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Reduction of Fe(III) in sediments by sulphate-reducing bacteria

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REDUCTION of ferric iron (Fe(III)) to ferrous iron (Fe(II)) is one of the most important geochemical reactions in anaerobic aquatic sediments because of its many consequences for the organic and inorganic chemistry of these environments[1]. In marine environments, sulphate-reducing bacteria produce H_2S, which can reduce iron oxyhydroxides[2] to form iron sulphides. The presence of siderite ($FeCO_3$) in marine sediments is anomalous, however, as it is unstable in the presence of H_2S. Previous work[3,4] has suggested a bacterial origin of siderite. Here we describe geochemical and microbiological studies which suggest that contemporary formation of siderite concretions in a salt-marsh sediment results from the activity of sulphate-reducing bacteria. We find that, instead of reducing Fe(III) indirectly through the production of sulphide, some of these bacteria can reduce Fe(III) directly through an enzymatic mechanism, producing siderite rather than iron sulphides. Sulphate-reducing bacteria may thus be an important and previously unrecognized agent for Fe(III) reduction in aquatic sediments and ground waters.

Nodular concretions of intimately mixed siderite and FeS are growing within sulphide-rich, Recent, salt-marsh sediments on the coast of Norfolk, UK[5]. The host mud around the concretions contain FeS but little siderite. Although modelled growth rates[6] for carbonate concretions imply that formation may take $>10^5$ years, these concretions are found in sediments that have been deposited in the last 50 years. This makes them uniquely suitable for investigations into the actual mechanisms of siderite concretion formation.

The formation of siderite in organic-rich marine mudrocks has previously been associated with the degradation of organic matter by anaerobic, methanogenic bacteria[7]. Carbonate resulting from this process has a characteristic positive isotope signature ($\delta^{13}C = +10$ to $+15$‰ PDB)[8]. However, the siderite concretions in the marsh have $\delta^{13}C$ values averaging -5.9‰ PDB, suggesting that the siderite is not formed under methanogenic conditions. An alternative microbial process that could favour siderite formation is microbial reduction of Fe(III). Most of the microbial Fe(III) reduction in aquatic sediments is proposed to result from the oxidation of important fermentation products such as acetate and hydrogen[1]. (The equations below illustrate the overall mass balance and may represent many contributory reactions).

$$4Fe_2O_3 + CH_3COO^- + 7H_2O \rightarrow 8Fe^{2+} + 2HCO_3^- + 15OH^- \quad (1a)$$

$$Fe_2O_3 + H_2 + H_2O \rightarrow 2Fe^{2+} + 4OH^- \quad (1b)$$

The production of ferrous iron, hydroxyl ions and bicarbonate should all favour siderite formation

$$Fe^{2+} + HCO_3^- + OH^- \rightarrow FeCO_3 + H_2O \quad (2)$$

To learn whether microbial Fe(III) reduction played a role in siderite formation, we analysed the microbial populations in a concretion and in two host-sediment samples from the marsh. The concretion was subspherical, ~7 cm in diameter and dark grey to black, its host an anoxic, black clayey-silt. As we cannot yet do all analyses on one sample, we have assumed that the mineralogy was similar to that of visually identical samples, which were indistinguishable from those reported previously from the same location (finely intergrown siderite, Mg-calcite and iron sulphide[5]).

Lipid biomarker analyses provides *in situ* measures of sedimentary microbial community structure and nutritional status without the requirement for isolation and growth of the microbiota[9]. Polar lipid fatty acids (PLFA) are maintained metabolically[10] and are an accurate measure of viable sedimentary microbial biomass[11]. Viable eubacterial biomass in the host-mud, measured as the PLFA per g dry weight of sediment, was

TABLE 1 Microbial biomarkers in the concretion and host mud

Parameter	Concretion	Host mud	Interpretation for the concretion, relative to host mud
PLFA (μg per g dry wt)	0.7 1.4-7.0*	4.8, 5.4	Two-thirds of the eubacterial biomass
PLDE (ng per g dry wt)	N.D.†	10, 12	No detectable methanogens
MK/PLFA (g per g)	0.27	0.06, 0.14	Much larger proportion of anaerobic respirers, relative to fermenters
UK/PLFA (g per g)	N.D.†	N.D.†	No detectable aerobes, in either
TL/PLFA (g per mg)	1.0	0.16, 0.22	More accumulated total lipid (former activity)
10-Methyl-16:0 (mol %)	2.0	2.4, 2.4	Less *Desulfobacter*
i17:1 (mol %)	1.5	0.8, 1.0	More *Desulfovibrio*

* Corrected for lower porosity.

† Not detected.

The concretion was packed in host anaerobic mud for transport to the laboratory. Despite this precaution it was found to have developed a brownish-orange rim as a result of atmospheric exposure. It was fractured with a solvent-cleaned chisel, and the outer orange layer discarded. The hard core of the concretion was pulverized with a mortar and pestle, and sieved through a 1-mm screen. Two samples of the host mud and the core were lyophilized. Lipid was extracted with a one-phase chloroform:methanol extractant, and fractionated into neutral lipid, glycolipid and polar lipid fractions with silicic acid. After derivatization, the eubacterial PLFA were analysed by capillary gas-chromatography/mass spectrometry (GC/MS)[9,13], and the archaebacterial PLDE and PLTE by supercritical fluid chromatography[14]. Ubiquinones and menaquinones were recovered from the neutral lipid fraction of the lipid extracts, separated on thin layer plates, recovered, and analysed by supercritical fluid chromatography. Bacteria grow in the water in pore space, and precipitation of concretionary cement in pore space in the mud reduces the volume of fluid available. Mineral and chemical analyses[5] suggest that porosity in the mud is 2-10 times greater than in the concretion, so we have multiplied the PLFA values measured in the latter by those factors (all other measures are relative) to allow comparison of microbial activities. Details of species identification are given in Fig. 1.

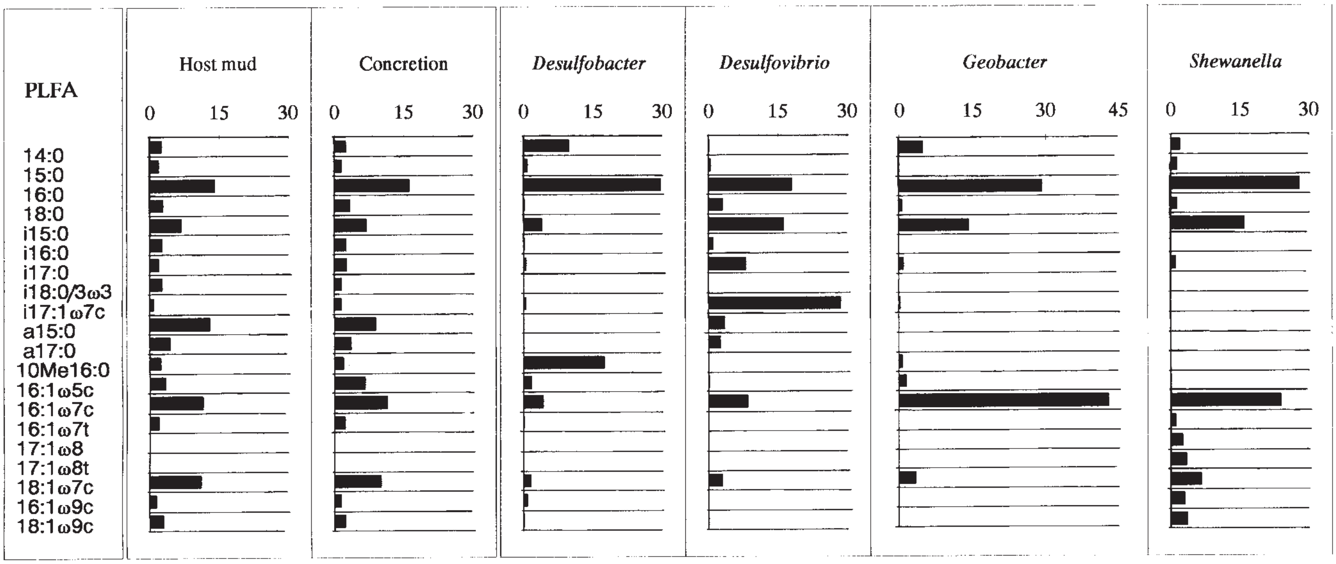

FIG. 1 Polar lipid fatty acid analyses (% of total). The significant (>2%) components measured in our samples are shown, together with analyses of two SRB cultures, typical of their genera (precision, ±0.3%). In addition to *Desulfobacter* and *Desulfovibrio*, we have included data for the dissimilatory Fe(III) reducers *Geobacter* and *Shewanella*[22]. The characteristic fatty acids for the two SRB examples are 10-methyl-palmitic PLFA (10 Me 16:0) and iso-17-carbon-monoenoic PLFA (i17:1ω7c), respectively. Some PLFA are not generically characteristic; with starvation, some bacteria increase the proportions of the specific monoenoic PLFA with the *trans* configuration[23]. Some of our samples show an increased ratio of the 16-and 18-carbon *trans* vaccenic acid to the *cis* isomer. Therefore certain of the data shown (16:1ω7c, 16:1ω7t, 18:1ω7c, 18:1ω9c) cannot be used to identify particular components of the microbial population.

similar to that found in an active subtropical estuary mud flat[12] (Table 1). The concretions had slightly lower viable biomass[13].

Polar lipid diether (PLDE) and polar lipid tetraether (PLTE) lipids are characteristic of the archaebacterial methanogens[14]. In the host mud, there was only a trace of PLTE at the minimum detectable limit and there was roughly a thousand times less PLDE than PLFA (Table 1). When compared to PLTE and PLDE analyses of several strains of methanogenic bacteria, these results suggest that the mud contained $<10^6$ methanogenic bacteria per gram. No PLTE or PLDE were detected in the concretion. These results further indicate that the formation of the siderite concretions is not associated with the activity of methanogenic bacteria.

Menaquinones (MK) are found in Gram negative anaerobes using Fe(III)[15] or sulphate[16] as a terminal electron acceptor as well as in Gram positive organisms, whereas ubiquinones are only found in microorganisms that can use oxygen as an electron acceptor. Fermentative eubacteria contain no respiratory quinones[16]. The MK/PLFA level in the concretion was two to three times higher than in the mud (Table 1). No ubiquinones were found in either the mud or the concretion. These results suggest that a high proportion of the microorganisms living in the concretion survive by anaerobic respiration such as Fe(III) or sulphate reduction.

Detailed analysis of the individual fatty acids gives more information on the types of microbial populations in the samples (Fig. 1). Some define specific components of the population, whereas others indicate nutritional status[9]. Cluster analysis of the PLFA data (Pearson's r correlation coefficient or euclidian metric) indicated the presence of two genera of sulphate-reducing bacterial (SRB), *Desulfobacter* and *Desulfovibrio.* The 10-methyl-palmitic PLFA (10 Me 16:0) is characteristic of *Desulfobacter*[17] and the iso-17-carbon-monoenic PLFA (i17:ω7c) is characteristic of the *Desulfovibrio*[18]. The concentrations of these biomarker PLFA (Table 1) imply more *Desulfovibrio* and less *Desulfobacter* within the concretion than in the mud.

Geobacter metallireducens (formerly, strain GS-15) and *Shewanella putrefaciens* are the only well characterized organisms known to couple the oxidation of fermentation acids and/or hydrogen to the reduction of Fe(III)[1]. The characteristic PFLA data gave no indication that either of these organisms was present in the mud or the concretion.

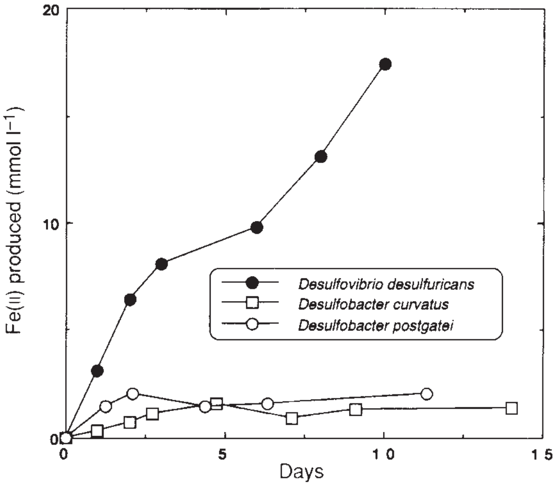

FIG. 2 Laboratory reduction of poorly crystalline Fe(III) oxide by *Desulfovibrio desulfuricans*, but not by *Desulfobacter postgatei* nor *Desulfobacter curvatus*. The inocula for all organisms were grown in a medium with sulphate as the electron acceptor and either H_2 (*D. desulfuricans*) or acetate (*D. postgatei* and *D. curvatus*) as the electron donor. Sulphate-grown cultures were used to inoculate (10% inoculum) similar medium in which the sulphate was replaced by a synthetic, poorly crystalline Fe(III) oxide (100 mmol l^{-1})[24] and the production of Fe(III) was measured over time[25]. The means of duplicate determinations on a representative of triplicate cultures are shown (precision, ±1 mmol l^{-1}). In the Fe(III)-containing media, the initial sulphate concentrations were 320, 740 and 440 μM for *D. desulfuricans*, *D. postgatei* and *D. curvatus*, respectively.

This, together with the *Desulfovibrio* enrichment, led us to investigate the possibility that SRB might be involved in Fe(III) reduction. In aquatic sediments, H_2 is likely to be the most important electron donor for *Desulfovibrio*

$$4H_2 + SO_4^{2-} \rightarrow S^{2-} + 4H_2O$$

whereas acetate is the most environmentally significant electron donor for *Desulfobacter*

$$CH_3COO^- + SO_4^{2-} \rightarrow S^{2-} + 2HCO_3^- + H^+$$

A pure culture of *Desulfovibrio desulfuricans* readily reduced Fe(III), whereas *Desulfobacter postgatei* and *Desulfobacter curvatus* did not (Fig. 2). The reduction of Fe(III) in the presence of *D. desulfuricans* could not be the result of indirect reduction of Fe(III) by sulphide because the initial sulphate concentration of only 320 μM could provide enough sulphide to reduce less than 5% of the Fe(III) that was reduced. Furthermore, all of the sulphate was reduced within the first day of the experiment whereas most of the Fe(III) reduction was after the first day. Several other species of *Desulfovibrio* also reduce Fe(III) oxide, and the Fe(III)-reducing enzyme has now been purified from *D. vulgaris* (D.R.L., E. J. P. Phillips, J. C. Woodward and E. E. Roden, unpublished data).

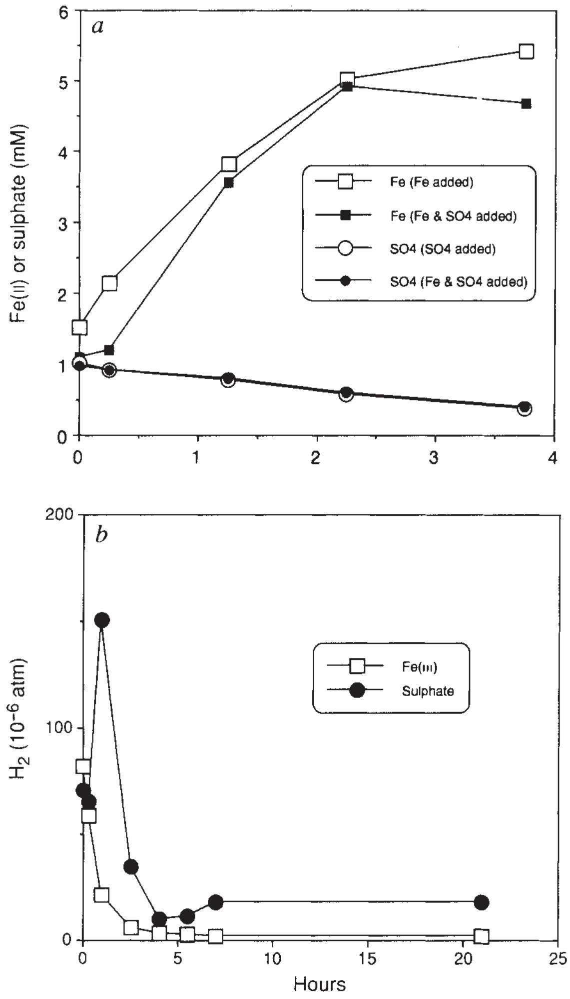

FIG. 3 H_2 oxidation coupled to Fe(III) reduction in *Desulfovibrio desulfuricans*. Under conditions of nonlimiting H_2 concentrations (*a*), *D. desulfuricans* reduced Fe(III) and sulphate simultaneously at rates comparable to Fe(III) and sulphate reduction when only one of the electron acceptors was provided. However, H_2 was metabolized at lower concentrations with Fe(III) than with sulphate (*b*).

METHODS. *D. desulfuricans* grown on H_2-sulphate medium were anaerobically collected by centrifugation and washed and re-suspended in 10 ml of anaerobic bicarbonate buffer ($NaHCO_3$, 25 mg; cysteine-HCl, 2.5 mg; pH 6.7) under N_2-CO_2 (80:20) in sealed 25-ml serum bottles. Cell protein concentrations were (*a*) 0.4 and (*b*) 0.2 mg ml^{-1}. H_2 was added to provide (*a*) 0.67 or (*b*) 10^{-4} atm. Where noted, sulphate and/or Fe(III) complexed with nitrilotriacetic acid were added from anaerobic stocks to provide initial concentrations of ~1 and 5 mM. Fe(II) and sulphate concentrations in aliquots were measured with ferrozine[25] and ion chromatography (precision ±1 mmol l^{-1}). H_2 was measured with a reduction gas analyser (precision, $\pm 5 \times 10^{-7}$ atm.).

To gain insight into the possible metabolism of Fe(III) and sulphate by *D. desulfuricans*, we investigated Fe(III) and sulphate reduction under limiting and nonlimiting H_2 concentrations (Fig. 3). Under nonlimiting conditions, sulphate and Fe(III) were reduced simultaneously (Fig. 3*a*). With sulphate as the electron acceptor, however, *D. desulfuricans* could not metabolize H_2 below $\sim 10^{-5}$ atm (in accordance with previous results[19]) whereas with Fe(III), H_2 was metabolized below 3×10^{-6} atm (Fig. 3*b*). This suggests that at the low concentrations of H_2 found in aquatic sediments, Fe(III) may be the preferred electron acceptor.

These findings suggest that the enrichment of *Desulfovibrio* within the siderite concretion may be an important feature, controlling growth of the concretion. Oxidation of H_2 coupled to reduction of Fe(III) (reaction 1*b*) favours the precipitation of siderite through the formation of Fe^{2+} and OH^-. The reaction does not produce bicarbonate. This is consistent with concretion formation in the marsh. Comparing the isotopic signature of concretion carbonate (−5.9‰) with that from organic matter oxidation (−25‰) and carbonate in sea water (0‰) suggests that most (~75%) of the carbonate comes from sea water. The additional bicarbonate (15%), derived from organic matter oxidation, could be due to the metabolism of as yet unknown Fe(III)-reducing microorganisms, or, given that the concretions contain some FeS, to the activity of SRB like *Desulfobacter*. We are currently investigating the reasons for the apparent localized, enhanced Fe(III) reduction by *Desulfovibrio* in the zone of the concretions.

SRB may be important catalysts for Fe(III) reduction in other sedimentary environments. They are abundant in the Fe(III) reduction zones of deep aquifers in the Atlantic Coastal Plain of the USA[20,21]. As there is no apparent sulphate reduction in these zones and the SRB cannot have been recently introduced to these deeply isolated environments, we speculate that the SRB must survive by reducing Fe(III). More intensive study of the microbiology of siderite concretions and other Fe(III)-reducing environments is needed. ☐

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