electrode, sulfate was produced in the presence of *D. propionicus* but not in the absence of cells (Fig. 4). Cells did not produce sulfate in the absence of the electrode.

**Implications.** The ability of *D. propionicus* to transfer electrons to Fe(III), AQDS, and electrodes has implications for anaerobic respiration in sedimentary environments. Only one other organism, the gram-positive bacterium *Desulfotonaculum reducens*, has been reported to grow with both sulfate and Fe(III) as terminal electron acceptors (27). *D. propionicus* is the first sulfate-reducing organism found to conserve energy to support growth from the reduction of insoluble Fe(III)-oxide, the most abundant form of microbially reducible Fe(III) in most sedimentary environments (12). Microorganisms that can use both sulfate and Fe(III) as electron acceptors may be most competitive at the interface between the zones of Fe(III) reduction and sulfate reduction in aquatic sediments where Fe(III) is still available but is not in sufficient concentrations to inhibit sulfate reduction (15, 18).

The ability of *D. propionicus* to use a graphite electrode as an electron acceptor provides an explanation for the consistent enrichment of closely related organisms in the family *Desulfobulbaceae* on electrodes harvesting electricity from marine sediments. As previously observed with such dissimilatory Fe(III)-reducing microorganisms as species within the family *Geobacteraceae* (2, 3), *Shewanella putrefaciens* (9, 10), *Clostridium butyricum* (23), *Rhodoferax ferrireducens* (4), *Aerobacter*...
monas hydrophila (24), and Geothrix fermentans (D. R. Bond and D. R. Lovley, unpublished data). *D. propionicus* did not require the addition of exogenous electron-shuttling compounds for electricity production. In addition, the finding that *D. propionicus* is able to oxidize elemental S⁰ with an electrode serving as the electron acceptor provides further insight into the biological mechanisms involved in current production by the marine sediment fuel cell. It has previously been shown that when Mn(IV) oxides are added to anoxic marine sediments containing sulfides, there is a production of sulfate that requires biological activity (1, 11). Mn(IV) is able to chemically oxidize sulfides to S⁰, which is then oxidized by microorganisms to sulfate with the reduction of Mn(IV). When a current-harvesting electrode is placed in anoxic sediments, similar processes are observed. Elemental S⁰ is known to precipitate on the anodes of marine sediment fuel cells as the result of abiotic sulfate oxidation at the anode surface (28), and elevated sulfate levels have been measured in sediments closest to the current-harvesting anode (28). These geochemical results coupled with the fact that S⁰ oxidation with an electrode serving as the electron acceptor was observed in pure-culture studies of *D. propionicus* suggests that the oxidation of elemental sulfur on the anode surface may be an important biological process in the marine sediment fuel cell.

However, the results also suggest that *Desulfobulbaceae* species are unlikely to play an important role in coupling the oxidation of organic matter to the reduction of the electrode in marine sediment fuel cells. *Desulfobulbaceae* are not known to oxidize acetate, which is likely to be the primary electron donor for electricity production. Rather, *D. propionicus* can only metabolize such organic acids as propionate, lactate, and pyruvate, which are less likely to be important extracellular intermediates in sediments (13). Furthermore, electron transfer to the electrode from the mixed metabolism of organic acids by *D. propionicus* is relatively inefficient; only ca. 25% of the electrons available from the incomplete oxidation of pyruvate, lactate, and propionate were transferred to the electrode surface. In contrast, several *Geobacteraceae* species can oxidize acetate and are able to quantitatively transfer all of the electrons available from the complete oxidation of organic acids to CO₂ to an electrode (2, 3). Therefore, the consistent enrichment of *Desulfobulbaceae* 16S rRNA gene sequences on current-harvesting marine anodes (2, 8, 28) is probably due to their ability to oxidize S⁰ on the electrode surface.

In summary, this study provides the first example of a sulfate-reducing organism that can also conserve energy to support growth via electron transfer to insoluble electron acceptors, such as Fe(III) oxide and electrodes. Further studies are warranted to determine whether the mechanisms involved in electron transfer by *D. propionicus* to these extracellular electron acceptors are similar to those in more well-studied Fe(III)-reducing microorganisms.

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