In situ silicification of an Icelandic hot spring microbial mat: implications for microfossil formation

S. Schultze-Lam, F.G. Ferris, K.O. Konhauser, and R.G. Wiese

Abstract: Transmission electron microscopy and energy-dispersive x-ray analysis revealed that filamentous phototrophic bacteria resembling *Chloroflexus aurantiacus* underwent rapid silicification in an Icelandic hot spring microbial mat. The mineralization associated with the cells occurred both extracellularly, within and on the external sheaths of the bacteria, and intracellularly, within the cytoplasm. The exceptional preservation of the bacterial sheaths is due to the presence of distinct mineral nucleation sites. This results in the production of silica casts of the bacteria, which bear a striking resemblance to microbial remains in ancient microfossil assemblages.

Résumé : Les analyses par microscopie électronique à transmission et par l'énergie dispersive des rayons-x ont révélé que les bactéries filamenteuses phototrophes, ressemblant à *Chloroflexus aurantiacus*, ont subi une silicification rapide au sein d'un tapis microbien de source thermale, en Islande. La minéralisation associée aux cellules se présente sous forme extracellulaire, à l'intérieur de et sur la membrane externe des bactéries, et sous forme intracellulaire, à l'intérieur du cytoplasme. La préservation exceptionnelle de la membrane des bactéries est due à la présence de sites de nucléation minérale dispersés. Le résultat est la production des moulages siliceux des bactéries, affichant une ressemblance remarquable avec les vestiges microbiens d'anciens assemblages microfossiles.
[Traduit par la rédaction]

Introduction

Fossil evidence of the Earth's earliest biosphere shows that complex communities of microorganisms were abundant and well distributed throughout the Precambrian Era. Among the richest beds of microbial fossils are those found in the Gunflint Iron Formation north of Lake Superior in Canada (Barghoorn and Tyler 1965), the early Archaean Apex chert of Australia (Schopf and Packer 1987; Schopf 1993), and the Riphean section of the Anabar uplift in Russia (Sergeev 1993); all ranging in age from 0.8 to 3.5 Ga. The distribution and types of microorganisms preserved as cellularly intact microfossils are remarkably similar to those in modern microbial mats, in which organic-rich layers formed by cyanobacteria and (or) other types of filamentous bacteria alternate with layers that are comprised of detrital inorganic sediment and authigenic mineral precipitates (Morse and Mackenzie 1990). These benthic microbial communities are considered to be analogous, and possibly homologous, with those that were preserved billions of years ago as stromatolites in silicified carbonates and in cherts. Although ancient microbial mats have been found to originate in a wide variety of aquatic environments, the microorganisms composing them were seldom preserved as structurally intact cells. Studies by Oehler and Schopf (1971) and Ferris et al.(1988) have shown that cellular preservation occurs through silicification. Because the highest concentrations of dissolved silica in modern-day aqueous surface environments are found in hot springs, it is likely that some microfossils developed in similar environments (Barghoorn and Tyler 1965; Oehler and Schopf 1971; Southgate 1986). Moreover, microbial mats can often be found lining the sides of pools and outflow channels of modern hot springs. The main mat-forming organisms are usually filamentous bacteria, which include cyanobacteria and other phototrophic bacteria that often have associated mineral precipitates (Ferris et al. 1986, 1987).

In this report, we provide a detailed description of in situ silicification and preservation of filamentous bacteria in a hot spring microbial mat community. Although silicification of unicellular bacteria has been studied previously in a laboratory setting (Oehler and Schopf 1971; Ferris et al. 1988; Urrutia and Beveridge 1993), the in situ silicification of a benthic microbial mat community has, to our knowledge, never been described before on a subcellular level. This high degree of resolution has allowed us to gain a unique insight into the processes that were probably responsible for the preservation of ancient microbial communities as microfossils.

Materials and methods

Samples of microbial mats were collected from an outflow channel of the geyser, Strokkur, on the Haukadalur Plain in

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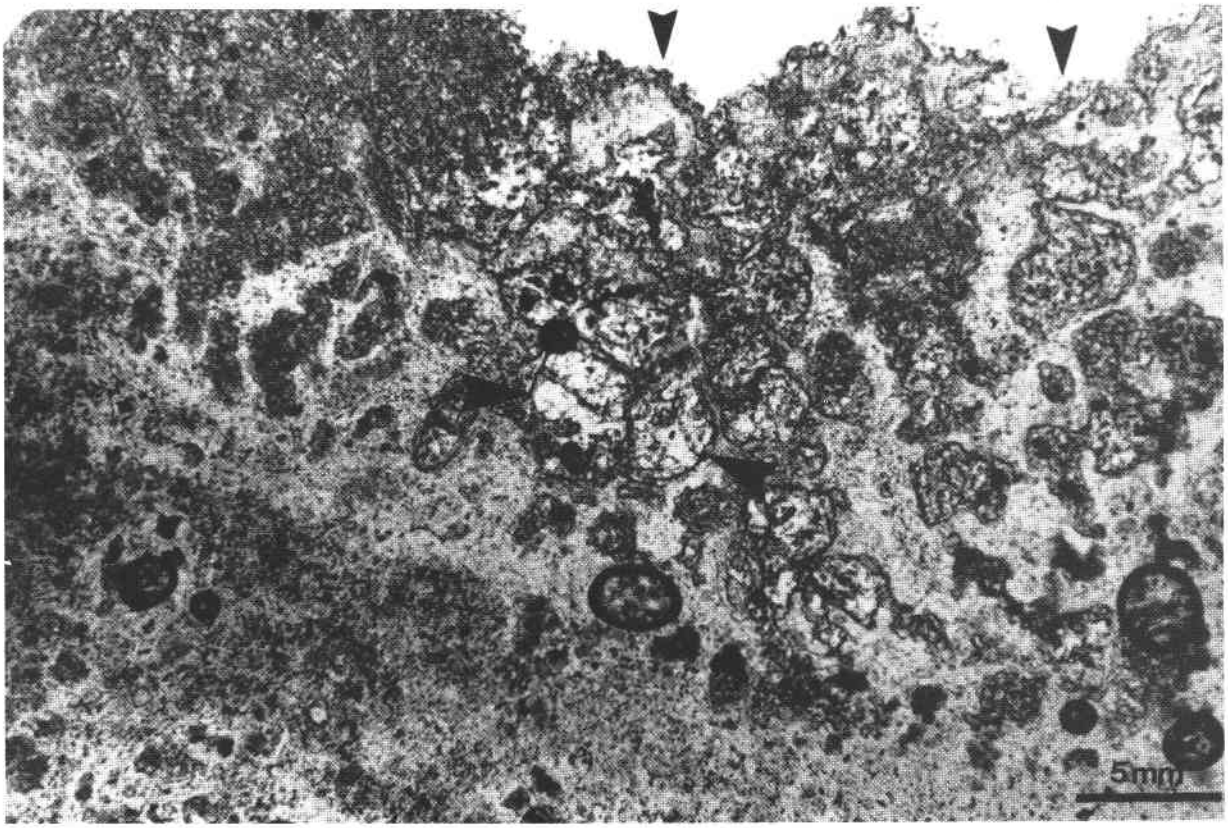
Fig. 1. Phase-contrast light micrograph of cross-sectioned mat. The darker areas at the top correspond to the higher proportion of organic material present in the form of *Chloroflexus* filaments. With depth, increasing silicification leads to a lighter appearance. Numerous cavities within the mat are spanned by bacterial filaments (arrowheads).



South Iceland, where they were observed growing as hard, finely laminated crusts on ledges within the channel. Upon collection, mat samples were fixed in aqueous glutaraldehyde (2% v/v final concentration) using spring water as the diluent.

In the laboratory, the mat samples were prepared for transmission electron microscopy (TEM) by dehydration in a graded series of ethanol solutions (25, 50, 75, and 100%) followed by 50:50 ethanol:acetone and 100% acetone (to increase miscibility with the embedding resin). All steps were carried out at room temperature for 15 min each. The samples were then left overnight in a mixture of 50:50 acetone : Epon 812 epoxy resin (Can-em) followed by immersion in 100% resin and polymerization at 60°C for 48 h. Thin sections (60 nm) were cut with a diamond knife on a Ultracut E ultramicrotome (Reichert-Jung) and collected on 200 mesh Formvar- and carbon-coated copper specimen grids (Marivac). Thin sections were viewed in a Philips EM300 transmission electron microscope, while elemental analysis of mineralized cells was performed by energy dispersive x-ray spectroscopy (EDS) using a Philips EM400 electron microscope equipped with a model LZ-5 light element detector and an exL multichannel analyzer (both from Link Analytical). To assess their degree of crystallinity, thin sections of the mineral precipitates were also subjected to selected area electron diffraction (SAED). The accelerating voltage was 100 keV with a beam current of 0.1 μA. Typical counting times for collection of EDS spectra were 100 s (live time).

For scanning electron microscopy (SEM), fixed samples were dehydrated in ethanol, as described earlier and freeze-dried in a carbon dioxide bomb. SEM was performed using a Hitachi S570 scanning electron microscope operating at 15 keV. Samples of mat material were also examined as cross sections by phase contrast light microscopy in order to gain an overall understanding of their structural organization.

Results

Examination of microbial mat cross sections by light microscopy revealed the structural organization of the mat; the predominantly darker colour of the top layers of the mat corresponded to a greater proportion of organic material than was present in the lower (older) more highly lithified layers (Fig. 1). This organic material consisted of abundant microbial filaments, approximately 1 μm in diameter, which made up the mat fabric. Numerous cavities were visible in the mat and were traversed by the filaments (Fig. 1). Detailed examination of filaments by phase microscopy, together with their appearance by TEM (describedbelow) indicated that this organism was likely a member of the bacterial genus *Chloroflexus*; an anaerobic bacterium that uses photosynthesis as its means of energy production and is typically found in hot springs.

Direct examination of *Chloroflexus* filaments in the Strokkur mat by SEM revealed that the filaments were randomly oriented and covered in abundant spherical precipitates, which were frequently seen to completely encrust the filaments

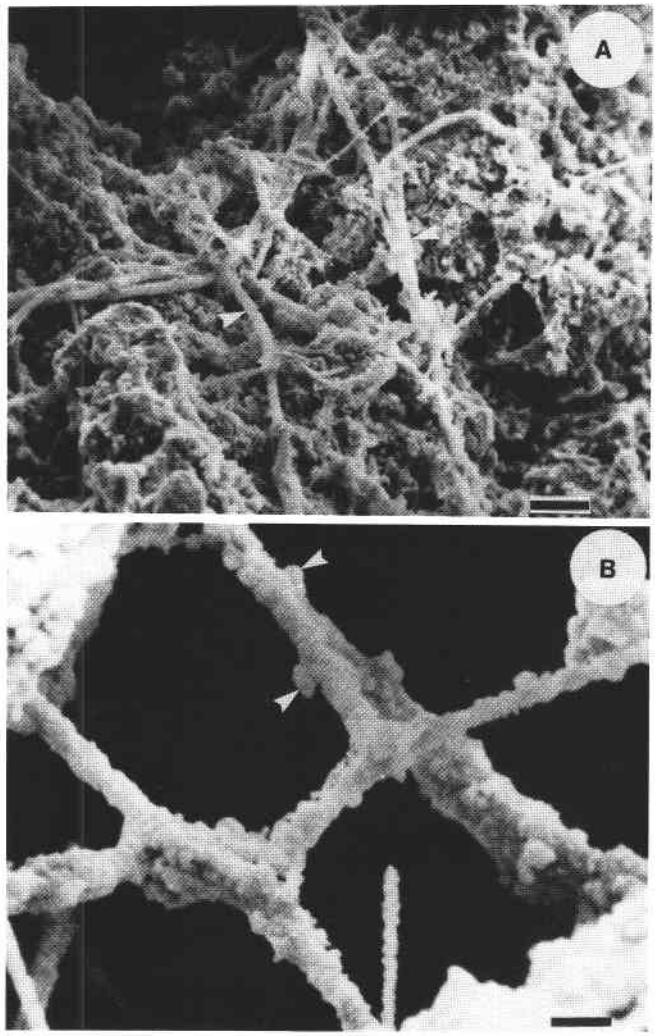
(Figs. 2A, 2B). TEM further allowed the cellular architecture and progressive mineralization of the *Chloroflexus* filaments to be seen (Figs. 3A–3D). The bacteria grew as filaments, which consisted of a long chain of cells growing within a cylindrical, tube-like sheath made up of fine, intertwined fibres. In cells relatively free from mineralization it could be seen that the intracellular membranes typical of cyanobacteria (which they otherwise resemble) were lacking (Fig. 3A). Instead, structures resembling the photosynthetic vesicles called chlorosomes found in the green bacteria, including *Chloroflexus*, were visible, confirming our identification of this organism.

The formation of spherical silica precipitates by the bacteria was observed both extracellularly, within and on the sheath exterior, and intracellularly, within the cytoplasm of the cells. The precipitates that formed in the cytoplasm (Fig. 3B) were considerably smaller than those that formed in the sheath, most likely due to physical constraints imposed by the density and structure of the cytoplasmic material. In many filaments, the silica crystallites appeared to merge (Fig. 3C) so that individual grains were no longer distinguishable (Fig. 3D). In all cases the initial precipitates had a spherical morphology and consisted of amorphous silica, as indicated by EDS (Fig. 4) and SAED. Eventually, of the original organic framework on which silica was precipitated, only the sheath remained recognizable. In these later stages, as the remains of cells became completely embedded, cytoplasmic and wall structure was lost, leaving behind a "cast" of the bacterial filament similar to those that exist in known microfossil assemblages.

Discussion

The dominant microorganisms in the Strokkur channel mats were filamentous bacteria closely resembling the green phototrophic bacterium, *Chloroflexus aurantiacus* (an anaerobic organism). These bacteria are commonly found in many hot spring environments, including the well-studied geyser pools of Yellowstone National Park, where they constitute the primary structural fabric of the mats (Doemel and Brock 1977). The filamentous microorganisms that dominate ancient microfossil assemblages are often identified as cyanobacteria, which are also phototrophic bacteria but with an aerobic phototrophic metabolism similar to that of higher plants and algae. However, on a general morphological level, especially when finer cytoplasmic details have been lost as a result of lithification (as in microfossils), green bacteria could easily be mistaken for cyanobacteria. The main ultrastructural difference between these two types of phototrophic prokaryotes is that *Chloroflexus* sp. have a smaller filament diameter and a simpler intracellular fine structure than cyanobacteria. In addition, the green bacteria belong to a more deeply rooted phylogenetic group than cyanobacteria and may represent a more ancient line of phototrophic prokaryotes (i.e., bacteria). It may be that our familiarity with the predominance of cyanobacteria in modern environments has led to a bias in the identification of an inordinately high number of microfossil organisms as cyanobacteria. This possibility has recently been addressed by Schopf (1993), who, after a systematic classification of organisms found in ancient microfossil assemblages, concluded that approximately 37% of the filamentous organisms in the early Archaean Apex chert were "probable bacteria" that resemble *Chloroflexus*, while the remaining 63% were "probable cyanobacteria" (Schopf 1993). Prior to Schopf's study, the green bacteria were largely ignored in considerations of microbial paleotaxonomy.

Fig. 2. (A) Scanning electron microscopic image showing the general topology of the Strokkur mat. Numerous *Chloroflexus* filaments can be seen traversing the mat surface (arrowheads). Scale bar = 10 μm. (B) Scanning electron micrograph showing a magnified view of the *Chloroflexus* filaments. Abundant silica crystallites (arrowheads) can be seen encrusting the filaments. Scale bar = 5 μm.



In the Strokkur geyser outflow channel, at the site of sample collection, the pH of the water varies with discharge from 8.3 to 9.3 and the temperature is 58°C. Dissolved silica is present at levels of approximately 476 ppm and should readily precipitate as amorphous SiO_2 under these conditions (Stumm and Morgan 1981). However, an important consideration raised by Reinhart (1980) is that, despite the high levels of dissolved Si in geyser waters, precipitates do not spontaneously form unless nucleation sites are provided for their deposition. The small size (approximately 1 μm diameter) and the presence of multiple reactive sites on bacterial surfaces allows these organisms to serve as heterogene-

Fig. 3. Transmission electron microscopic images of 60 nm thin sections of mat samples from the Strokkur geyser outflow channel showing progressive stages in the mineralization of *Chloroflexus* filaments. (A) Spherical silica crystallites (arrowheads) have formed at discrete sites within the sheath (S) itself as shown by the presence of holes (H) left behind as mineral grains were torn away during sectioning of the sample. Note that at this stage, no mineral precipitates are present on or within the cells (star) and structures resembling chlorosomes are visible as roughly ovoid structures immediately within the cell wall. (B) Silicification of the cell interior has occurred; an important prerequisite for preservation of cellular structure in the geological record (S and H designated as in A). (C) As silicification progresses, the round precipitates appear to merge, forming a matrix of silica in which the sheath (S) is embedded. Here, and in D, the silica matrix has shattered during sectioning, giving it a fractured appearance. (D) Eventually, all that remains is a sheath within a matrix of amorphous silica (in this case the sheath is seen in cross section rather than in longitudinal section as in A–C). Scale bar for all panels = 1 μm.

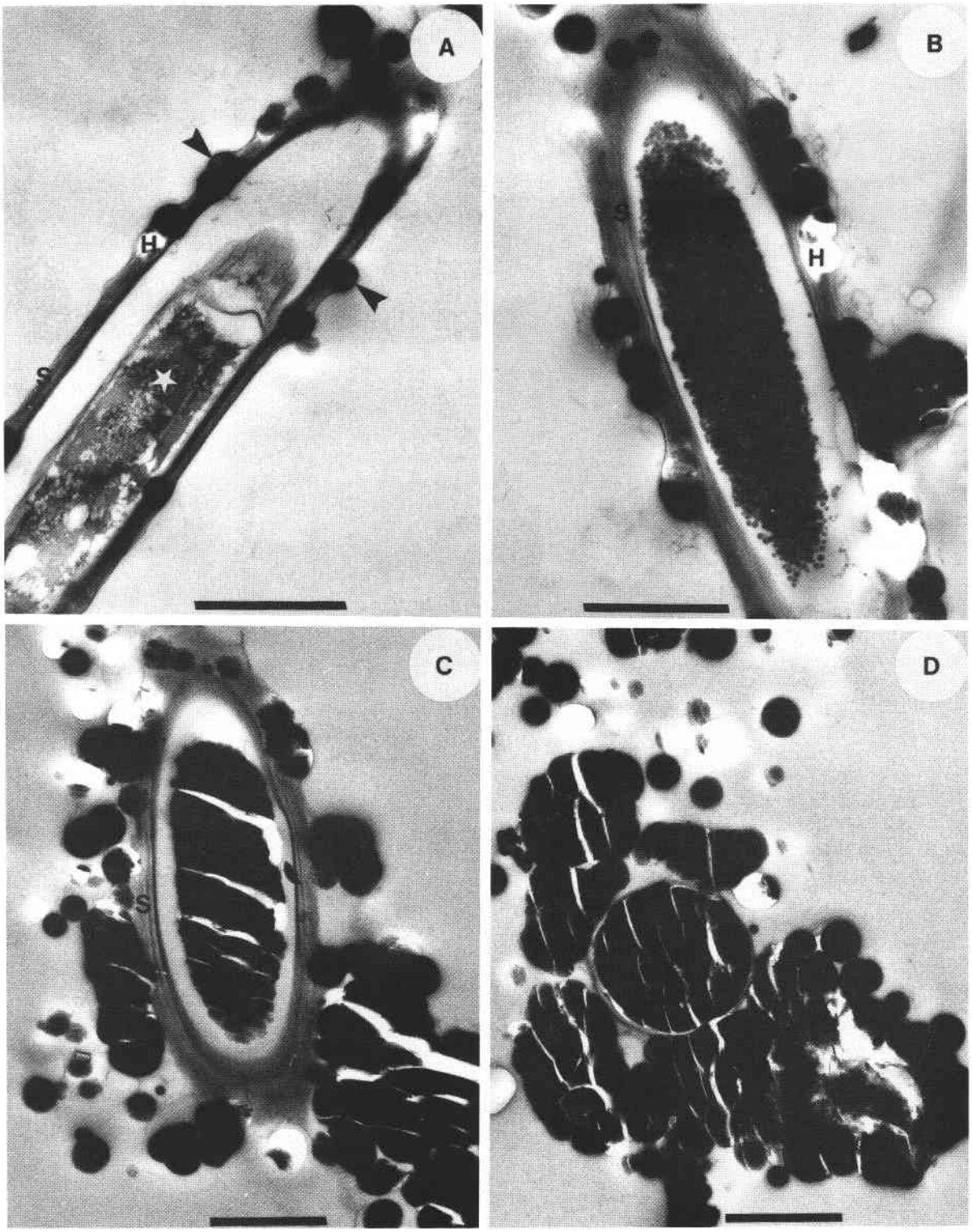
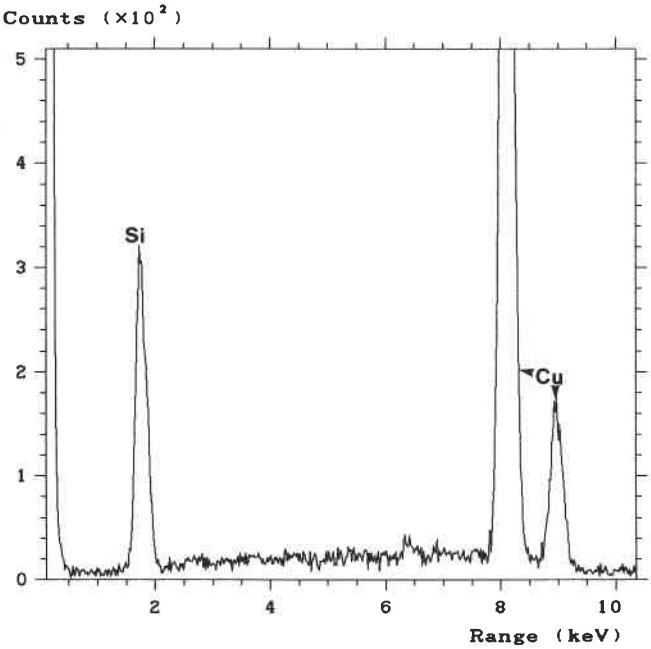


Fig. 4. Energy dispersive x-ray spectrum showing the elemental composition of the precipitates associated with *Chloroflexus* filaments in the Strokkur mat. At all stages of mineralization (as shown in Fig. 3) the precipitates consisted of SiO_2 with no other elements present at detectable levels. The Cu signal is due to the presence of the copper specimen support grid.



ous nucleation templates for mineral formation (Beveridge 1989; Schultze-Lam et al. 1992). The essential role of these ubiquitous organisms as nucleation sites for mineral deposition has been demonstrated in many diverse environments including hot springs (Ferris et al. 1986, 1987; Beveridge 1989; Schultze-Lam et al. 1993). Moreover, previous studies on the precipitation of silicate minerals by bacteria have shown that the association between bacterial walls and silica occurs via hydrogen bonding between wall hydroxyl groups and polysilicic acid. Silica crystallites are formed by subsequent hydrolysis and polymerization of the bound silicic acid (Rinehart 1980; Urrutia and Beveridge 1993).

Based on our observations, we believe that silica is initially precipitated on the outer surface and within the fibrous matrix of the extracellular sheath. It is likely that mineral formation remains localized in the sheath until after the cells' death, since it is reasonable to assume that the presence of abundant mineral precipitates on or in the cell itself would be a serious detriment to the physiological activities of the cell. The sheath is made of a network of intertwined fibres, and it is possible that while the cells remain metabolically active, mineralized sheath material is shed and replaced by new material in order to avoid interference of mineral precipitates with ion exchange reactions that are important for metabolism. Such shedding behaviour has been noted for the proteinaceous S-layer of the unicellular cyanobacterium, *Synechococcus* GL24, which becomes encrusted with calcite ($CaCO_3$) in its natural lake water habitat (Thompson et al. 1990; Schultze-Lam et al. 1992).

The silica precipitates formed within the cells and on the sheath surrounding the filaments as well as within the fibrous sheath material itself, distending it somewhat (see Fig. 3). This structural perturbation of the sheath, together with the recognition of intracellular mineralization, has not previously been observed. Bacterial mineralization in other environments seems to be limited to epicellular mineral formation, implying that nucleation occurs on surface-exposed sites only (Beveridge 1989; Schultze-Lam et al. 1993). However, in the Strokkur mat, the cells were mineralized not only externally but also internally, the latter being an important prerequisite for the preservation of filamentous cellular morphology within a mineral matrix.

The observation of silicification in a natural environment is of paramount significance to taphonomic studies of microfossils and represents an important link between observational studies of ancient microfossil assemblages and laboratory experiments of microbial silicification. We have found that, while in situ silicification of filamentous green bacteria occurs rapidly, little or no structural detail of the cytoplasm is preserved. Rapid silicification is regarded as a prerequisite to preservation of cell structure as a microfossil. Furthermore, in such an advanced stage of mineralization the sheaths of green bacteria like *Chloroflexus* closely resemble those of cyanobacteria. Our results provide support for Schopf's proposal that many microfossils may have been mistakenly identified as cyanobacteria. Early in Earth's history microbial mats may have had a significantly higher proportion of phototrophic bacteria than of cyanobacteria. This has important implications for oxygenation of the atmosphere and the role of cyanobacteria in this process in addition to provoking a reevaluation of the paleoecology of ancient microfossil assemblages and evolution of oxygenic photosynthesis.

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