Enzymatic iron and uranium reduction by sulfate-reducing bacteria

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ABSTRACT

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The potential for sulfate-reducing bacteria (SRB) to enzymatically reduce Fe(III) and U(VI) was investigated. Five species of *Desulfovibrio* as well as *Desulfobacterium autotrophicum* and *Desulfobulbus propionicus* reduced Fe(III) chelated with nitrilotriacetic acid as well as insoluble Fe(III) oxide. Fe(III) oxide reduction resulted in the accumulation of magnetite and siderite. *Desulfobacter postgatei* reduced the chelated Fe(III) but not Fe(III) oxide. *Desulfobacter curvatus*, *Desulfomonile tiedjei*, and *Desulfotomaculum acetoxidans* did not reduce Fe(III). Only *Desulfovibrio* species reduced U(VI). U(VI) reduction resulted in the precipitation of uraninite. None of the SRB that reduced Fe(III) or U(VI) appeared to conserve enough energy to support growth from this reaction. However, *Desulfovibrio desulfuricans* metabolized H_2 down to lower concentrations with Fe(III) or U(VI) as the electron acceptor than with sulfate, suggesting that these metals may be preferred electron acceptors at the low H_2 concentrations present in most marine sediments. Molybdate did not inhibit Fe(III) reduction by *D. desulfuricans*. This indicates that the inability of molybdate to inhibit Fe(III) reduction in marine sediments does not rule out the possibility that SRB are important catalysts for Fe(III) reduction. The results demonstrate that although SRB were previously considered to reduce Fe(III) and U(VI) indirectly through the production of sulfide, they may also directly reduce Fe(III) and U(VI) through enzymatic mechanisms. These findings, as well as our recent discovery that the S°-reducing microorganism *Desulfuromonas acetoxidans* can reduce Fe(III), demonstrate that there are close links between the microbial sulfur, iron, and uranium cycles in anaerobic marine sediments.

Introduction

The oxidation of organic matter coupled to the reduction of Fe(III), Mn(IV), or U(VI) is an important process affecting the organic and inorganic geochemistry of anaerobic marine sediments. In many coastal marine sediments Fe(III) and/or Mn(IV) reduction are important, and sometimes the dominant, processes for anaerobic organic matter oxidation (Aller et al., 1986; Sørensen and Jørgensen, 1987; Aller, 1988; Hines et al., 1991; Canfield et al., 1993). Even in deep sea sediments in which anaerobic oxidation processes are less quantitatively important for organic matter oxidation, there still are often extensive zones in which organic matter oxidation is coupled to Fe(III) or Mn(IV) reduction (Froelich et al., 1979).

Reduction of Fe(III) and Mn(IV) in marine sediments results in the dissolution of insoluble Fe(III) and Mn(IV) oxides with the release of soluble Fe(II) and Mn(II) and the production of Fe(II)- and Mn(II)-bearing minerals such as magnetite (Karlin et al., 1987), siderite (Coleman et al., 1993) and Mn(II) carbonates (Middelburg et al., 1987). The reduction of soluble U(VI) to insoluble U(IV) in anaerobic marine sediments is the most significant global sink for dissolved uranium (Veeh, 1967; Anderson et al., 1989; Klinkhammer and Palmer, 1991).

Despite its geochemical significance, the mechanisms for metal reduction in marine sediments have not been investigated in detail. At one time it was considered that much of the Fe(III) reduction in aquatic sediments was the result of nonenzy-

matic, strictly chemical reactions in which Fe(III) was reduced either as the result of the development of a "low redox potential," or by nonenzymatic reactions with organic compounds or H_2 (Lovley, 1991). More recent studies have suggested that, in freshwater sediments, neither of these nonenzymatic mechanisms is quantitatively significant (Lovley, 1991; Lovley et al., 1991b). Instead, most of the Fe(III) reduction results from Fe(III)-reducing bacteria (FeRB) enzymatically coupling the oxidation of organic compounds and H_2 to the reduction of Fe(III).

FeRB can also enzymatically reduce U(VI) and Mn(IV) (Lovley, 1991). In a manner similar to Fe(III) reduction, enzymatic reduction of U(VI) is likely to be a much more important process than previously proposed abiotic mechanisms for U(VI) reduction in aquatic sediments (Lovley et al., 1991a; Lovley and Phillips, 1992a). Elucidation of the relative contributions of enzymatic and nonenzymatic processes for Mn(IV) reduction have been complicated by the fact that Fe(II) produced from Fe(III) reduction can rapidly reduce Mn(IV), making it uncertain whether microorganisms are directly reducing Mn(IV) or indirectly reducing Mn(IV) through the reduction of Fe(III) (Lovley, 1991).

Studies on the metabolism of organic matter in freshwater aquatic sediments and aquifers in which organic matter was being oxidized to carbon dioxide with the reduction of Fe(III), as well as studies with pure cultures of FeRB have suggested that Fe(III) reduction is catalyzed by the cooperative activity of a microbial food chain (Fig. 1). In this model, small amounts of Fe(III) may be reduced by microorganisms fermenting sugars and amino acids. However, FeRB which can couple the oxidation of important fermentation products (acetate, H_2), long-chain fatty acids, or aromatic compounds to the reduction of Fe(III) catalyze most of the Fe(III) reduction in the sediments.

It seems reasonable to suspect that a similar microbial food chain with marine FeRB filling in the various roles may account for the oxidation of organic matter coupled to Fe(III) reduction in marine sediments. In marine sediments there is, at least theoretically, a greater potential for nonenzymatic reduction of Fe(III) to be more important than it is in freshwater. This is because sulfate-reducing bacteria (SRB) can reduce the sulfate that is abundant in seawater to sulfide which will nonenzymatically reduce Fe(III) (Goldhaber and Kaplan, 1974; Pyzik and Sommer, 1981). However, geochemical data suggests that there is often no
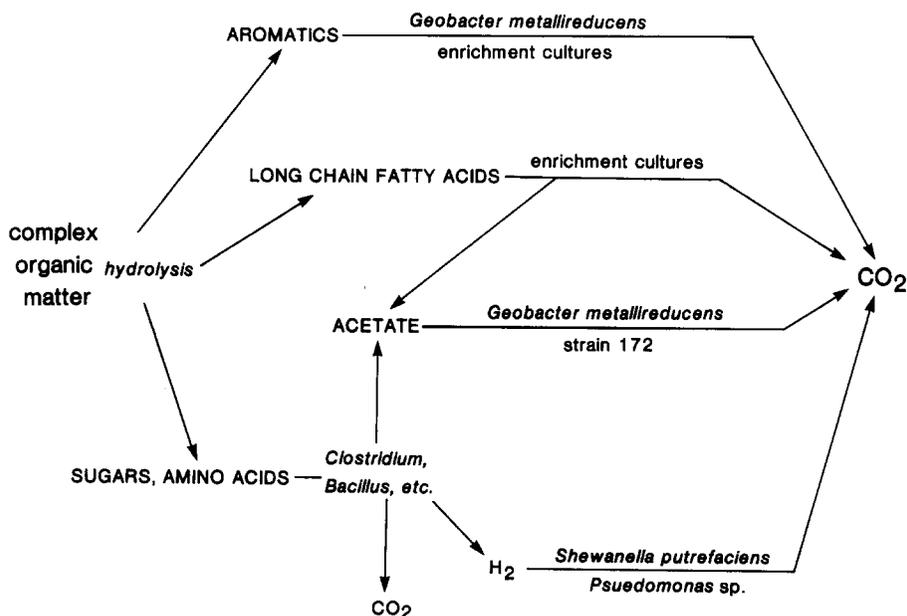

Fig. 1. Model for coupling the oxidation of organic matter to the reduction of Fe(III) in freshwater aquatic sediments and aquifers.

sulfate reduction in the zones of marine sediments in which Fe(III) is being reduced (Lovley, 1991). Furthermore, inhibiting the production of sulfide by selectively inhibiting microbial sulfate reduction did not inhibit Fe(III) reduction in a variety of marine and estuarine sediments or enrichment cultures (Sørensen, 1982; Tugel et al., 1986; Lovley and Phillips, 1987a; Canfield, 1989; Canfield et al., 1993). This suggests that enzymatic Fe(III) reduction by FeRB is the predominant mechanism for Fe(III) reduction in marine sediments.

One FeRB, strain BrY, which can grow at marine salinities was recently described (Caccavo et al., 1992). BrY oxidizes H_2 with the reduction of Fe(III). It can also incompletely oxidize lactate to acetate and carbon dioxide with Fe(III) as the electron acceptor. In addition to Fe(III), BrY can use O_2, Mn(IV), U(VI), fumarate, thiosulfate, or trimethylamine n-oxide as an electron acceptor.

Although FeRB and SRB were previously considered to be distinct microbial populations (Lovley and Phillips, 1987b), recent studies have demonstrated that some organisms which use sulfate or $S°$ as their electron acceptor also have the ability to reduce Fe(III), Mn(IV), or U(VI). Analysis of the 16S rRNA sequence of the freshwater, acetate-oxidizing, Fe(III) reducer *Geobacter metallireducens* demonstrated that its closest known relative was the marine microorganism, *Desulfuromonas acetoxidans* (Lovley et al., 1993a). This organism was previously known for its unique ability to couple the oxidation of acetate to the reduction of $S°$ (Pfennig and Biebl, 1976). However, *D. acetoxidans* can also couple the oxidation of acetate to the reduction of Fe(III) or Mn(IV) (Roden and Lovley, 1993).

G. metallireducens and *D. acetoxidans* are in the delta proteobacteria which also includes all of the Gram-negative SRB (Devereux et al., 1989). Fe(III) was reduced in cell suspensions of two SRB, *Desulfovibrio desulfuricans* and *Desulfotomaculum nigrificans* (Jones et al., 1984), but the physiological and environmental significance of this Fe(III) reduction was not examined. For example, from the experimental details it was not clear how fast a given quantity of the SRB were reducing the Fe(III) or whether the Fe(III) reduction was enzymatically catalyzed. Lipid analysis of siderite

concretions forming in salt marsh sediments indicated that they were enriched with *Desulfovibrio* species and it was demonstrated that *Desulfovibrio* species could enzymatically reduce Fe(III) (Coleman et al., 1993; Lovley et al., 1993b). *D. desulfuricans* can also enzymatically reduce U(VI) to U(IV) (Lovley and Phillips, 1992a,b).

The purpose of the studies reported here was to further investigate the potential for reduction of Fe(III) and U(VI) reduction by *Desulfovibrio* and related SRB in order to learn more about which organisms might be involved in the dissolution of Fe(III) oxides and the formation of reduced iron and uranium minerals in marine sediments.

Materials and methods

Source of organisms and culturing techniques

All of the SRB were purchased from the American Type Culture Collection (ATCC), Rockville MD, USA or the German Collection of Microorganisms (DSM), Braunschweig, Germany with the exception of *Desulfomonile tiedjei* which was a gift from Joseph Suflita, University of Oklahoma (Table 1). With the exception of *Desulfotomaculum acetoxidans*, all of these organisms are gram negative. The SRB were cultured under strict anaerobic conditions in bicarbonate-buffered media with a gas phase of N_2/CO_2 (80:20). Nutrients, trace minerals, salts, vitamins, and electron donors were added in the form and amount specified in the recipes referenced in Table 1. In the media for the *Desulfovibrio* species *desulfuricans*, *vulgaris*, and *baculatus* L-cysteine (0.25 g/l) was added as a reductant from a concentrated anaerobic stock. The media for all other cultures was reduced by the addition of sodium sulfide (0.4 g/l). For studies on growth with Fe(III) as the electron acceptor, chloride salt was substituted for the major sulfate salt and approximately 100 mmol/l of a synthetic poorly crystalline Fe(III) oxide (Lovley and Phillips, 1986a) was added. SRB were routinely cultured in 10 ml volumes in anaerobic pressure tubes. In order to grow cells for studies with cell suspensions, 100 ml was cultured in 160 ml serum bottles.

TABLE 1

SRB tested and culture conditions

Organism	Source	Culture number	Medium	Electron donor	Incubation temperature (°C)
Desulfovibrio desulfuricans	ATCC	29577	Lovley and Phillips (1992)	lactate or H₂[a]	35°
Desulfovibrio vulgaris	ATCC	29579	Lovley and Phillips (1992)	lactate	30°
Desulfovibrio baculatus	DSM	1741	Lovley and Phillips (1992)	lactate	30°
Desulfovibrio sulfodismutans	DSM	3696	DSM 386	lactate	30°
Desulfovibrio baarsii	DSM	2075	DSM 208	butyrate, caproate, octanoate	35°
Desulfobacterium autotrophicum	DSM	3382	DSM 383	H₂	25°
Desulfobulbus propionicus	DSM	2032	DSM 194	propionate	30°
Desulfobacter curvatus	ATCC	43919	ATCC 1648	acetate	30°
Desulfobacter postgatei	DSM	2034	DSM 193	acetate	30°
Desulfomonile tiedjei	J. Suflita		DeWeerd et al. (1990)	pyruvate	30°
Desulfotomaculum acetoxidans	DSM	771	DSM 124	acetate	30°

[a]For growth on H_2 the yeast extract and proteose peptone were omitted and 5 mM acetate was provided as a carbon source.

Cell suspension experiments

For cell suspensions, cells were harvested under N_2–CO_2 and resuspended in anaerobic bicarbonate buffer. In all instances the buffer contained sodium bicarbonate (2.5 g/l) and for organisms requiring higher salt concentrations (D. postgatei, D. curvatus and D. autotrophicum) the buffer was supplemented with NaCl, $MgCl_2$, CaCl, and KCl in amounts consistent with that in the growth medium. The cells were centrifuged again and then resuspended under N_2–CO_2 in the appropriate bicarbonate buffer. Aliquots of the cell suspension were then added to buffer (10 ml) in 25 ml serum bottles which, unless specified otherwise, had been amended with an excess of the organic electron donor (5–10 mM) or H_2 (0.67 atm) of the same electron donor(s) as in the growth medium.

For studies on Fe(III) reduction, the buffer also contained 10 mM Fe(III) chelated with nitrilotriacetic acid [Fe(III)–NTA] prepared as previously described (Roden and Lovley, 1993). In one experi-

ment (Fig. 2) reduction of 10 mM Fe(III) citrate was also evaluated. For studies on U(VI) reduction, approximately 100 μM U(VI)–acetate was provided as the electron acceptor. For the studies on H_2 uptake with sulfate (1 mM) as the electron
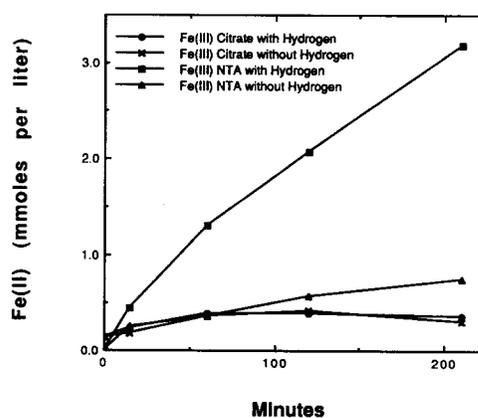

Fig. 2. Fe(III) reduction by cell suspensions (0.1 mg protein/ml) of Desulfovibrio desulfuricans with H_2 as the electron donor and Fe(III)–citrate or Fe(III)–NTA as the electron acceptor.

acceptor, the buffer was also amended with 0.25 g of L-cysteine–HCl per liter, as this is necessary to maintain sulfate-reducing activity (Lovley and Phillips, 1992a). Bottles for H_2 threshold uptake studies were amended with ca. 1×10^{-4} atmospheres of H_2 after the addition of cells. For the studies on potential molybdate inhibition of Fe(III) reduction a final concentration of 1 or 10 mM sodium molybdate was added from a concentrated anaerobic solution.

Reduction of Fe(III) and U(VI) and H_2 uptake were monitored by removing liquid aliquots [Fe(III) or U(VI) reduction] or headspace samples (H_2 uptake) with a syringe and needle over time.

Analytical techniques

HCl-extractable Fe(II) was measured with ferrozine as previously described (Lovley and Phillips, 1986b). U(VI) concentrations were measured under anaerobic conditions as previously described (Lovley et al., 1991a; Gorby and Lovley, 1992) with a Kinetic Phosphorescence Analyzer. Samples for sulfate determination were filtered (Gelman Acrodisc; pore diameter, 0.2 μm) and analyzed on a Dionex ion chromatograph. H_2 was quantified with a gas chromatograph and a reduction gas analyzer (Lovley and Goodwin, 1988). Protein was determined by the method of Lowry et al. (Lowry et al., 1951) with bovine serum albumin as a standard. Cell counts were by epifluorescence microscopy (Hobbie et al., 1977). For X-ray diffraction analysis, the precipitates were dried and prepared under anaerobic conditions and analyzed as previously described (Lovley and Phillips, 1992a).

Results

Potential for Fe(III) and U(VI) reduction by SRB

The finding that *D. desulfuricans* could reduce Fe(III) oxide (Coleman et al., 1993) was surprising because our initial studies with this organism indicated that it could not reduce Fe(III) citrate, the soluble Fe(III) form that we have routinely used for screening organisms for their ability to reduce Fe(III) (Lovley and Phillips, 1988; Lovley et al., 1989). When retested with Fe(III) citrate, cell

suspensions of *D. desulfuricans* still did not reduce Fe(III) citrate, but readily reduced Fe(III)–NTA when a suitable electron donor (H_2) was provided (Fig. 2). Other studies have suggested that citrate may be toxic to sulfate- and sulfur-reducing microorganisms (Lovley and Phillips, 1992a; Roden and Lovley, 1993). Thus, Fe(III)–NTA was used as the soluble Fe(III) form in screening other SRB. *D. desulfuricans* can readily reduce the soluble U(VI)–carbonate complex that is formed when U(VI) acetate is added to bicarbonate buffer (Lovley and Phillips, 1992a,b) and thus this form of U(VI) was added for screening other SRB.

Desulfovibrio species other than *D. desulfuricans* also actively reduced Fe(III) and U(VI). For example, *D. vulgaris* and *D. baculatus* reduced both Fe(III) (Fig. 3) and U(VI) (Fig. 4) at rates comparable to those observed with *D. desulfuricans*. When differences in the concentrations of cell proteins are considered, the initial rates of Fe(III) reduction for the other *Desulfovibrio* species and for *D. autotrophicum* were comparable to those for *D. vulgaris* and *D. baculatus*. The other SRB tested either reduced Fe(III) more slowly, or not at all. The only other SRB that reduced U(VI) were *D. sulfodismutans* and *D. baarsii* which both reduced
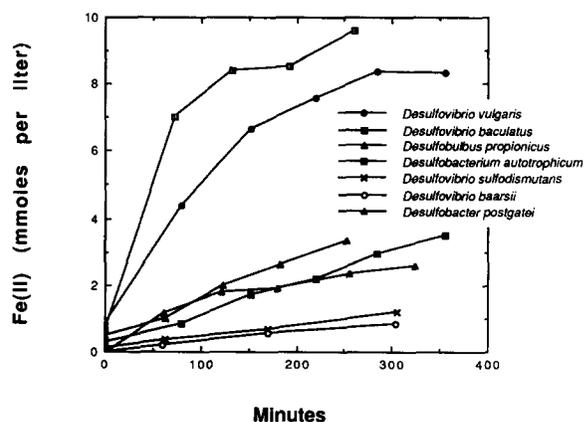
Minutes

Fig. 3. Rates of Fe(III)–NTA reduction by cell suspensions of various SRB with the electron donor(s) listed in Table 1. Cell protein concentrations (mg/ml) for the various organisms were: *Desulfovibrio vulgaris* (0.3), *Desulfovibrio baculatus* (0.5), *Desulfobulbus propionicus* (0.08), *Desulfobacterium autotrophicum* (0.03), *Desulfovibrio sulfodismutans* (0.02), *Desulfovibrio baarsii* (0.1), *Desulfobacter postgatei* (0.2). Other SRB tested which showed no Fe(III) reduction were: *Desulfobacter curvatus*, *Desulfomonile tiedjei*, and *Desulfotomaculum acetoxidans*.
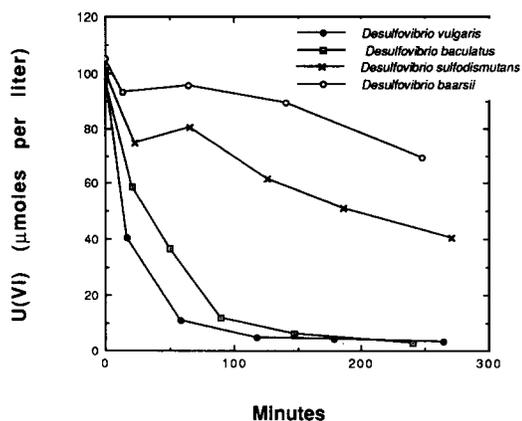
Fig. 4. Rates of U(VI) reduction by cell suspensions of various SRB with the electron donors listed in Table 1. Cell protein concentrations (mg/ml) for the various organisms were: *Desulfovibrio vulgaris* (0.1), *Desulfovibrio baculatus* (0.1), *Desulfovibrio sulfodismutans* (0.06), and *Desulfovibrio baarsii* (0.1). Other SRB tested which showed no U(VI) reduction were: *Desulfobulbus propionicus*, *Desulfobacterium autotrophicum*, *Desulfobacter postgatei*, *Desulfobacter curvatus*, *Desulfomonile tiedjei*, and *Desulfotomaculum acetoxidans*.

Fig. 5. Growth, and Fe(III) and sulfate reduction by *Desulfovibrio desulfuricans* inoculated into medium with H_2 as the electron donor, Fe(III) oxide as the electron acceptor, and acetate as a carbon source.

U(VI) relatively slowly (Fig. 4). Studies in which the electron donor was omitted demonstrated that the rates of metal reduction that were observed were dependent upon the presence of the electron donor (data not shown).

With the exception of *Desulfobacter postgatei*, all of the SRB that reduced Fe(III)–NTA, reduced poorly crystalline Fe(III) oxide when first inoculated into a medium in which the sulfate was replaced with Fe(III) oxide (data not shown). However, reduction of Fe(III) oxide did not appear to yield energy to support growth for most of these organisms as the capacity to reduce Fe(III) oxide was lost on subsequent transfer (10% inoculum) into Fe(III) oxide medium.

D. desulfuricans could continue to be transferred (over 7 transfers of 10% inoculum) in Fe(III) oxide medium with H_2 as the electron donor and acetate as a carbon source. However, all of the cell growth was associated with the initial reduction of the small amount of sulfate that was present in the medium from the addition of the trace mineral mixture (Fig. 5). The 300 μM sulfide that was presumably produced from the reduction of the sulfate would be enough to reduce, at most 0.6 mM Fe(II). After the sulfate was depleted, *D.*
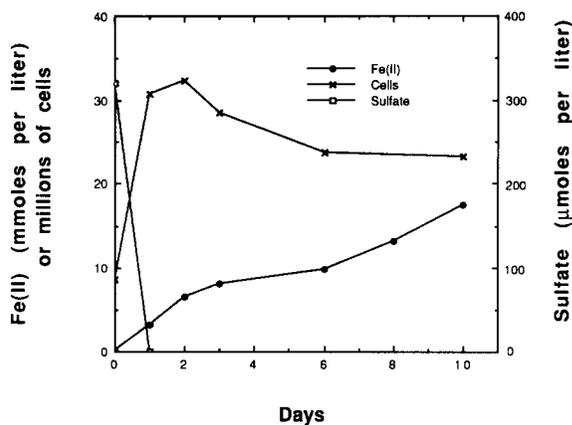
desulfuricans reduced another 14 mmoles per liter of Fe(III), but there was no cell growth. This indicated that Fe(III) reduction did not provide energy to support cell growth. Previous attempts to grow *D. desulfuricans* with U(VI) as the electron acceptor were unsuccessful (Lovley and Phillips, 1992a) and we were also unable to grow *D. vulgaris* with U(VI).

Mineral formation as the result of Fe(III) and U(VI) reduction

During the reduction of poorly crystalline Fe(III) oxide by the SRB, the reddish brown Fe(III) precipitate was converted to a black, often magnetic precipitate (Fig. 6) that looked similar to the ultrafine-grained magnetite- and siderite-containing precipitate that forms during Fe(III) oxide reduction by *G. metallireducens* under similar conditions (Lovley et al., 1987; Lovley, 1990). X-ray diffraction analysis of the precipitate formed during Fe(III) reduction by *D. desulfuricans* confirmed that it was comprised of magnetite and siderite.

Previous studies have demonstrated that U(VI) reduction by *D. desulfuricans* results in the extracellular precipitation of uraninite (Lovley and Phillips, 1992a). X-ray diffraction of the precipitate that formed during U(VI) reduction by a *D. vulgaris* cell suspension indicated that it also was comprised of uraninite.
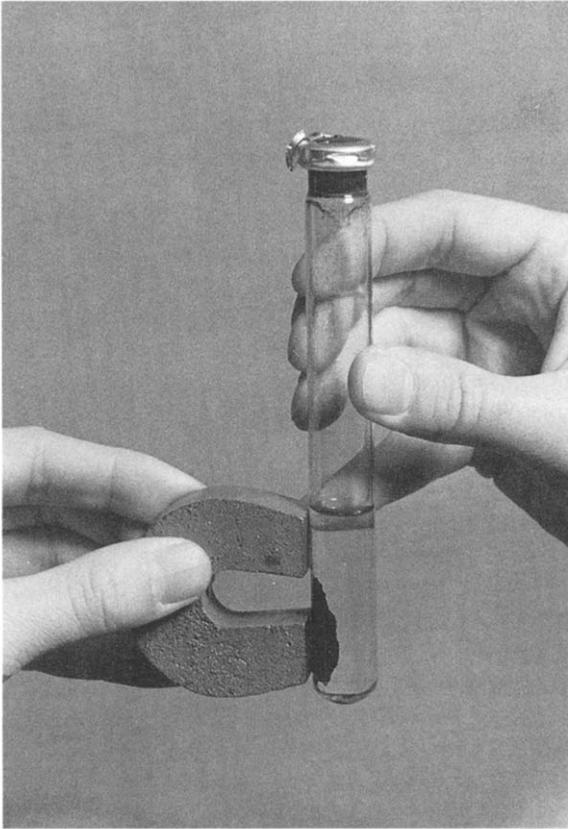
Fig. 6. Accumulation of magnetic precipitate as the result of *Desulfovibrio desulfuricans* reducing Fe(III) oxide.

H_2 threshold

A previous study demonstrated that *D. desulfuricans* could metabolize H_2 down to lower concentrations with Fe(III) as the electron acceptor than with sulfate, suggesting that electron flow may be diverted preferentially to Fe(III) at the low concentrations of H_2 found in most sediments (Coleman et al., 1993). The minimum threshold for H_2 uptake with U(VI) as the electron acceptor was also lower than that for sulfate (Fig. 7). The U(VI) threshold is typically one-half the threshold with Fe(III) as the electron acceptor.

Effect of molybdate on Fe(III) reduction

Since molybdate has been used as a metabolic inhibitor to try to elucidate the potential contribution of microbial sulfate reduction to Fe(III) reduc-
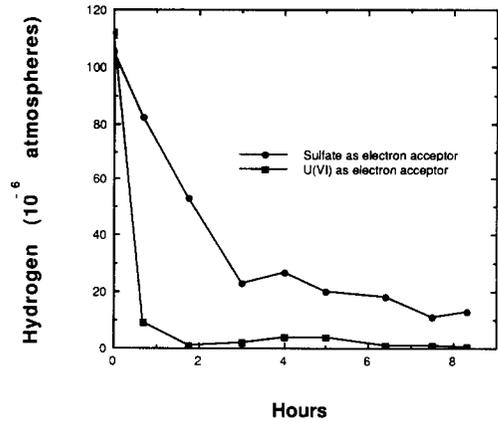

Fig. 7. H_2 uptake by cell suspensions of *Desulfovibrio desulfuricans* with U(VI) or sulfate as the electron acceptor.

tion in sediments (Sørensen, 1982; Lovley and Phillips, 1987a; Canfield, 1989; Canfield et al., 1993), the affect of molybdate on Fe(III) reduction by *D. desulfuricans* was evaluated. Molybdate had no affect on the rate of Fe(III) reduction by *D. desulfuricans* (Fig. 8).

Discussion

SRB have long been thought to be important agents in the reduction of metals in aquatic sediments. However, their role was considered to be an indirect one in which the sulfide produced from sulfate reduction nonenzymatically reduced metals. The studies reported here, along with other recent reports (Lovley and Phillips, 1992a; Coleman et al.,
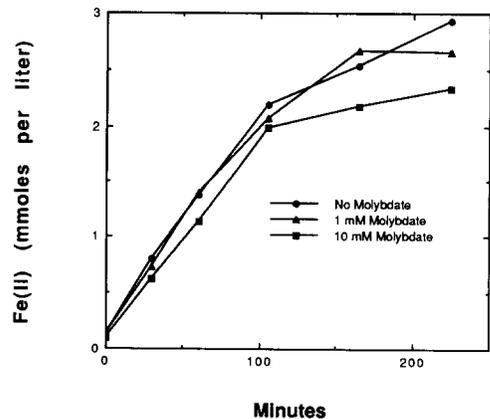

Fig. 8. Effect of molybdate on Fe(III)–NTA reduction by cell suspensions of *Desulfovibrio desulfuricans*.

1993; Roden and Lovley, 1993; Lovley et al., 1993b) have indicated that SRB may also reduce metals directly through an enzymatic process. Although previous studies have suggested that the microbial populations involved in Fe(III) and sulfate reduction are separate, often competing, populations (Lovley and Phillips, 1987b), the results presented here demonstrate that the same organisms can be involved in both processes. As detailed below, this metabolism has several important implications for the geochemistry of marine sediments.

Mechanism for Fe(III) and U(VI) reduction by SRB

Several lines of evidence suggest that the Fe(III) and U(VI) reduction in the presence of SRB is enzymatically catalyzed. The reduction of Fe(III) and U(VI) by washed cell suspensions in sulfate-free buffer demonstrates that the SRB are not indirectly reducing Fe(III) and U(VI) through the production of sulfide. The fact that physiological electron donors are required for metal reduction demonstrates that the reduction is not the result of the nonenzymatic interaction of metals with cellular material. Previous studies have demonstrated that none of the electron donors used in these studies can, by themselves, directly reduce Fe(III) without enzymatic mediation (Lovley et al., 1991b).

More detailed studies have recently elucidated an enzymatic mechanism for Fe(III) and U(VI) reduction by $D.$ $vulgaris$ (Lovley et al., 1993b). The c_3 cytochrome of $D.$ $vulgaris$ reduces Fe(III) and U(VI) as well as several other metals. The physiological electron donor for c_3 is hydrogenase (LeGall and Fauque, 1988). When electrons are provided to c_3 with a combination of hydrogenase and H_2, poorly crystalline Fe(III) oxide is reduced with the release of soluble Fe(II) and the formation of magnetite and siderite. U(VI) is reduced to U(IV) which precipitates as uraninite. c_3 may be the metal reductase in other $Desulfovibrio$. However, the finding that $Desulfomonile$ $tiedjei$, which has been reported to contain a low molecular weight cytochrome that is similar to c_3 (DeWeerd et al., 1990), did not reduce Fe(III) or U(VI),

demonstrates that the presence of a low molecular weight c-type cytochrome does not always ensure that the organism can reduce metals.

Cytochrome c_3 is also the $S°$ reductase in $Desulfovibrio$ species (Fauque et al., 1979). In contrast to Fe(III) or U(VI) reduction, $S°$ reduction can yield energy to support cell growth (Biebl and Pfennig, 1977). The apparent inability of $Desulfovibrio$ species to conserve energy to support growth from Fe(III) or U(VI) reduction is similar to O_2 reduction in that c_3 also reduces O_2 but $Desulfovibrio$ species can not grow with O_2 as the sole electron acceptor (Postgate, 1984). However, some ATP may be generated during O_2 reduction (Dilling and Cypionka, 1990). Further studies are required to determine if there is any energy conservation from Fe(III) or U(VI) reduction.

Role of SRB in Fe(III) and U(VI) reduction in marine sediments

The results presented here demonstrate the potential for SRB to reduce Fe(III) and U(VI), but the environmental significance of their activity is as yet unknown. There are likely to be many other organisms capable of Fe(III) and U(VI) reduction in marine sediments. For example, two organisms which can serve as models for enzymatic reduction of Fe(III) in marine sediments are $Desulfuromonas$ $acetoxidans$ (Roden and Lovley, 1993) and strain BrY (Caccavo et al., 1992). In contrast to the SRB, both $D.$ $acetoxidans$ and BrY can conserve energy to support growth from Fe(III) reduction. At least superficially, this would be expected to give these organisms a competitive advantage over SRB in competing for organic compounds and H_2 in Fe(III)-reducing environments.

However, this is not necessarily the case. Only one study has attempted to describe the distribution of FeRB in marine sediments. In that study (Coleman et al., 1993), lipid analyses indicated that siderite concretions within a salt marsh were enriched with $Desulfovibrio$ species relative to the surrounding sediment. Assuming that the concretion represented a zone of enhanced Fe(III) reduction, this suggested that $Desulfovibrio$ species were the important catalysts for Fe(III) reduction. These

results demonstrate that the factors controlling the distribution of specific FeRB in marine sediments may be complex.

The relative contribution of SRB to Fe(III) reduction in marine sediments could be readily determined if there was an inhibitor that would selectively inhibit Fe(III) reduction by SRB. Molybdate inhibits sulfate reduction by SRB in sediments (Oremland and Capone, 1988), but did not inhibit Fe(III) reduction by *D. desulfuricans*. This suggests that molybdate can not be used to differentiate between Fe(III) reduction by SRB and other organisms in sediments. Thus, the finding that molybdate does not inhibit Fe(III) reduction in marine sediments (Sørensen, 1982; Canfield, 1989; Canfield et al., 1993) gives no information on the type of microorganisms involved and only implies that Fe(III) reduction is through a direct enzymatic mechanism rather than through sulfide generation.

The apparent inability of SRB to conserve energy to support growth from U(VI) reduction is not likely to place SRB at a competitive disadvantage with other FeRB which can grow with U(VI) as the sole electron acceptor (Lovley and Phillips, 1992a). This is because at the low concentrations of U(VI) in marine pore waters, the amount of growth by U(VI) reduction is expected to be minimal. Other factors, such as the availability of Fe(III) oxides that will support the growth of FeRB that are not SRB, are likely to be most important in determining the relative importance of SRB in U(VI) reduction.

Mechanisms for segregation of the zones of Fe(III), U(VI), and sulfate reduction

Sulfate reduction often takes place in a sediment zone that is distinct from the zone of Fe(III) reduction (Reeburgh, 1983). It was previously concluded that sulfate reduction may be prevented in the Fe(III) reduction zone because FeRB outcompete SRB for important electron donors such as acetate and H_2 (Lovley and Phillips, 1987b). When microbially reducible Fe(III) oxides are abundant, FeRB maintain the concentrations of these electron donors too low to support sulfate reduction (Lovley and Phillips, 1987b). The finding that

D. desulfuricans can metabolize H_2 at lower concentrations with Fe(III) as the electron acceptor than with sulfate (Coleman et al., 1993) suggests that the competition between Fe(III) reduction and sulfate reduction is not necessarily just an intercellular competition between distinct populations of FeRB and SRB but may also result, at least in part, from an internal competition between different electron transport pathways within SRB (Fig. 9).

D. desulfuricans' minimum threshold for H_2 uptake with U(VI) as the electron acceptor is comparable to that for Fe(III) which suggests that SRB may be able to enzymatically reduce U(VI) in the Fe(III) reduction zone, prior to significant sulfide accumulation. This finding is consistent with the suggestion that U(VI) is reduced within the Fe(III) reduction zone of sediments (Cochran et al., 1986; Lovley et al., 1991a).

The zones of sulfate reduction and metal reduction in marine sediments are not always well segregated, especially in non-steady state situations such as heavy bioturbation. When H_2 concentrations are not limiting, *D. desulfuricans* reduces sulfate simultaneously with Fe(III) (Coleman et al., 1993) or U(VI) (Lovley and Phillips, 1992a). Thus SRB may catalyze both sulfate and metal reduction at the same time in some sediments.

Formation of Fe(II) and U(IV) bearing minerals

Ultrafine-grained magnetite is produced in some pelagic marine sediments in which the geochemical evidence suggests Fe(III) reduction is the predominant terminal electron accepting process (Karlin et al., 1987). The mechanisms for this magnetite production have not been elucidated but it is consistent with the accumulation of ultrafine-grained magnetite during Fe(III) oxide reduction in cultures of dissimilatory Fe(III) reducing microorganisms (Lovley et al., 1987; Lovley, 1990). The results presented here demonstrate that SRB also have the potential to contribute to magnetite formation in anaerobic marine sediments.

The results also demonstrate that Fe(III) oxide reduction by SRB can lead to the production of siderite. This further supports the hypothesis (Coleman et al., 1993) that Fe(III) reduction by
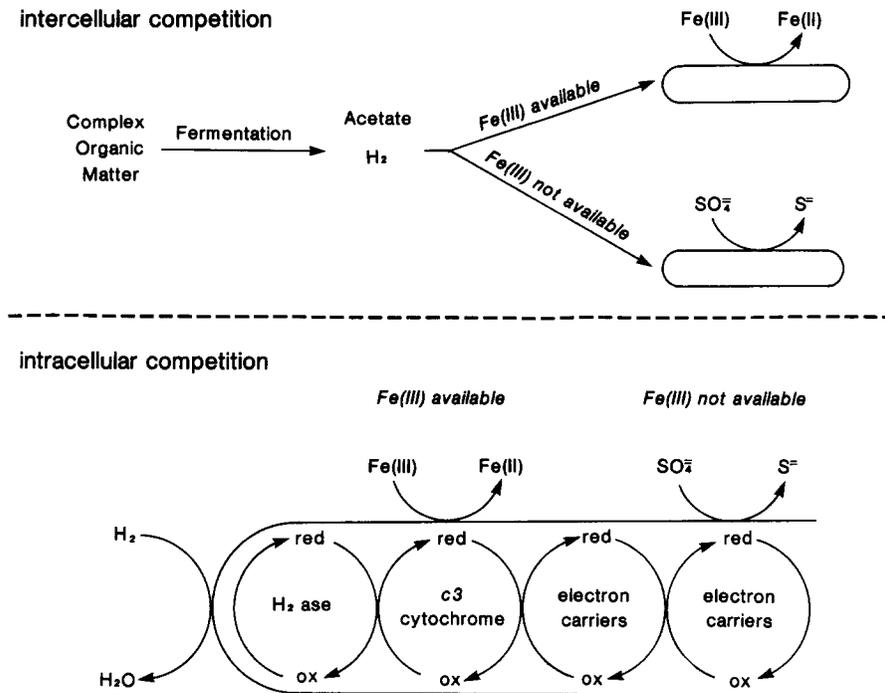
Fig. 9. Intercellular and intra-cellular mechanisms potentially responsible for the inhibition of sulfate reduction in the presence of microbially reducible Fe(III) oxides in sediments.

SRB is responsible for the formation of siderite concretions in marine sediments.

The formation of uraninite during U(VI) reduction by SRB is a potential mechanism for the well-known (Veeh, 1967; Anderson et al., 1989; Klinkhammer and Palmer, 1991) sequestration of uranium in anaerobic marine sediments. Although nonenzymatic reduction of U(VI) by sulfide has been the traditional explanation for U(VI) reduction in anaerobic sediments, recent studies have demonstrated that enzymatic reduction of U(VI) by U(VI)-reducing microorganisms is the more likely mechanism (Lovley et al., 1991a; Lovley and Phillips, 1992a).

Model for organic matter oxidation coupled to Fe(III) reduction in marine sediments

There is much less information about the microbiology of organic matter oxidation coupled to microbial Fe(III) reduction in marine sediments than there is for freshwater sediments and aquifers. However, if it is assumed that the overall pattern

of organic matter oxidation in marine sediments is similar to that for freshwater sediments, then a model similar to that described for freshwater environments (Fig. 1) can begin to be devised for marine environments (Fig. 10). It is assumed that fermentative microorganisms such as *Clostridia*, *Bacillus*, etc. would metabolize fermentable sugars and amino acids to fermentation products in marine sediments as they do in freshwater environments. However, several species of *Desulfovibrio*, *D. autotrophicum*, and strain BrY may replace the H_2-oxidizing, Fe(III)-reducing microorganisms that have been used as models for this metabolism in freshwater sediments. *Desulfuromonas acetoxidans* serves as the marine model for oxidation of acetate coupled to Fe(III) reduction. Marine microorganisms which oxidize aromatic compounds or long-chain fatty acids with the reduction of Fe(III) have yet to be described.

It must be emphasized that although the results presented here demonstrate that a variety of SRB can reduce Fe(III) and U(VI), it is not known if these microorganisms are the dominant Fe(III)-
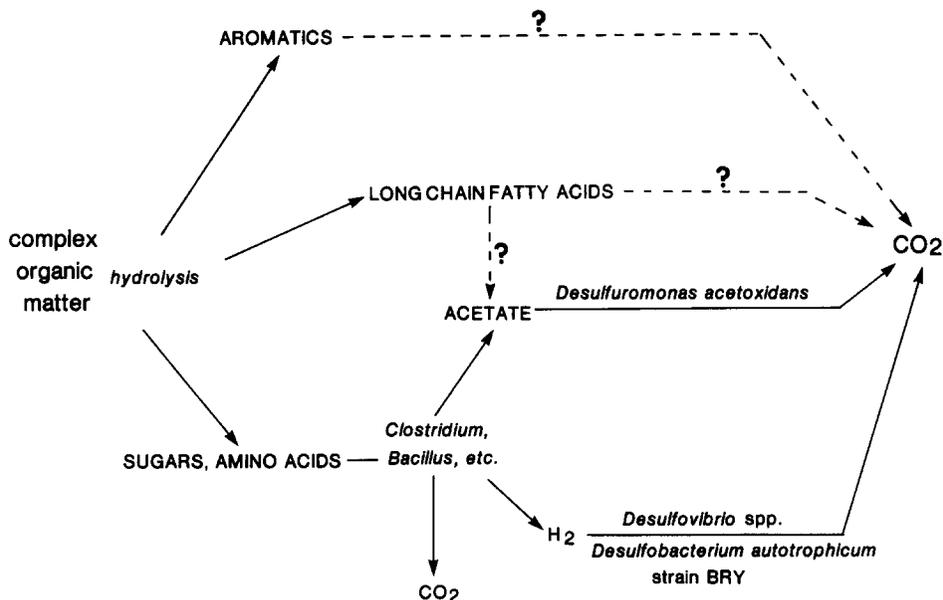
Fig. 10. Preliminary model for coupling the oxidation of organic matter to the reduction of Fe(III) reduction in marine sediments. The model is based on the model for freshwater environments (Fig. 1) but with substitution of known marine FeRB for freshwater FeRB.

and U(VI)-reducing microorganisms in marine sediments. Even for freshwater sediments that have been studied more intensively, there are only a few Fe(III)- and U(VI)-reducing microorganisms which can serve as models for these reactions. SRB could potentially even be important in Fe(III) reduction in freshwater. For example, SRB are abundant in the zones of deep aquifers of the Atlantic Coastal Plain in which Fe(III) reduction is the terminal electron accepting process (Chapelle and Lovley, 1992; Murphy et al., 1992). These SRB are probably gaining energy for survival from Fe(III) reduction in order to survive for such long periods in these isolated subsurface environments in which there is no detectable sulfate reduction. Such findings emphasize the need for more intensive study of community structure of Fe(III)-reducing environments. Determination of which microbial populations catalyze most of the Fe(III) and U(VI) reduction in marine sediments might aid in better understanding the factors that control the rate and extent of these processes and may further elucidate some aspects of the complex interaction of the iron and sulfur cycles in these environments.

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