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EXPERIMENTAL ARTICLES

High-Temperature Microbial Sulfate Reduction Can Be Accompanied by Magnetite Formation

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Abstract—The hyperthermophilic sulfate-reducing archaeon *Archaeoglobus fulgidus* was found to be capable of lithoautotrophic growth on medium containing molecular hydrogen, sulfate, and amorphous Fe(III) oxide. During the growth of this microorganism, amorphous Fe(III) oxide was transformed into black strongly magnetic precipitate rich in magnetite, as shown by Moessbauer studies. Experiments involving inhibition of microbial sulfate reduction and abiotic controls revealed that magnetite production resulted from chemical reactions proceeding at elevated temperatures (83°C) between molecular hydrogen, amorphous Fe(III) oxide, and sulfide formed enzymatically in the course of dissimilatory sulfate reduction. It follows that magnetite production in this system can be characterized as biologically mediated mineralization. This is the first report on magnetite formation as a result of activity of sulfate-reducing microorganisms.

Key words: magnetite, sulfate reduction, microbial reduction of Fe(III), *Archaeoglobus fulgidus*.

According to current concepts, bacterial formation of magnetite can be caused by activities of magnetotactic [1], dissimilatory iron-reducing [2], and Fe(II)-oxidizing [3] microorganisms. Laboratory cultures of dissimilatory iron reducers are able to convert amorphous Fe(III) oxide to magnetite under suitable physicochemical conditions. Meanwhile, it is only the reduction of Fe(III) to Fe(II) that is believed to be enzymatic, whereas the subsequent formation of minerals is thought to proceed without enzyme participation [4]. The biogeochemical iron and sulfur cycles are coupled, particularly, through the activities of dissimilatory sulfate-reducing microorganisms. Sulfide, a metabolite of this physiological group of prokaryotes, can react with Fe(III) compounds to form elemental sulfur and various iron sulfides [5, 6]. Iron sulfides formed as a result of the activities of mesophilic sulfate reducers in laboratory cultures were identified as mackinawite and gregite when cultivation was performed in the presence of soluble Fe(II) compounds [7, 8], as pyrite when the medium contained amorphous iron oxide [9], and as pyrrhotine when growth occurred in the presence of hematite [10]. Several sulfate-reducing bacteria are known to carry out enzymatic reduction of Fe(III), and their activities can lead to the formation of siderite concretions [11]. Inorganic chemical synthesis of magnetite is possible when Fe^{2+} ions and Fe(III) oxides are both present in the reaction system and can be accelerated by increasing temperature and pH [12, 13]. Until now, there were no reports of magnetite formation through the activities of sulfate-reducing microorganisms.

Studying the capacity of sulfate reducers for dissimilatory reduction of ferric iron, we found that the hyperthermophilic sulfate-reducing archaeon *Archaeoglobus fulgidus* formed magnetic precipitate when cultured on amorphous Fe(III) oxide in the presence of sulfate. In the present work, the composition of this magnetic precipitate is reported along with the conditions and possible mechanism of its formation.

MATERIALS AND METHODS

Archaeoglobus fulgidus VC-16^T (DSM 4304^T) was obtained from the German Collection of Microorganisms (DSMZ), Braunschweig, Germany, and routinely cultured on the recommended medium with lactate and sulfate (DSMZ medium 399 [http://www.dsmz.de]). The medium for culturing *A. fulgidus* in the presence of ferric iron, used in experiments on abiotic Fe(III) reduction, was composed of the following (in grams per liter of distilled water): NaCl, 18.0; $MgCl_2 \cdot 6H_2O$, 0.4; KCl, 0.34; NH_4Cl, 0.25; $CaCl_2$, 0.11; $K_2HPO_4 \cdot 3H_2O$, 0.18; $Fe(NH_4)_2(SO_4)_2 \cdot 7H_2O$, 0.002; $NaHCO_3$, 5.0; and 1 ml of trace element solution [14]. The medium was prepared anaerobically by boiling and cooling under a CO_2 flow (100%, high-purity) and dispensed by 10 ml under an H_2 or N_2 flow (100%) in Hungate tubes (17 ml) equipped with rubber stoppers and screw caps. The medium contained no reducing reagents or organic substances unless otherwise indicated. Fe(III) was added as amorphous oxide in a concentration of

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Table 1. Growth, Fe(II) concentration, and solid phase composition in *A. fulgidus* cultures grown under different conditions*

Cultivation conditions			Features of grown cultures**			
Electron donor	SO_4^{2-}, mM	Changes in cultivation medium	Growth***, 10^7 cells/ml	Fe(II), mM	Relative Fe(III) content of the solid phase, %	Relative Fe_3O_4 content of the solid phase, %
H_2	0	–	<0.1	<0.05	100 ± 1.7	0 ± 0
H_2	0.5	–	1.1	10.3	80.6 ± 2.3	19.4 ± 2.1
H_2	2.0	–	1.1	11.9	70.9 ± 2.9	29.1 ± 2.1
H_2	14.0	–	1.2	15.4	54.3 ± 1.6	45.7 ± 1.4
Lactate + H_2	2.0	–	1.9	10.3	92.1 ± 2.3	7.9 ± 1.4
Lactate + H_2	14.0	–	10.2	22.9	77.9 ± 3.1	22.2 ± 2.0
None	14.0	N_2 (100%) in the gas phase	<0.1	<0.05	not determined	
H_2	14.0	no $NaHCO_3$	<0.1	<0.05	not determined	
H_2	2.0	+ Na_2MoO_4 (20 mM)	<0.1	<0.05	not determined	

* The inoculum for these experiments was obtained on medium with H_2, amorphous Fe(III) oxide, and 2 mM sulfate after at least five serial 5% culture transfers. The average values for three independent experiments are given.

** After 7 days of cultivation at 83°C.

*** The maximum cell density observed.

90 mmol/l. Amorphous Fe(III) oxide was obtained by titration of a $FeCl_3$ solution to pH 9.0. The medium was sterilized by autoclaving at 135°C for 1 h. The pH of the medium measured at 20°C was 6.9–7.0. Sulfate (in the form of $MgSO_4 \cdot 7H_2O$) and sulfide (in the form of $Na_2S \cdot 9H_2O$) were added to the medium in different concentrations from sterile solutions. For experiments to study the effect of CO_2/HCO_3^- on growth, the medium was prepared under a flow of N_2 (100%) and, if needed, a sterile solution of $CO_2/NaHCO_3$ (pH 7.5) was added to a final concentration of $NaHCO_3$ in the medium of 2 g/l. The organism was cultured at 83°C.

The cells were enumerated in an L4 fluorescence optical microscope (LOMO, Russia). Fe(II) was determined with 2,2-dipyridyl [15] following a 24-h extraction in 0.6 M HCl. Magnetic properties of the sediment were determined with a permanent magnet. Moessbauer studies were carried out as previously described [16]. The sediment was collected and dried to constant weight in a desiccator filled with N_2 (100%) at 60°C.

RESULTS

Archaeoglobus fulgidus grew on a medium containing sulfate and amorphous Fe(III) oxide with molecular hydrogen as a sole electron donor (Table 1). Even though the maximum number of cells never exceeded 2×10^7 cells/ml, the cultures were capable of sustainable growth under such conditions in the course of at least six subsequent 5% transfers. After 2–6 days of cultivation, the amorphous Fe(III) oxide was converted to black magnetic precipitate rich in Fe(II). *A. fulgidus* failed to grow unless in the presence of sulfate (concentrations tested, 0.5–14 mM). No growth or change in the appearance of Fe(III) amorphous oxide was noted in media free of both H_2 and HCO_3^-. The microorganisms failed to grow and to form Fe(II) in media containing molybdate, which is a specific inhibitor of dissimilatory sulfate reduction enzymes. The maximum cell density was found to increase when sodium lactate (16 mM) was added to the medium as a supplementary electron donor and a source of carbon. The pH of the medium did not show much variation in the course of cultivation.

The precipitates formed during the growth of microorganisms were studied by Moessbauer spectroscopy. The obtained spectra were processed and analyzed by recovering the ultrafine parameter distribution function of partial Moessbauer spectra [16]. As seen from figure, the Moessbauer spectrum of the original amorphous hydrate was a quadrupole doublet with ultrafine parameters corresponding to the Fe^{3+} high-spin state in the octahedral surrounding. The addition of sulfate to the culture medium was found to give rise to a partial spectrum in the Moessbauer spectrum of the sediment with a quasi-continuous distribution of ultrafine magnetic fields, indicating the formation of magnetite, which is known to lack complete crystalline coordination [17]. The relative magnetite content increased with the initial sulfate concentration and decreased when lactate was added.

To determine the composition of the mineral phase formed in reduction of amorphous Fe(III) oxide by sulfide, in control tests, a solution of $Na_2S \cdot 9H_2O$ was injected into tubes with sterile uninoculated medium to

obtain different final sulfide concentrations (Table 2). The controls were run at 20 and 83°C with a gas phase composed of H_2 (100%) or N_2 (100%). Moessbauer studies showed that magnetite was formed only at 83°C in the test variants with hydrogen and with a sulfide concentration equal to 2.0 or 10.0 mM (the magnetite content of the solid phase being, respectively, 68 ± 3 and 52 ± 4%). In other controls, the reduction of Fe(III) was not coupled with magnetite formation. In the same tests, the formation of magnetite was accompanied by the formation of small quantities of siderite. The formation of siderite was also noted at 20°C in the presence of hydrogen and 10 mM sulfide. In the absence of molecular hydrogen, magnetite and siderite failed to form even in test variants with significant reduction of Fe(III).

DISCUSSION

Our findings show that magnetite can be formed at elevated temperatures from amorphous Fe(III) oxide in the presence of molecular hydrogen and sulfide produced enzymatically via microbial sulfate reduction. The following chemical reactions are likely to take place in the system under study:

Sulfate reduction to sulfite by molecular hydrogen:

$$SO_4^{2-} + H^+ + 4H_2 \longrightarrow HS^- + 4H_2O.$$

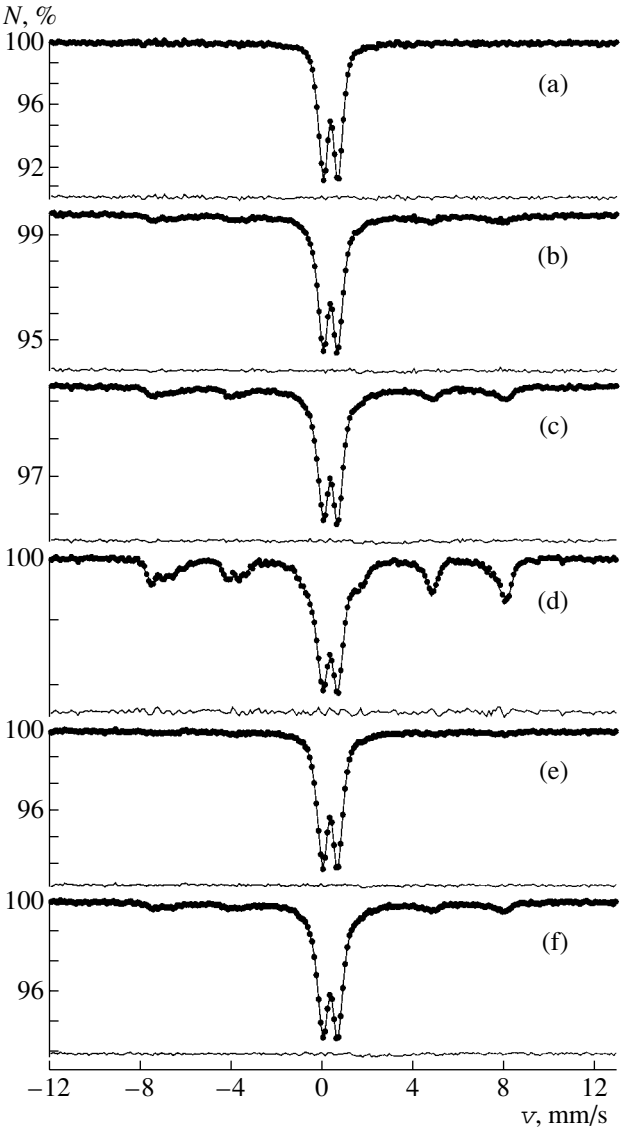
Fe(III) reduction by sulfide to yield Fe(II) and elemental sulfur:

$$2Fe(OH)_3 + HS^- + 5H^+ \longrightarrow 2Fe^{2+} + S^0 + 6H_2O.$$

Magnetite formation from Fe(III) and Fe(II):

$$2Fe(OH)_3 + Fe^{2+} \longrightarrow Fe_3O_4 + 2H^+ + 2H_2O.$$

Obviously, all processes taking place in the system are not described by these equations. For example, magnetite was not formed in abiotic controls unless in the presence of molecular hydrogen. It can be supposed that H_2 enters into reaction with the surface of Fe(III) oxide and thus hinders fast formation of insoluble iron sulfides, which allows the dissolved Fe^{2+} to react with the amorphous Fe(III) oxide. The results of abiotic experiments with uninoculated medium show that Fe(III) can be reduced in significant amounts (8–12 mM) even at fairly low sulfide concentrations (0.5–2.0 mM) both at 20 and 83°C and this process does not require the presence of molecular hydrogen. The fact that the concentration of ferrous iron exceeds the concentration of sulfide can be explained, apparently, by the cyclic mechanism of Fe(III) reduction brought about by partial reductant regeneration on the surface of iron oxide. It should be noted that the data on the Fe(II) content relate only to the HCl-extracted iron and, given that the degree of ferrous iron extraction varies with the mineral, ought not be compared on a purely quantitative basis. The important fact is that formation of magnetite requires the presence of molecular hydrogen and elevated temperature.



Moessbauer spectra of ^{57}Fe in the solid phase formed in growth of *A. fulgidus* with H_2 as the electron donor at a sulfate content of the solution equal to (a) 0, (b) 0.5, (c) 2.0, and (d) 14.0 mM and with H_2 + lactate as the electron donor at a sulfate content of (e) 2.0 and (f) 14 mM.

The hyperthermophilic archaeon *Archaeoglobus fulgidus* was described as a sulfate reducer able to grow organotrophically on sulfate and thiosulfate and lithotrophically only with thiosulfate [18]. Even though cell suspensions of *A. fulgidus* were previously shown to be able to reduce soluble forms of Fe(III) [19], the capacity of *A. fulgidus* to grow with Fe(III) serving as an electron acceptor was never demonstrated [20]. In our experiments, *A. fulgidus* grew lithoautotrophically with molecular hydrogen as an electron donor in the presence of sulfate and amorphous Fe(III) oxide. The fact that no stable growth is possible without sulfate or in the presence of both sulfate and molybdate suggests that *A. fulgidus* grows by reducing sulfate rather than

Table 2. Fe(II) content and the solid phase composition in uninoculated medium under various conditions*

Conditions			Characteristics of the medium after 7 days			
Gas phase	S^{2-}, mM	*T*, °C	Fe(II), mM	Relative content of Fe(III), %	Relative content of siderite, %	Relative content of Fe_3O_4, %
H_2	0	83	<0.05	100 ± 2	0	0
H_2	0.5	83	8.2	100 ± 2	0	0
H_2	2.0	83	12.9	28 ± 3	4 ± 1	68 ± 3
H_2	10.0	83	24.3	45 ± 2	3 ± 1	52 ± 2
H_2	0	25	<0.05	100 ± 2	0	0
H_2	0.5	25	<0.05	100 ± 2	0	0
H_2	2.0	25	9.6	100 ± 2	0	0
H_2	10.0	25	20.7	91 ± 1	9 ± 1	0
N_2	0	83	<0.05	100 ± 1	0	0
N_2	0.5	83	9.3	100 ± 1	0	0
N_2	2.0	83	9.7	100 ± 1	0	0
N_2	10.0	83	12.9	100 ± 1	0	0
N_2	0	25	<0.05	100 ± 1	0	0
N_2	0.5	25	<0.05	100 ± 1	0	0
N_2	2.0	25	10.0	100 ± 1	0	0
N_2	10.0	25	21.1	100 ± 1	0	0

* In uninoculated medium with H_2 at 83°C in the presence of 14 mM $MgSO_4$, the concentration of Fe(II) after 7 days was less than 0.05 mM.

Fe(III). It follows then that magnetite is formed in the reaction of the produced sulfide with Fe(III) in the presence of hydrogen, as in the case of an abiotic system.

The obtained evidence suggests that magnetite can be formed as a result of microbial sulfate reduction. This occurs in a chemical reaction of amorphous Fe(III) oxide, molecular hydrogen, and enzymatically produced sulfide. Hence, this process can be characterized as a biologically mediated mineralization. *A. fulgidus* is known to inhabit deep-sea and shallow-water hydrothermal vents as well as high-temperature oil reservoirs and, therefore, magnetite in these systems can arise as a result of activities of sulfate-reducing prokaryotes. The transformation of amorphous Fe(III) oxide to magnetite should also be taken into account in laboratory practice, where until now the formation of magnetite was regarded as direct evidence of the activities of iron-reducing microorganisms.

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