Sorption of Fe (Hydr)Oxides to the Surface of *Shewanella putrefaciens*: Cell-Bound Fine-Grained Minerals Are Not Always Formed De Novo

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Shewanella putrefaciens, a gram-negative, facultative anaerobe, is active in the cycling of iron through its interaction with Fe (hydr)oxides in natural environments. Fine-grained Fe precipitates that are attached to the outer membranes of many gram-negative bacteria have most often been attributed to precipitation and growth of the mineral at the cell surface. Our study of the sorption of nonbiogenic Fe (hydr)oxides revealed, however, that large quantities of nanometer-scale ferrihydrite (hydrous ferric oxide), goethite (α -FeOOH), and hematite (α -Fe₂O₃) adhered to the cell surface. Attempts to separate suspensions of cells and minerals with an 80% glycerin cushion proved that the sorbed minerals were tightly attached to the bacteria. The interaction between minerals and cells resulted in the formation of mineral-cell aggregates, which increased biomass density and provided better sedimentation of mineral Fe compared to suspensions of minerals alone. Transmission electron microscopy observations of cells prepared by whole-mount, conventional embedding, and freeze-substitution methods confirmed the close association between cells and minerals and suggested that in some instances, the mineral crystals had even penetrated the outer membrane and peptidoglycan layers. Given the abundance of these mineral types in natural environments, the data suggest that not all naturally occurring cell surface-associated minerals are necessarily formed de novo on the cell wall.

Bacterial cells are often associated with fine-grained Fe precipitates in natural environments (16, 17, 21, 22, 38, 49). Attraction of soluble metals to bacterial surfaces and subsequent development of bound minerals have been demonstrated by studies of metal sorption (7, 8, 14); bacterial walls are known to serve as templates for nucleation and growth of metal precipitates (5). Yet bacteria are frequently attracted to and adhere to large mineral substrates, such as iron oxides and silicates (24, 52).

Minerals vary a great deal in size. In natural environments where bacteria are active, such as soils, sediments, rivers, and lakes, Fe minerals are generally nanometer size rather than micrometer size (12, 20, 38). Nanophase hematite has even been investigated as a possible component of Martian soil (32, 33). Smaller mineral particles are more chemically reactive than large particles since they have extremely high surface-to-volume ratios and (generally) poorer crystallinity, and this greater reactivity implies that the rate of biotic and abiotic reduction is higher (40). The small size may be due to weathering processes or, for authigenic minerals, to the presence of atypical ions that disrupt crystal growth (12). Laboratory studies of microbe-mineral interactions have, however, often used synthetic minerals that are relatively large (diameter, $>1\ \mu\text{m}$) or fine minerals immobilized on a support (19, 24, 31). While large minerals are important for studying the initiation of bio-film growth or bacterial adhesion forces, their total surface area in natural environments is probably small compared to that of free fine-grained particles, which cycle actively via erosion, mixing, and transport processes (12). Furthermore, once fine-grained Fe minerals attach to bacterial surfaces, the

charge characteristics of the cells are probably altered, which in turn affects flocculation and sedimentation properties.

Bacteria usually have a net negative surface charge at a circumneutral pH due to acidic functional groups (3, 11). However, Fe (hydr)oxide minerals (a term which includes both Fe oxides and Fe hydroxides) are positively charged at pH values below ~ 8 to 9 (12). Hence, electrostatic interactions favor the approach of bacteria and minerals; the hydrophobicity of either substrate increases the van der Waals interaction (52). Whether an association takes place also depends on steric factors between a mineral face and bacterial ligands. Whereas the size of Fe minerals varies a great deal, the size of bacteria is relatively fixed at about 1.5 to 2.5 μm^3 or less under the nutrient-deficient conditions found in many natural environments (4). The curved surfaces of bacteria, which are independent of cell shape, may restrict the organisms to tangential associations with larger Fe minerals (24). Very small minerals, on the other hand, can surround and enmesh a cell, as shown by electron microscope studies of minerals that have precipitated on cell walls (6).

Shewanella spp. are facultatively anaerobic, iron-reducing bacteria commonly found in natural aquatic and sedimentary environments (13, 35). They reduce soluble or mineral-bound Fe³⁺ by a dissimilatory mechanism in which Fe³⁺ serves as an electron acceptor for the membrane-bound electron transport chain during respiration (36). Attachment to Fe (hydr)oxides is thought to be necessary for reduction to occur (1), although a mutant strain of *Shewanella alga* that attaches poorly also reduced Fe³⁺ (10). *Shewanella putrefaciens*, however, attaches to Fe (hydr)oxides under both aerobic and anaerobic conditions (24). Grantham et al. (24) used atomic force microscopy to detect etch patterns made by *S. putrefaciens*, and their study indicated that the cells dissolved the Fe beneath them.

Because of the potential of fine-grained Fe (hydr)oxides to

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sorb to *Shewanella* cells in natural environments, we investigated the sorption capability of these compounds under laboratory conditions to determine the magnitude and possible mechanism of the interaction; the term sorption is used here because the precise uptake mechanism (i.e., surface interactions or penetration of the mineral below the outer membrane) is not entirely clear. We used synthetic ferrihydrite and nanocrystalline goethite (both hydroxides) and hematite (an oxide) as examples of fine-grained minerals; a much larger goethite was included for comparison. These minerals are commonly found in natural sediments and soils and are important reactive agents in Fe cycling (12, 34).

MATERIALS AND METHODS

Fe minerals. Ferrihydrite was prepared by adding 1 M NaOH to 2 M $\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$ until the pH was 7 (i.e., the Fe/OH ratio was ~ 1). The precipitate was washed four times in sterile deionized water. Analysis by X-ray diffraction and transmission electron microscopy (TEM) showed that the precipitate was a two-line ferrihydrite consisting of strongly aggregated particles that were about 3 nm in diameter.

Nanometer-size crystals of goethite and hematite and microcrystalline goethite were prepared by hydrolysis of $\text{Fe}(\text{NO}_3)_3$ salts, as described previously (23). For nanogoethite, 0.7 mol (282.8 g) of $\text{Fe}(\text{NO}_3)_3 \cdot 9\text{H}_2\text{O}$ was dissolved in 350 ml of 2 M HNO_3 , and then the preparation was diluted with 1.4 liters of ultrapure water; 1 M NaOH was then added to obtain an OH/Fe molar ratio of 2 and a pH of 1.8. After 60 days the precipitate was washed three times and dialyzed in sterile deionized water. The microgoethite was prepared by the same method, except that an initial OH/Fe molar ratio of 4 and an initial pH of 12.9 were used. Characterization of these goethite minerals in a previous study by X-ray diffraction, TEM, Mössbauer spectroscopy, and a dissolution technique showed that synthesis at pH 1.8 resulted in pure goethite crystals about 20 nm long (c-axis) (23). Similar characterization of the product resulting from the alkaline goethite synthesis procedure showed multidomain crystals that were about 800 nm long (23).

The hematite was prepared by hematite method 1 described by Schwertmann and Cornell (43), and the product was washed and dialyzed as described above for goethite. The synthesis resulted in diamond-shaped crystals about 30 nm in diameter. The mineralogy was confirmed by selected area electron diffraction (SAED).

All of the minerals were prepared by using sterile techniques and were checked for microbial contamination by plating on Trypticase soy agar (TSA) (Becton Dickinson). In addition, the minerals were kept at 4°C as suspensions; they were not dried since drying increases the strong aggregation to which nanominerals are prone.

Bacteria. Cultures of *S. putrefaciens* CN32 were provided by Y. Gorby of the Pacific Northwest National Laboratory in Richland, Wash., and cells were maintained on TSA plates. Metal sorption studies and lipopolysaccharide (LPS) analysis (using the sodium dodecyl sulfate-polyacrylamide gel electrophoresis silver staining method [51]) were performed with aerobically grown cultures.

Mineral sorption experiments. To closely observe sorption of minerals to bacteria, single colonies from TSA plates were inoculated into a defined mineral medium (DM) consisting of trace element salts, 3.9 mM PO_4^{3-} , 20 mM lactate, and 4.5 mM 1,4-piperazinediethanesulfonic acid (PIPES) buffer (pH 6.5). Cells were grown to the mid-exponential phase (optical density at 560 nm [OD_{560}], 0.1) under aerobic conditions, washed twice with 4.5 mM PIPES buffer, and resuspended in flasks containing the trace element solution at an OD_{560} of 0.4. Ferrihydrite, goethite, and hematite were added to obtain suspension concentrations of 0.4, 0.6, and 0.6 mg/ml, respectively ([Fe], approximately 4 mM). The flasks were swirled gently for 30 min at 100 rpm. Samples were prepared for TEM as whole mounts with no stain and as thin sections of conventionally embedded and freeze-substituted preparations (9).

Sorption experiments were also conducted under anaerobic conditions. To do this, we prepared bottles that contained 60 ml of DM; these bottles were degassed with N_2 , sealed, and autoclaved. They were placed in an anaerobic chamber (Coy Laboratory Products), in which all further manipulations took place. Sterile, anaerobic, degassed suspensions of ferrihydrite, nanogoethite, microgoethite, or hematite were injected into the bottles to obtain a [Fe] of 4 mM. Cultures that were grown aerobically in DM and had reached the mid-exponential to late exponential growth phase (OD_{560} , 0.2) were degassed with H_2/Ar (5:95) and inoculated into DM suspensions. The initial concentrations

were approximately 2×10^7 CFU/ml, as determined by plate counting. The reduction potentials of suspensions in the anaerobic chamber were determined with an electrode (Corning combination electrode), and samples for TEM were taken when the reduction potentials were less than 0 mV. Samples were checked for the presence of Fe^{2+} at this time by the ferrozine method (28) using 0.5 M HCl extracts. Whole-mount samples for TEM were prepared in a glove box and were exposed to O_2 for ~ 30 s as they were moved from an airtight jar to the sample holder and inserted into the microscope. Likewise, embedded samples were prepared under anaerobic conditions until the plastic polymerization step.

Separation of cells with sorbed minerals from mineral-free cells. Since the density of bacteria with sorbed minerals was higher than the density of free cells, the two types of cells were separated by using a glycerin step gradient and centrifugation. Suspensions of cells were prepared in DM so that the OD_{560} were 0.70 to 0.75, and Fe minerals were added to the cell suspensions so that the mineral-bound Fe concentrations were approximately 4 mM. Two milliliters of each cell-mineral suspension was carefully layered on a 10-ml 80% (vol/vol) glycerin cushion in a glass centrifuge tube and then centrifuged at $3,300 \times g$ for 20 min. The two liquid layers and the pellet were checked for the presence of cells by the plate count and streak plate methods, respectively, as well as by TEM. To determine the fractionation of Fe, the suspension interface layer and the glycerin layer were carefully removed with a pipette and treated with an equal volume of concentrated (36%) HCl to dissolve the mineral-bound Fe. The pellet was then dissolved by treating it with 6 M HCl. The Fe contents of the HCl extracts were measured by graphite furnace atomic absorption spectroscopy by using a Perkin-Elmer model 2380 atomic absorption spectrophotometer with an HGA-400 graphite atomizer. The same density separation method was used for suspensions containing Fe oxide without cells.

TEM. Cells and minerals were prepared for TEM and were examined as previously described (27). No negative stains were used for whole mounts and for most thin sections so that the sorbed mineral phases could be attributed to only preformed minerals. Once this was done, some thin sections were stained with 2% (wt/vol) uranyl acetate and then with lead citrate (9) to determine the structures to which the minerals were attached. All imaging was performed with a Philips EM300 TEM operating at 60 kV with a liquid nitrogen cold trap.

RESULTS

Separation by glycerin centrifugation. After centrifugation, the amount of mineral in the cell-mineral suspension above the glycerin cushion was particularly reduced (Fig. 1), and the suspension contained relatively mineral-free bacteria, which was reflected by the low Fe concentrations in this layer (Table 1). Preparations containing only minerals also had low Fe concentrations in the upper layer. The glycerin layer contained few bacteria ($<10^2$ CFU/ml for nanogoethite and hematite treatments, no cells for the ferrihydrite treatment) and little Fe. The pellet consisted of densely coated bacteria and free aggregated minerals. After centrifugation of minerals alone, the upper fluid phase also had a low [Fe], but the glycerin had a significantly higher [Fe] compared to the preparations containing bacteria and minerals. Although the pellets at the bottoms of the tubes contained most of the minerals, some pellet material was also smeared along the sides of the tubes. For samples containing a cell-mineral suspension, streaking of the pellet revealed the presence of cells, which was confirmed by TEM. That the mineral was not simply enmeshed in exopolysaccharide was shown by the close contact between the cells and the minerals (Fig. 2). Plate counting showed that the numbers of cells (CFU) in the cell-mineral suspensions decreased after centrifugation from an initial value of $2.9 \times 10^9 \pm 0.1 \times 10^9$ CFU/ml to $6.5 \times 10^7 \pm 4.4 \times 10^7$ CFU/ml for ferrihydrite, from an initial value of $1.8 \times 10^9 \pm 0.1 \times 10^9$ CFU/ml to $1.0 \times 10^6 \pm 0.7 \times 10^6$ CFU/ml for hematite, and from an initial value of $1.9 \times 10^9 \pm 0.2 \times 10^9$ CFU/ml to $7.9 \times 10^6 \pm 7.1 \times 10^6$ CFU/ml for nanogoethite (based on average values for three replicates). These decreases are statistically

FIG. 1. Density separation of cell-mineral or mineral suspensions through a glycerin cushion. (A) Before centrifugation. An upper layer containing suspended cells and minerals or only minerals is above an 80% glycerin solution. (B) After centrifugation. A mineral pellet has formed at the bottom and partially on the side of the tube, and few particles are suspended in either the suspension layer or the glycerin layer.

significant, as determined by two-tailed *t* test analyses of the data ($P < 0.05$). Pure cells were not attached to the glass of the centrifuge tubes, whereas the pellet consistently contained large amounts of cells and Fe (hydr)oxide (Fig. 2). More than 95% of the mineral Fe was detected in the pellet (Table 1). Based on the CFU and [Fe] values, it appears that attachment to cells resulted in more effective separation of the Fe (hydr)oxide from the liquid phases.

Since we could easily separate the unsorbed minerals and the cells with sorbed minerals from the clean cells by density centrifugation, the mineral association was not the result of random association during preparation of the samples for viewing. The cells were effective sorbents of all our freshly prepared fine-grained minerals; about 90% of the cells had

FIG. 2. Thin section of a portion of a pellet formed during centrifugation through a glycerin cushion of suspended *S. putrefaciens* CN32 cells with nanogoethite.

minerals attached to them when approximately 200 cells were examined by TEM using the whole-mount method. This percentage compared well with observations made by TEM before separation was attempted.

Aerobic conditions. Suspensions of cells and minerals prepared under aerobic conditions rapidly formed flocs that settled out once agitation was stopped. TEM observation of unstained whole mounts (Fig. 3) and thin sections of flocs that were conventionally embedded (Fig. 4 and 5) showed that minerals were clustered on the outer periphery of intact cells and, in some cases, attached to flagella (Fig. 3). Not all of the cells sorbed minerals; we often observed cells with mineral-saturated surfaces next to cells that were relatively mineral free (Fig. 4), suggesting that mineral sorption may reflect certain discrete stages in cellular physiology (such as cell age or viability) or some other factor (such as cell surface composition). The patchwork pattern of mineral sorption on individual cells may also have been due to heterogeneous distribution of charges on the cell surface (44). It is possible, though, that the paucity of minerals on some cells was due to the thinness and angle of the thin section.

Fine-grained hematite and goethite minerals. When preparations were monitored by TEM for 15 min to 3 days, it was apparent that only short exposures were necessary to obtain substantial binding of goethite and hematite to the cell surfaces. The attachment was so strong even after such short exposures that the minerals occasionally penetrated the outer membrane (Fig. 6), which was confirmed by stereoinaging (unpublished results). Crystals appeared within the periplasmic space and even penetrated the plasma membrane (Fig. 7), suggesting that the peptidoglycan layer had been pierced, which is remarkable in light of this layer's elasticity and strength (53). It is possible, though, that the small minerals had somehow managed to pass through strategically placed holes in

TABLE 1. Fractionation of Fe in the mineral or cell-mineral suspension interface, the glycerin cushion, and the pellet after centrifugation for preparations containing Fe (hydr)oxide with and without *S. putrefaciens* CN32 cells

Treatment	Fe (% of total) ^a		
	Suspension	Glycerin	Pellet
Ferrihydrite	0.7 ± 0.0	7.0 ± 0.1	92.3 ± 3.4
Hematite	3.1 ± 0.2	2.8 ± 0.2	94.1 ± 4.0
Nanogoethite	5.3 ± 0.3	11.4 ± 0.5	83.3 ± 6.2
Ferrihydrite + cells	3.3 ± 0.2	1.0 ± 0.0	95.7 ± 5.1
Hematite + cells	1.0 ± 0.1	0.6 ± 0.0	98.4 ± 3.9
Nanogoethite + cells	1.2 ± 0.1	1.0 ± 0.0	97.8 ± 5.0

^a Concentrations were determined by graphite furnace atomic absorption spectrophotometry. The values are means ± standard deviations based on three replicates.

FIG. 3. Unstained whole mount showing CN32 cells after 30 min of exposure to hematite in suspension. (Inset) Mineral aggregates (small arrows) attached to a flagellum (large arrow).

the murein network, such as the holes which have been modeled (39). Although suspensions of the cells were plated on TSA and the cultures remained viable, it was difficult to determine if the penetrated bacteria were alive. Interestingly, their cytoplasm had the same consistency as the cytoplasm of unpenetrated cells in thin sections, indicating that nucleic acids and proteins had not leaked out. Because centrifugation must be used to pellet cells for fixation, it is possible that centrifugal shear forces propelled the particles into the cells. Results obtained for uncentrifuged whole-mount cells and stereoinaging of these cells, however, also support the hypothesis that penetration occurred without the help of shear forces. Furthermore, there were no indications of cellular collapse, which would be expected if the balloon-like cell wall were abruptly pierced with minerals.

Ferrihydrite. Of all the cell-mineral relationships, the spatial relationship between cells and ferrihydrite was the most difficult to assess. This was because ferrihydrite had the strongest tendency to aggregate and the aggregates behaved as a larger mineral in suspension. However, thin sections of samples prepared by freeze-substitution revealed that ferrihydrite became tightly associated with cells and, like hematite and goethite, sometimes breached the outer membrane (Fig. 8).

Microgoethite. Cells exposed to the larger mineral goethite sorbed fewer crystals, and these mineral particles did not penetrate the cell wall. The crystals tended to align so that their long axes were parallel to the long axis of the cell or tangential to the cell's curves; it is possible that crystals originally attached

at the poles were sheared off during centrifugation. It appeared that the cells preferred to retain small or broken crystals (Fig. 9).

Anaerobic conditions. We found that all cultures reached anaerobic conditions after 1 day, as indicated by the negative values for the reduction potentials. At this time there was not yet significant reduction of Fe by the bacteria, because Fe^{2+} was not detected at levels higher than the value obtained for the cell-free control. A previous experiment showed that Fe reduction began after 5 days under identical culture conditions. The bacteria presumably used any remaining traces of O_2 and organic molecules freed from dead cells as electron acceptors before reducing Fe^{3+} ; the sizes of cell populations were reduced from 2×10^7 to 6×10^6 CFU/ml or less 1 day after inoculation. In spite of differences in the culture method, the relationship of minerals to the cells was identical to what we observed under aerobic conditions. Cell poles appeared to be particularly attractive to the fine minerals under both aerobic and anaerobic conditions.

LPS analysis. We determined by sodium dodecyl sulfate-polyacrylamide gel electrophoresis and silver staining that *S. putrefaciens* CN32 contained only rough LPS in the outer membrane. This result is identical to previous results obtained for the same strain (A. A. Korenevsky and T. J. Beveridge, Abstr. 100th Gen. Meet. Am. Soc. Microbiol., abstr. Q-251, 2000).

DISCUSSION

Bacteria have surfaces that are reactive with metals largely due to the dissociation of protons from carboxyl and phospho-

FIG. 4. Stained thin section of CN32 cells with associated nanogoethite after 30 min of exposure to the mineral. The large arrows indicate the cell wall, and the small curved arrows indicate goethite.

FIG. 5. Stained thin section of a CN32 cell with associated hematite (arrows) after 30 min of exposure to the mineral.

ryl group constituents of cell wall macromolecules (14). It is well known that anionic organic molecules form ionic and covalent bonds with Fe (hydr)oxides which are extensive enough to reverse the dominantly positive charges on the min-

FIG. 6. Thin section showing the cell envelope after 30 min of exposure to nanogoethite. The arrows indicate sites where the mineral appears to penetrate the outer membrane.

FIG. 7. Thin section at a cell pole after 30 min of exposure to hematite. The arrows indicate sites where the mineral appears to penetrate the outer membrane (large arrows) and peptidoglycan (small arrow). A fragment of polar membrane (PM) is also visible.

erals (12, 42, 48, 50), just as metal adsorption can reverse the negative charges on bacterial cells (11). The cell walls of gram-negative bacteria have outer membranes that are rich in LPS, which is highly anionic and makes the cells attractive to cationic metal species (15, 41). Ferris and Beveridge (15) observed metal binding on *Escherichia coli* K-12 and found that the phosphoryl residues in the outer membrane were the most likely binding sites for metals; a similar conclusion was reached by Langley and Beveridge (27) for *Pseudomonas aeruginosa*. *E. coli* K-12 has only rough LPS (i.e., no O side chains). Because *S. putrefaciens* CN32 also contains only rough LPS in its outer membrane, it is thought that the phosphoryl groups bind metals and preformed minerals to the greatest extent. Phosphate is known to form surface complexes by ligand exchange on Fe (hydr)oxides (2, 37). Because surface complex formation on a solid (hydr)oxide phase in solution is analogous to complex formation in homogeneous systems (47), by extrapolation the functional groups of metal (hydr)oxide minerals should react by a similar mechanism with the organic phosphate functional groups on the cell surface. In the case of minerals attaching to cells, steric factors have a strong influence as well. Another factor is the hydrophobicity of the attaching surfaces. The surface of *S. putrefaciens* CN32 is more hydrophobic than the surfaces of *E. coli* and *P. aeruginosa* (unpublished results determined by hexadecane partitioning), whereas Fe (hydr)oxides are hydrophilic. Attractive forces between hydrophilic and hydrophobic charged surfaces are dominated by the electrostatic interaction force (52); under the conditions used in our experiments, the net interaction between negatively charged CN32 cells and positively charged Fe(hydr)oxides is attractive.

The preferential attraction of the minerals to cell poles war-

FIG. 8. Thin section of a freeze-substituted CN32 cell after 30 min of exposure to ferrihydrite. The arrows indicate sites on the cell wall where ferrihydrite appears to be attached to the outer membrane.

rants further study. A similar phenomenon was found by Grantham et al. (24), who observed etch patterns on Fe mineral substrata after anaerobic incubation that indicated apical attachment of the cells. Sonnenfeld et al. (45) found that the negative charge was concentrated at the cell poles in *Bacillus subtilis*, a gram-positive organism, and we are currently investigating whether *S. putrefaciens* has a similar charge distribution. A study of the *S. putrefaciens* CN32 surface by electrostatic force microscopy revealed an overall heterogeneous distribution of charge that did not exhibit anomalies at the cell poles (44); however, the cells used in that study were dried in air, and the results may not be easily extrapolated to a fluid system.

In situ studies of Fe minerals and cells have proven that an intimate association takes place. For example, using TEM, Southam and Beveridge (46) observed clusters of *Thiobacillus* spp. closely surrounded by dense aggregates of fine, fractured, nonbiogenic chalcopyrite in mine tailings. Minerals not only attach to the cell wall; TEM studies of samples from Lake Lugano revealed cells enmeshed in a network of exopolysaccharide densely studded with Fe-rich granules approximately 10 to 50 nm in diameter that appeared to have grown on the polysaccharide mesh (38). Many studies of bacterium-mineral interactions in situ have assumed or concluded, however, that the fine precipitates associated with cell surfaces formed by biomineralization processes, without strong supporting evidence (17, 18, 22, 25, 29, 30). Comparisons of TEM images obtained by previous researchers to the cells with sorbed minerals that we produced in this study revealed many similarities.

FIG. 9. Thin section of a CN32 cell after 30 min of exposure to microgoethite. The arrows indicate sites where the mineral is attached to the cell.

In particular, the closeness of the association can be deceiving. For example, Fortin et al. (22) observed cells and minerals located near a hydrothermal vent system. They observed that minerals appeared to be rooted in the cell wall and took this observation as evidence that nucleation of the mineral on the cell occurred. We noticed, however, a similar close association between our fine minerals and cell walls after less than 30 min of contact. While it has been proven under laboratory conditions that cells accumulate soluble metal ions and can form precipitates (7, 8, 26, 27), it is not clear that conditions at the cell surface in natural environments result in the formation of only the crystalline minerals that were observed in the studies mentioned above. The formation of Fe (hydr)oxides is affected in a complex way by many factors, including temperature, pH, reduction potential, and common natural substances, particularly metals, organics, Si, and P (12). Cells create microenvironments at their surfaces by concentrating some molecules, excluding others, and releasing exudates and by-products of metabolism; the net effect is highly specific to the environment and the organism that it supports. While there is no doubt that biomineralization does take place, it is apparent from our study that sorption of preformed nanominerals can also occur. We presume that in natural environments both this process and the genesis of true biominerals occur together, sometimes sharing similar reactive surface sites on a cell. The affinity of bacterial surfaces for preformed minerals does not diminish the importance of biomineralization. Rather, it demonstrates another route for the cycling of Fe and extends the biogeochemical phenomena of bacteria.

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