POSSIBLE ROLE OF MICROBIAL POLYSACCHARIDES IN NONTRONITE FORMATION

MASATO UESHIMA AND KAZUE TAZAKI

Department of Earth Sciences, Kanazawa University, Kanazawa, Ishikawa 920-1192, Japan

Abstract—Nontronite and microbes were detected in the surface layers of deep-sea sediments from Iheya Basin, Okinawa Trough, Japan. Nontronite, an Fe-rich smectite mineral, was embedded in acidic polysaccharides that were exuded by microbial cells and electron microscopy showed that the nontronite layers were apparently oriented in the polysaccharide materials. We propose that the formation of nontronite was induced by the accumulation of Si and Fe ions from the ambient seawater and that extracellular polymeric substances (EPS) served as a template for layer-silicate synthesis. Experimental evidence for this hypothesis was obtained by mixing a solution of polysaccharides (dextrin and pectin) with ferrosiliceous groundwater. After stirring the mixture in a sealed vessel for two days, and centrifuging, Fe-rich layer silicates were identified within the precipitate of both the dextrin and pectin aggregates, whereas rod-shaped or spheroidal Si-bearing iron hydroxides were found in the external solution. Microbial polysaccharides would appear to have affected layer-silicate formation.

Key words—Biomineralization, Extracellular Polysaccharides, Microbes, Micromorphology, Microstructure, Nontronite.

INTRODUCTION

There is good evidence to suggest that microbes are involved in, and contribute to, global elemental cycling (*e.g.* Fisk *et al.,* 1998; Tazaki, 1999). The roles of sulfate-reducing microbes in sulfur and arsenic cycling (*e.g.* Fortin *et al.,* 1996, Newman *et al.,* 1998) and of lichens and mycorrhizal fungi in mineral weathering (Jongmans *et al.,* 1997; Barker and Banfield, 1996; Barker *et al.,* 1998) are well established. The precipitation of clayey materials and specific metals, such as Fe and Mn, in microbial mats has also been reported (Tazaki, 1997; Akai *et al.,* 1999).

An important process in microbe-mineral interactions is biomineralization, *i.e.* microbially-mediated synthesis of minerals. In this regard, the work of Schultze-Lam *et al.* (1992, 1996a, 1996b) has shown that polymeric substances exuded by microbial cells including the S-layer, mucopolysaccharides, capsules, *etc.* often provide nucleation sites and possibly a favorable chemical microenvironment for biomineralization. Similarly, Tashiro and Tazaki (1999) have determined that the layer of extracellular polymeric substances (EPS) surrounding microbial cells could act as a template in the formation of iron hydroxides.

Clays are widespread in both terrestrial and marine sediments. Nontronite, an Fe-rich dioctahedral smectite, is a common clay constituent of deep-sea sediments from the Red Sea, Galapagos, Mariana, NE Pacific, and other hydrothermal sites (Cole and Shaw, 1983; Singer *et al.,* 1984; Singer and Stoffers, 1987). Nontronite deposits are found not only near hydrothermal vent openings, but also on the sea-floor where the temperatures range from 2 to 50°C (Juniper and Tebo, 1995). The formation of nontronite may be bio-logically mediated because the morphology of the mineral from the sea-floor is ordered spheroids or tubes of a few μm in diameter, similar to that of microbes (Köhler *et al.,* 1994; Fortin *et al.,* 1998). However, it is difficult to establish if nontronite formation is microbially-mediated or not. Non-microbial mechanisms of nontronite formation have also been reported. From laboratory synthesis experiments, nontronite precipitation is achieved at surface temperatures by nucleation under oxidizing conditions followed by ageing under reducing conditions (Harder, 1976). From observation of deep-sea sediments, nontronite formation mechanisms might include the alteration of volcanic rock fragments and glasses, low-temperature reaction of Fe hydroxides with biogenic silica, and direct precipitation from hydrothermal fluids (Cole and Shaw, 1983).

Electron microscopy was combined with various staining techniques to investigate the role of microbial EPS in the formation of layer-silicate clays. The process of biomineralization is proposed in both chemical and structural terms. In addition, we have examined the synthetic formation of nontronite using a mixture of polysaccharides (dextrin and pectin) with Fe- and Si-rich groundwater.

MATERIAL AND METHODS

Sampling location

Deep-sea sediments in the Iheya Basin. Brownish deep-sea sediments were collected from the Natsushima seamound in the Iheya Basin (27°34.5′N, 127°08.5′E), Middle Okinawa Trough, southwest Japan, during a dive of the deep-sea vessel SHINKAI 2000 (Uyeda, 1987; Kimura *et al.,* 1988). The pH of

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the sediments was 7.1–7.4 and the temperature was 6–7°C (Gamo *et al.,* 1987). The sediments contained nontronite, amorphous silica and Fe oxyhydroxides (Masuda *et al.,* 1987). According to Masuda (1995), the nontronite could have been formed by diagenesis or dissolution-precipitation after deposition of Fe-Si oxides. Some of the same sediments described by Masuda (1995) were used in this investigation. After the sampling, 50 mg of the brownish fine particles were dispersed in 1 mL of distilled de-ionized water. The pH of the suspension was 7.2 and the electrical conductivity (EC) was 0.33 mS/cm.

Groundwater at the Kakuma River. Groundwater flowing through the Onma formation was collected at the Kakuma River in Kanazawa, Japan. Microbial mats composed of amorphous Si-bearing iron hydroxides are found around the outlet of the groundwater (Tashiro and Tazaki, 1999). The pH and the EC ranged from 6.0–6.5 and 0.40 mS/cm (Tashiro and Tazaki, 1999). Bacteria and other microorganisms synthesize extracellular polymeric layers which are thought to be a complex mixture composed primarily of extracellular polysaccharides (Barker *et al.,* 1997). To investigate the possible role of polysaccharides in mineral formation from Fe and Si in the solution, 1000 mL of the groundwater were mixed with 100 mL of a 3, 5 and 10 wt.% aqueous solution of neutral pH polysaccharide (dextrin) or acidic polysaccharide (pectin). The groundwater was not sterilized because amorphous (Fe, Si) oxide particles will precipitate from the solution during sterilization. The mixtures were stirred for two days with a magnetic stirrer. Blank experiments were also carried out in which 1000 mL of the groundwater alone and 1000 mL of distilled de-ionized water were mixed with 100 mL of dextrin solution and stirred. The beakers containing the groundwater or the polysaccharide solution were either sealed using a polyethylene film or left unsealed during stirring. These products were collected by centrifugation and mounted on Cu grids for examination by transmission electron microscopy (TEM).

Characterization methods

X-ray powder diffraction (XRD) analyses were carried out using a Rigaku Rint 1200 system X-ray diffractometer with CuKα radiation generated at 40 kV and 30 mA and scanned at a speed of 1°/min from 2° to 65°2θ. Randomly- and preferentially-oriented samples were prepared for XRD analysis to determine the mineral composition of the sediment. The powdered material was inserted directly into an Al holder and pressed gently to give a random orientation. To obtain preferentially oriented samples, a portion of the sediment suspension was pipetted onto a glass slide and dried. To identify the clay minerals, the samples were

expanded with ethylene glycol and heated at 600°C for 2 h (Moore and Reynolds, 1997).

The suspensions were dispersed ultrasonically, mounted on glass slides, and examined using an optical microscope. Microbial cells in the suspension were stained with 0.01 μg/mL DAPI (4'6-diamidino-2-phenylindole) dye, specific for DNA, and 0.01 wt.% AO (acridine orange) dye, specific for DNA and RNA, using a 0.2 mL capacity dropper (Hobbie *et al.,* 1977; Porter and Feig, 1980). The DAPI- and the AO-stained samples were examined under an epifluorescence microscope equipped with specific filters needed to identify the presence of DNA and RNA. Ruthenium red is a hexavalent cation complex that can bind strongly to acidic polymers (Beveridge, 1989). To 1 mL of the ultrasonically-treated suspension, 0.05 mL of 1 wt.% ruthenium-red solution was added. The stained material was examined using a conventional optical microscope and transmitted light.

After passing the sediment clay suspensions through a Nuclepore® (pore size: 0.6 μmφ) filter, the residues on the filters were freeze dried without prior ultrasonic treatment and examined using a scanning electron microscope (SEM) equipped with energy-dispersive X-ray spectroscopy (EDX). For EDX analysis, the freeze-dried samples were mounted on a stub and were coated with carbon and, in some instances, were coated with gold. The SEM observations were carried out using a JEOL JSM-5200LV SEM operated at an accelerating voltage of 15 kV. Qualitative analyses were performed using a Philips EDAX PV9800 STD EDX attached to the SEM.

Samples with and without the ultrasonic treatment were mounted on a Cu grid for TEM examination. To avoid clumping during drying, excess water was withdrawn from the solution using a filter paper before examination. The ruthenium-red-stained material was also mounted on a Cu grid. The TEM and high-resolution TEM (HRTEM) observations were completed on a JEOL 2000EX TEM operated at accelerating voltages of 100, 120, 160 and 200 kV.

RESULTS

Structural characteristics of minerals on the surface of the sediments

Figure 1 shows the XRD patterns of the oriented sample before any treatment, after exposure to ethylene glycol, and after heating at 600°C for 2 h. The 13.4 Å peak of the untreated specimen (Figure 1A) shifted to 17.2 Å after ethylene glycol treatment (Figure 1B) and to 10.3 Å after heating at 600°C (Figure 1C). The d(060) reflection of the randomly-oriented sample had a spacing of 1.52 Å. These observations indicate that the mineral is a smectite. The d spacings of smectites and the sediment are shown in Table 1.
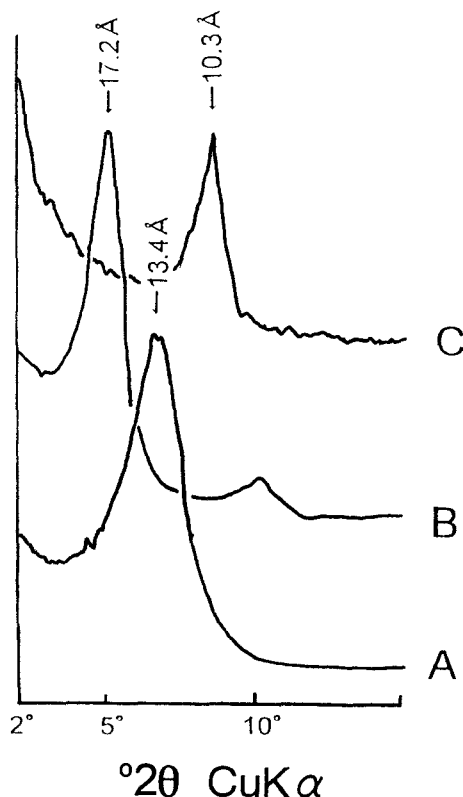
Figure 1. X-ray powder diffraction patterns of nontronite from the Iheya Basin sediments: (A) untreated, (B) after ethylene-glycol treatment, (C) after heating for 2 h at 600°C.

Table 1. The *d*-spacing of smectites and sediment sample.

	060	001	001 (E.G.[1] treatment)
Montmorillonite	1.49–1.50 Å	14–15 Å	17 Å
Beidellite	1.49–1.50 Å	14–15 Å	17 Å
Nontronite	1.52 Å	14–15 Å	17 Å
Saponite	1.52–1.54 Å	14–15 Å	17 Å
Hectorite	1.52–1.54 Å	14–15 Å	17 Å
Stevensite	1.52–1.54 Å	14–15 Å	17 Å
Sediment sample	1.52 Å	14–15 Å	17 Å

[1] E.G.—ethylene glycol.

Surface micromorphology of microbes and minerals on the sediment

Microbial colonies were observed in aggregates obtained from the surface of the sediment using epifluorescence microscopy with DAPI and AO staining (not shown). The aggregates were composed of coccus and rod-shaped microbes a few μm in size. Optical microscopic observation with ruthenium-red staining showed that the aggregates are composed of fibrous, coccus and rod-shaped microbes (not shown). The red stain indicates the presence of acidic polysaccharides. Most of the microbes were stained by ruthenium-red suggesting the presence of acidic polysaccharides (Barker *et al.*, 1997).

Figure 2 shows SEM images of aggregates on the surfaces of the sediments. The surface of the spheroidal aggregates have a honeycomb structure (Figure 2A) and are composed mainly of Si and Fe with small amounts of Mg and Ca (Figure 2B), consistent with the presence of nontronite. Figure 3 shows microbial aggregates composed of coccus and bacillus
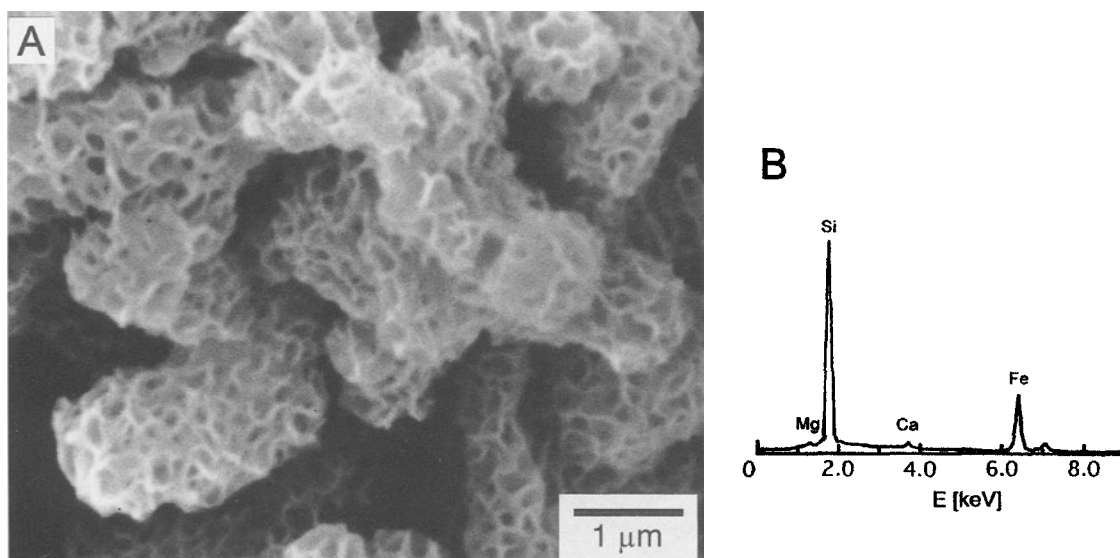

Figure 2. SEM image of nontronite aggregates (A) and the EDX spectrum (B) showing the elemental composition of the honeycomb precipitates.
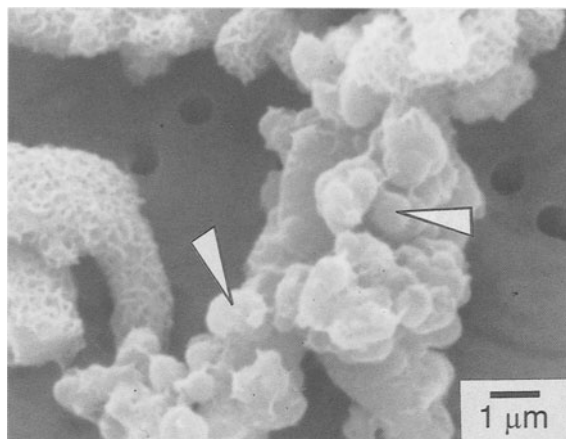
Figure 3. SEM image of microbial cells in the sediments. The presence of filmy materials on the surface of cocci or bacilli microbes of ~1 μm diameter (arrows) suggests a primitive stage of the honeycomb precipitation.
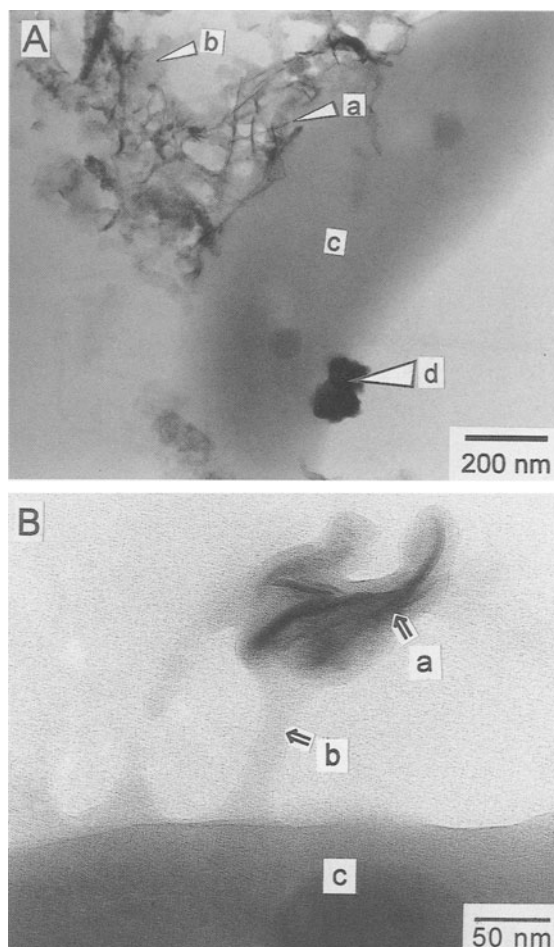

Figure 4. (A) TEM image of microbes obtained from the ultrasonically-treated solution containing (a) linear, extremely thin materials within (b) the EPS (c) exuded from microbial cell, and (d) amorphous hydroxide materials on outer EPS. (B) HRTEM image of microbial cell obtained from the ultrasonically-treated solution showing (a) poorly-developed nontronite particle within (b) the EPS exuded from (c) a microbial cell surface.

covered with a filmy material, suggesting that the precipitates initially form on the surface of microbes.

Mineral formation in the acidic polysaccharides associated with microbial cell surfaces

To remove the honeycomb structure from microbial surfaces, the sediments were ultrasonified for 3 min. Figure 4 shows HRTEM images of the nontronite apparently formed or trapped within the polymeric material exuded from microbial cell walls. Extremely thin nontronite can be seen within the EPS material (Figure 4A) as well as a poorly-ordered nontronite (Figure 4B). In contrast, only rod-shaped particles of amorphous Fe hydroxides were observed outside the EPS. Figure 5 shows nontronite layers oriented in the same direction of elongation as the EPS. Staining by ruthenium-red revealed that the EPS consists of acidic polysaccharides (Figure 5A).

Experimental synthesis of the precursor nontronite using a ferrosiliceous groundwater with polysaccharides

Particles of varied morphology were produced during the synthesis experiments. An SEM/EDX analysis indicates that the particles were mainly composed of Fe and Si. Films and rod-shaped materials were observed in the sealed groundwater system containing >5% polysaccharide. The films were apparently formed within the high dextrin concentration area, whereas the rod-shaped materials were formed outside this area. Layered structures with $d(001)$ spacings of 15.0 and 14.1 Å, similar to smectites (Table 1), were observed in the dextrin films (Figure 6B). In the sealed groundwater system with <3% polysaccharide and in the unsealed groundwater system with polysaccharides, spheroidal Fe particles were formed. The struc-

tures of particles synthesized from groundwater and dextrin or pectin are shown in Table 2.

DISCUSSION

The observations above indicate that the particle morphologies varied due to contact with the atmosphere and polysaccharides. Iron hydroxides were formed around the outlet of the groundwater, whereas nontronite formed in the deep sea. Moreover, the TEM observations of the deep sea sediments indicate that nontronite only formed within the EPS material. These results suggest that nontronite, not Fe-rich hydroxide, forms preferentially within the polymeric materials.
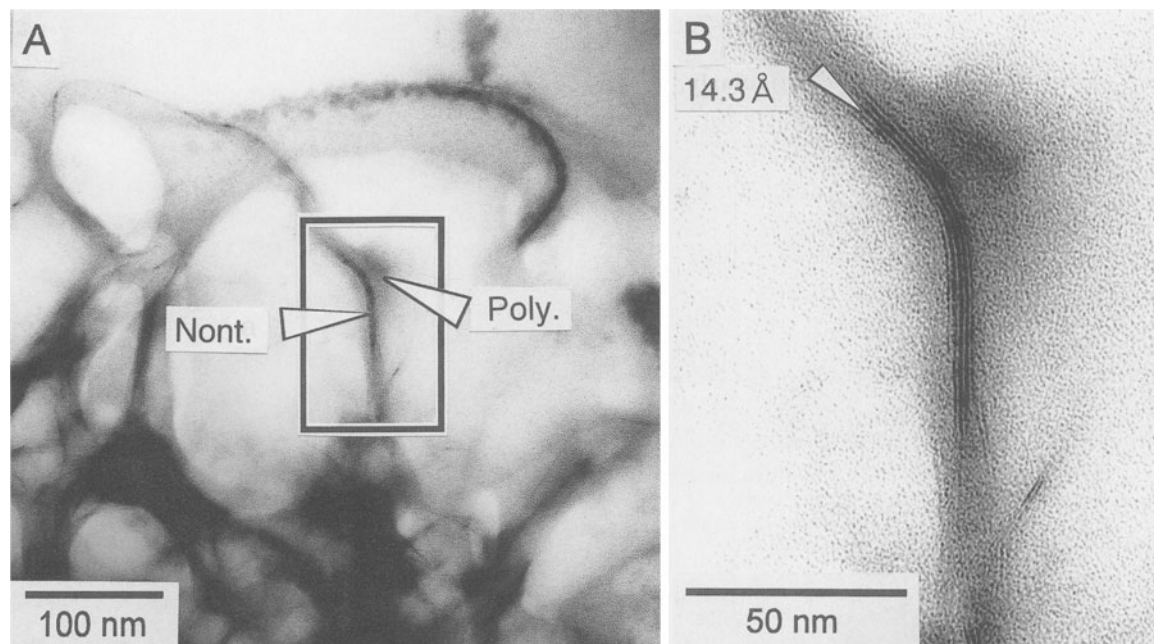
Figure 5.   TEM images of EPS stained with ruthenium red on outer cell wall. (A) The lattice images were aligned in the same direction of elongation as the EPS. (B) Ultrastructure of nontronite with d(001) of 14.3 Å within the EPS. The EPS stained with ruthenium red is composed of acidic polysaccharides.
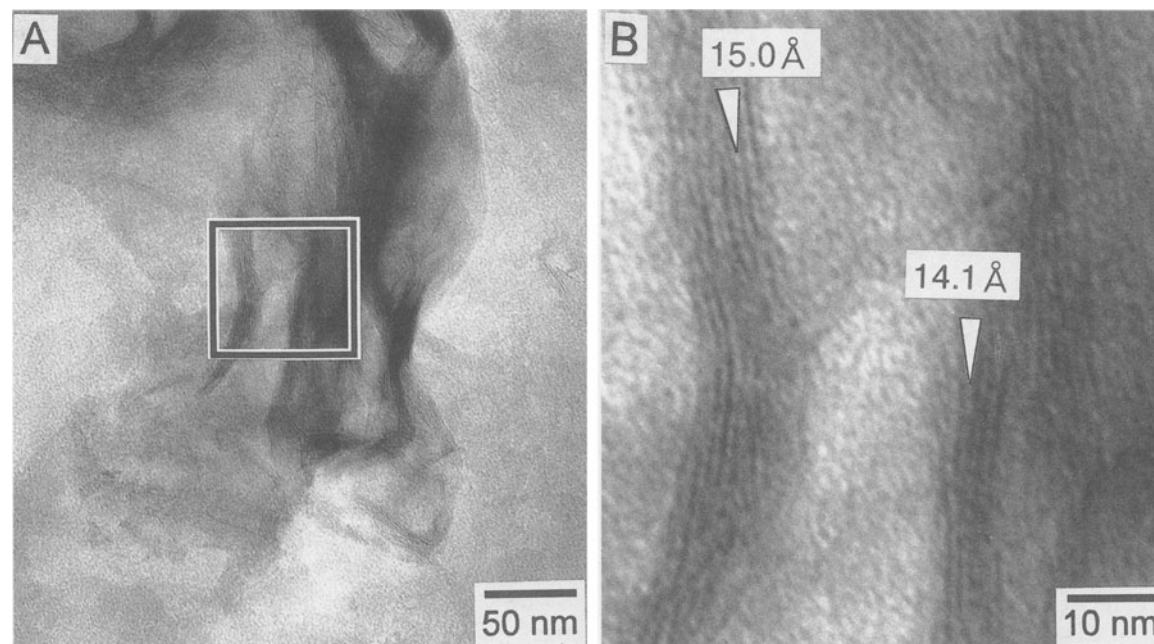

Figure 6.   (A) TEM image of polymeric aggregates synthesized in a mixture of groundwater and 10 wt.% dextrin solution sealed in a polyethylene film after 2 days' ageing showing layer-silicate minerals. (B) High-magnification image of layered materials with d-spacings of 15.0 and 14.1 Å within the aggregates. Note the close similarity to the nontronite formed in the deep-sea sediment (Figure 5).

Table 2. Structures of particles synthesized from groundwater and dextrin.

Added polysaccharide	Concentration wt%	pH	Structure of formed particles	
			Unsealed system	Sealed system
Dextrin	3	7.6	spheroidal	spheroidal
(neutral)	5	7.5	spheroidal	layered
	10	7.3	spheroidal	layered
Pectin	3	6.5	spheroidal	spheroidal
(acidic)	5	6.2	spheroidal	layered

Nontronite formation within extracellular polymeric substances (EPS)

Besides nontronite, TEM observations revealed the presence of amorphous Fe hydroxides (Figure 4d). However, nontronite formed within the EPS on bacterial cells, whereas amorphous Fe hydroxides formed outside the EPS. The EPS consist of several kinds of organic compounds, including polysaccharides, lipids, proteins and organic acids (Barker *et al.*, 1997; Fortin *et al.*, 1997; Sleytr and Beveridge, 1999) that apparently can act as a template for nontronite formation.

Interaction between dissolved silica and organic polysaccharide polymer

The results of the synthesis experiments support the idea that the chain structure of polysaccharides can affect layer silicate orientation. Thus, very fine, oriented particles with lattice images of 15.0 and 14.1 Å were formed within the dextrin films (Figure 6). These particles were not observed in the absence of polysaccharides (dextrin and pectin), when polysaccharide concentrations were low, or under oxidizing conditions.

These two results suggest that in ambient conditions, both the bacterial EPS material and the synthetic polysaccharides give rise to different conditions for crystal growth. Because many organic molecules have a low symmetry (*i.e.* one dimension) relative to alkali halides, crystals formed on organic molecules are sometimes oriented (Aizenberg *et al.*, 1999). Polysaccharide polymers have chain structures which might act as a template for layered silicate formation. If the molecules are oriented, crystals nucleated on the molecules could be also oriented. However, the nucleation mechanism is not clear. In order to clarify the actual interactions between polysaccharide polymers and the crystallization of Fe and silica at the molecular level, further studies such as Si-NMR or XAFS will be needed.

Some organic substances may act as inhibition agents for mineralization or degradation of minerals. Organic acids and humic substances can inhibit the formation of short-range ordered aluminosilicates (allophane, imogolite) (Inoue and Huang, 1984, 1990). Mineral weathering can be accelerated in the presence of organic acids (Ueshima and Tazaki, 1998; Welch *et al.*, 1999) or siderophores (Liermann *et al.*, 2000). In contrast, acting as an 'ionic binder' can be considered another possible role for organic substances. Polymeric molecules seem particularly favorable for ion accumulation because of their high surface area. Bacterial cells composed of peptidoglycan can act as a nucleation site for mineralization (Ferris *et al.*, 1986; Urrutia and Beveridge, 1993, 1994, 1995). Moreover, Urrutia and Beveridge (1993) suggested a cation bridging mechanism in which multivalent metal cations complex with organic fabric (*e.g.* COO–) that in turn bridges with ionic silicates to form large aggregates.

In the case of an interaction between clays and biofilms for particle binding, mica grains were precipitated on the outer bacterial cell wall with a tangential orientation which suggests that metal cations may have served as cation bridges (Konhauser *et al.*, 1998). Theng and Orchard (1995) also suggested that multivalent cations may have served as cation bridges in the interaction between clays and microbial EPS. As a result of this study, we now believe it is possible that such cation bridging of Fe and Si at the molecular level might also mediate layered silicate formation.

Nontronite can form in the presence of $Fe(OH)_2$ and/or $Mg(OH)_2$ under strict reducing conditions (Eh = −600, −200 mV) at 3 and 20°C (Harder, 1976, 1978), or by ageing of coprecipitated gels of silica and $FeSO_4$ under initially reducing, then oxidizing conditions (Decarreau and Bonin, 1986; Decarreau *et al.*, 1987). In this study, however, the Eh was initially −60 mV and then it was changed to 250 mV for 2 days. The polysaccharides may act as a catalyst for nontronite formation.

The groundwater from the Onma formation contains 23 ppm Fe and 16 ppm dissolved silica. However, no nontronite was found in submerged sediments and microbial mats at the groundwater outlet, only amorphous Si-bearing Fe hydroxides were found (Tashiro and Tazaki, 1999). The question arises as to why nontronite does not form in terrestrial water systems. In this instance, the formation of Fe hydroxides can be mediated by Fe-oxidizing microbes (*Toxothrix* sp. and *Gallionella ferruginea, etc.*). Here, the ferrous ions may be preferentially oxidized to layered silicates by binding to the polysaccharide polymers. Nontronite formation would require sufficient time as well as a template to orient the tetrahedral silicate sheet on the polysaccharide polymers. Near hydrothermal vents in the deep sea, Fe was present as oxides, carbonates, sulfides/sulfates, and smectite-type phyllosilicates (Badaut *et al.*, 1992; Köhler *et al.*, 1994; Juniper and Tebo, 1995). On the basis of our investigation, polysaccharide molecules of microbial origin may be involved in the formation of Fe-rich layer silicates.

CONCLUSIONS

This study suggests the mediation of microbial poly-saccharides in biomineralization. The major conclusions of our investigation can be summarized as follows: (1) Deep-sea ferrisiliceous sediments from the Iheya Basin, Okinawa Trough, Japan contain nontronite and microbes. The microbes exude extracellular polymeric substances (EPS) that are mainly composed of acidic polysaccharides. Nontronite layers appeared to form and grow within the EPS materials, whereas amorphous Fe hydroxides formed outside the EPS. We propose that EPS served as a template for layer silicate synthesis. (2) Iron-rich layer silicates also form in a stirred mixture of dextrin or pectin and ferrosiliceous groundwater solution. The Fe-rich layer silicates occur within the polysaccharide (dextrin and pectin) materials, whereas rod-shaped or spheroidal Si-bearing Fe hydroxides are observed on the outside of the poly-saccharide materials. Microbial polysaccharides possibly have a role in layer silicate formation.

ACKNOWLEDGMENTS

REFERENCES

Aizenberg, J., Black, A.J. and Whitesides, G.M. (1999) Control of crystal nucleation by patterned self-assembled monolayers. *Nature*, **398**, 495–498.

Akai, J., Akai, K., Ito, M., Nakano, S., Maki, Y. and Sasagawa, I. (1999) Biologically induced iron ore at Gunma iron mine, Japan. *American Mineralogist*, **84**, 171–182.

Badaut, D., Decarreau, A. and Besson, G. (1992) Ferri-pyrophyllite and related Fe^{3+}-rich 2:1 clays in recent deposits of Atlantis II Deep, Red Sea. *Clay Minerals*, **27**, 227–244.

Barker, W.W. and Banfield, J.F. (1996) Biologically versus inorganically mediated weathering reactions: relationships between minerals and extracellular microbial polymers in lithobiontis communities. *Chemical Geology*, **132**, 55–69.

Barker, W.W., Welch, S.A. and Banfield, J.F. (1997) Geomicrobiology of silicate mineral weathering. Pp. 391–428 in: *Geomicrobiology: Interactions between Microbes and Minerals* (J.F. Banfield and K.H. Nealson, editors). Reviews in Mineralogy, **35.** Mineralogical Society of America, Washington, D.C.

Barker, W.W., Welch, S.A., Chu, S. and Banfield, J.F. (1998) Experimental observation of the effect of bacteria on aluminosilicate weathering. *American Mineralogist*, **83**, 1551–1563.

Beveridge, T.J. (1989) The structure of bacteria. Pp. 1–65 in: *Bacteria in Nature: a Treatise on the Interaction of Bacteria and their Habitats* (E.R. Leadbetter. and J.S. Poidexter, editors). Plenum, New York.

Cole, T.G. and Shaw, H.F. (1983) The nature and origin of authigenic smectites in some recent marine sediments. *Clay Minerals*, **18**, 239–252.

Decarreau, A. and Bonnin, D. (1986) Synthesis and crystallogenesis at low temperature of Fe(III)-smectites by evolution of coprecipitated gels: Experiments in partially reducing conditions. *Clay Minerals*, **21**, 861–877.

Decarreau, A., Bonnin, D., Badaut-Trauth, D., Couty, R. and Kaiser, P. (1987) Synthesis and crystallogenesis of ferric smectite by evolution of Si-Fe coprecipitates in oxidizing conditions. *Clay Minerals*, **22**, 207–223.

Ferris, F.G., Beveridge, T.J. and Fyfe, W.S. (1986) Iron-silica crystallite nucleation by bacteria in a geothermal sediment. *Nature*, **320**, 609–611.

Fisk, M.R., Giovannoni, S.J. and Thorseth, I.H. (1998) Alteration of oceanic volcanic glass: Textural evidence of microbial activity. *Science*, **281**, 978–980.

Fortin, D., Davis, B. and Beveridge, T.J. (1996) Role of *Thiobacillus* and sulfate-reducing bacteria in iron biocycling in oxic and acidic mine tailings. *FEMS Microbiology Ecology*, **21**, 11–24.

Fortin, D., Ferris, F.G. and Beveridge, T.J. (1997) Surface-mediated mineral development by bacteria. Pp. 161–180 in: *Geomicrobiology: Interactions between Microbes and Minerals* (J.F. Banfield and K.H. Nealson, editors). Mineralogical Society of America.

Fortin, D., Ferris, F.G. and Scott, S.D. (1998) Formation of Fe-silicates and Fe-oxides on bacterial surfaces in samples collected near hydrothermal vents on the Southern Explorer Ridge in the northeast Pacific Ocean. *American Mineralogist*, **83**, 1399–1408.

Gamo, T., Ishibashi, J., Sakai, H., Kodera, M., Igarashi, G., Ozima, M., Akagi, T. and Masuda, A. (1987) Geochemistry of hydrothermal solutions in the Okinawa Trough: Report on Dive of the SHINKAI 2000. *JAMSTECTR Deepsea Research*, 213–224 (in Japanese with an English abstract).

Harder, H. (1976) Nontronite synthesis at low temperatures. *Chemical Geology*, **18**, 169–180.

Harder, H. (1978) Synthesis of iron layer silicate minerals under natural conditions. *Clays and Clay Minerals*, **26**, 65–72.

Hobbie, J.E., Daley, R.J. and Jasper, S. (1977) Use of Nuclepore Filters for counting bacteria by fluorescence microscopy. *Applied and Environmental Microbiology*, **33**, 1225–1228.

Inoue, K. and Huang, P.M. (1984) Influence of citric acid on the natural formation of imogolite. *Nature*, **308**, 58–60.

Inoue, K. and Huang, P.M. (1990) Perturbation of imogolite formation by humic substances. *Soil Science Society of America Journal*, **54**, 1490–1497.

Jongmans, A.G., van Breemen, N., Lundström, U., van Hees, P.A.W., Finlay, R.D., Srinivasan, M., Unestam, T., Giesler, R., Melkerud, P.-A. and Olsson, M. (1997) Rock-eating fungi. *Nature*, **389**, 682–683.

Juniper, S.K. and Tebo, M. (1995) Microbe-metal interactions and mineral deposition at hydrothermal vents. Pp. 219–253 in: *The Microbiology of Deep-sea Hydrothermal Vents* (D.M. Karl, editor). CRC Press, New York.

Kimura, M., Uyeda, S., Kato, Y., Tanaka, T., Yamano, M., Gamo, T., Sakai, H., Kato, S., Izawa, E. and Oomori, T. (1988) Active hydrothermal mounds in the Okinawa Trough backarc basin, Japan. *Tectonophysics*, **145**, 319–324.

Köhler, B., Singer, A. and Stoffers, P. (1994) Biogenic nontronite from marine white smoker chimneys. *Clays and Clay Minerals*, **42**, 689–701.

Konhauser, K.O., Fisher, Q.J., Fife, W.S., Longstaffe, F.J. and Powell, M.A. (1998) Authigenic mineralization and detrital clay binding by freshwater biofilms: the Brahmani River, India. *Geomicrobiology Journal*, **15**, 209–222.

Lierman, L.J., Karinowski, B.E., Brantley, S.L. and Ferry, J.G. (2000) Role of bacterial siderophores in dissolution of

hornblende. *Geochimica et Cosmochimica Acta,* **64,** 587–602.

Masuda, H. (1995) Iron-rich smectite formation in the hydrothermal sediment of Iheya Basin, Okinawa Trough. Pp. 509–521 in: *Biogeochemical Processes and Ocean Flux in the Western Pacific* (H. Sakai and Y. Nozaki, editors). Terra Scientific Publishing Company, Tokyo.

Masuda, H., Ishibashi, J., Kato, Y., Gamo, T. and Sakai, H. (1987) Oxygen isotope ratio and trace element composition of hydrothermal sediments from Okinawa Trough, collected with SHINKAI 2000, Dive 231. *JAMSTECTR Deepsea Research,* 225–231 (in Japanese with an English abstract).

Moore, D.M. and Reynolds, R.C. (1997) *X-ray Diffraction and the Identification and Analysis of Clay Minerals. 2nd edition.* Oxford University Press, Oxford, New York, 378 pp.

Newman, D.K., Ahmann, D. and Morel, F.M.M. (1998) A brief review of microbial arsenate respiration. *Geomicrobiology Journal,* **15,** 255–268.

Porter, K.G. and Feig, Y.S. (1980) The use of DAPI for identifying and counting aquatic microflora. *American Society of Limnology and Oceanography,* **25,** 943–948.

Schultze-Lam, S., Ferris, F.G., Sherwood-Lollar, B. and Gerits, J.P. (1996a) Ultrastructure and seasonal growth patterns of microbial mats in a temperate climate saline-alkaline lake: Goodenough Lake, British Columbia, Canada. *Canadian Journal of Microbiology,* **42,** 147–161.

Schultze-Lam, S., Fortin, D. and Beveridge, T.J. (1996b) Mineralization of bacterial surfaces. *Chemical Geology,* **132,** 171–181.

Schultze-Lam, S., Harauz, G. and Beveridge, T.J. (1992) Participation of a cyanobacterial S-layer in fine-grain mineral formation. *Journal of Bacteriology,* **174,** 7971–7981.

Singer, A. and Stoffers, P. (1987) Mineralogy of a hydrothermal sequence in a core from the Atlantis II Deep, Red Sea. *Clay Minerals,* **22,** 251–267.

Singer, A., Stoffers, P., Hellar-Kallai, L. and Szafranek, D. (1984) Nontronite in a deep sea core from South Pacific. *Clays and Clay Minerals,* **32,** 375–383.

Sleytr, U.B. and Beveridge, T.J. (1999) Bacterial S-layers. *Trends in Microbiology,* **7,** 253–259.

Tashiro, Y. and Tazaki, K. (1999) The primitive stage of microbial mats comprising iron hydroxides. *Earth Science,* **53,** 27–35 (in Japanese with an English abstract).

Tazaki, K. (1997) Biomineralization of layer silicates and hydrated Fe/Mn oxides in microbial mats: An electron microscopical study. *Clays and Clay Minerals,* **45,** 203–212.

Tazaki, K. (1999) Architecture of biomats reveals history of geo-, aqua-, and bio-systems. *Episodes,* **22,** 21–25.

Theng, B.K.G. and Orchard, V.A. (1995) Interactions of clays with microorganisms and bacterial survival in soil: a physicochemical perspective. Pp. 123–143 in: *Environmental Impact of Soil Component Interactions—Metals, other Inorganics, and Microbial Activities, Volume II* (P.M. Huang, J. Berthelin, J.-M. Bollag, W.B. McGill and A.L. Page, editors). Lewis Publishers, CRC Press, Florida.

Ueshima, M. and Tazaki, K. (1998) Bacterial bioweathering of K-feldspar and biotite in granite. *Journal of the Clay Science Society of Japan,* **38,** 68–82 (in Japanese with an English abstract).

Urrutia, M.M. and Beveridge, T.J. (1993) Mechanism of silicate binding to the bacterial cell wall in *Bacillus subtilis. Journal of Bacteriology,* **175,** 1936–1945.

Urrutia, M.M. and Beveridge, T.J. (1994) Formation of fine-grained metal and silicate precipitates on a bacterial surface *(Bacillus subtilis). Chemical Geology,* **116,** 261–280.

Urrutia, M.M. and Beveridge, T.J. (1995) Formation of short-range ordered aluminosilicates in the presence of a bacterial surface *(Bacillus subtilis)* and organic ligands. *Geoderma,* **65,** 149–165.

Uyeda, S. (1987) Active hydrothermal mounds in the Okinawa back-arc trough. *EOS, Transactions American Geophysical Union,* **68,** 737.

Welch, S.A., Barker, W.W. and Banfield, J.F. (1999) Microbial extracellular polysaccharides and plagioclase dissolution. *Geochimica et Cosmochimica Acta,* **63,** 1405–1419.