Biosilicification: the role of cyanobacteria in silica sinter deposition

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INTRODUCTION

The contribution of micro-organisms to amorphous silica precipitation in modern geothermal hot-spring environments has been the topic of intense study in the last three to four decades. Here, we present a review on the field and laboratory studies that have specifically addressed bacterial silicification, with a special focus on cyanobacterial silicification. Studies related to the biogenic silicification processes in diatoms, radiolarians and sponges are not discussed, despite the fact that, in the modern oceans (which are undersaturated with respect to silica), the diagenetic 'ripening' of such biogenic silica controls the global silica cycle (Dixit *et al*., 2001). It is well-known that the amorphous silica in these organisms (particularly in size, shape and orientation) is controlled primarily by the templating functions of glycoproteins and polypeptides (e.g. silaffin and silicatein). For information on these issues, we refer the reader to the extensive reviews by Simpson & Volcani (1981), Kröger *et al*. (1997, 2000), Baeuerlein (2000), Perry & Keeling-Tucker (2000), Hildebrand & Wetherbee (2003) and Perry (2003). In addition, in terrestrial environments, a large pool of amorphous silica is cycled through higher plants (grasses and trees) that are believed to use silicification as a protection mechanism against pathogens and insects. Information on these processes can be found in the papers by Chen & Lewin (1969), Sangster & Hodson (1986) and Perry & Fraser (1991).

In this review, we will focus solely on microbial silicification processes, which have been studied extensively in active geothermal hot-spring environments. These are characterized by geothermal waters supersaturated with respect to amorphous silica

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derived from water–rock interaction at depth. The link between microbes and the surface manifestations of sinter formation (both carbonate- and silica-based) was first documented in Yellowstone National Park at the end of the 19th century (Weed, 1889). Since that time, active geothermal systems have been studied widely, due to their importance as geothermal energy sources and as a proxy to understanding the formation of epithermal ore deposits, which constitute the deep-seated hydrothermal features beneath active systems. In the last quarter of the 20th century, a multitude of studies have been carried out to quantify the formation of silica and carbonate terraces in active systems, with a view towards understanding whether micro-organisms play an active or passive role in their formation (Walter *et al*., 1972; Ferris *et al*., 1986; Schultze-Lam *et al*., 1995; Cady & Farmer, 1996; Konhauser & Ferris, 1996; Jones *et al*., 1998, 2001; Konhauser *et al*., 2001; Mountain *et al*., 2003). Most of these studies have focused on the relationships between microbes and the resulting morphology and structure of modern siliceous sinters. They provide insights into the driving forces for sinter formation in contemporary deposits and are thus relevant to processes in Archaean and early Proterozoic settings, where microbes may have become encased and thus preserved as microfossils (Konhauser, 2000; Cady, 2001; Toporski *et al*., 2002).

There have also been numerous experimental laboratory microbial silicification studies. In single-step batch experiments, it has been shown clearly that the affinity of aqueous silica to bind to a microbial surface is low, regardless of whether the micro-organisms are equilibrated with solutions supersaturated or undersaturated with respect to amorphous silica (Fein *et al*., 2002; Phoenix *et al*., 2003; Yee *et al*., 2003). Such single-step experiments do not reliably mimic the processes leading to the significant silica accumulation observed in hot springs. Other experimental studies have used high concentrations of either organosilicon solvents, such as tetraethylorthosilicate, or inorganic silica concentrations and/or a variety of temperatures and pressures to induce silicification in the presence of micro-organisms and demonstrated that a complex interplay exists between the precipitation of silica and the formed textures and structures (e.g. Oehler & Schopf, 1971; Leo & Barghoorn, 1976; Walters *et al*., 1977; Francis *et al*., 1978; Ferris *et al*., 1988; Westall *et al*., 1995; Konhauser *et al*., 2001; Toporski *et al*., 2002; Mountain *et al*., 2003). These studies offered important insights into the diagenetic-related fossilization processes and sinter textural development, but they cannot provide mechanistic data pertaining to molecular-level interactions between micro-organisms and silica accumulating in environments such as hot springs or the ancient oceans.

Many studies of microbial silicification in active hot springs have shown that silicification rates are rapid, but that the silicification process is controlled by purely abiotic driving forces [i.e. boiling, cooling, evaporation, waves and splash; see Mountain *et al*.

(2003) and references therein]. Microscopic analysis of silicified micro-organisms from active hot springs shows that the microbial surface may act as a nucleation site for silica precipitation (Schultze-Lam *et al*., 1995; Konhauser & Ferris, 1996; Jones *et al*., 2000; Phoenix *et al*., 2000; Mountain *et al*., 2003). Recent studies that exposed cyanobacteria repeatedly to freshly prepared, supersaturated, polymerizing silica solution (a pseudo-flow-through setting) have shown that extensive biomineralization, similar to that observed in hot springs, can be induced (Phoenix *et al*., 2000; Benning & Mountain, 2004; Benning *et al*., 2004a, b), with similar structures and textures to those observed in the field (Benning & Mountain, 2004; Fig. 1). Based on detailed microscopic and, more recently, spectroscopic measurements of samples from such laboratory experiments, it is now believed that the accumulation of amorphous silica on the surface of cyanobacteria is controlled solely by silica nanoparticle aggregation, but that the contribution of the microbial sheaths or cell walls in this aggregation process is considered significant (see below; Phoenix *et al*., 2000; Benning *et al*. 2004a, b). Lastly, recent studies by van der Meer *et al*. (2002) and Pancost *et al*. (2005) have shown that specific biomarker lipids can be preserved in natural modern silica sinters. Such biomarker studies can provide insight into the complex community structure of thermophilic and hyperthermophilic micro-organisms (including both archaea and bacteria) that are present during silica sinter formation. The knowledge of what biomolecules remain preserved in the rock record may provide a means to extrapolate back in time and thus to better understand processes in ancient rocks.

In the following pages, we describe the current understanding of the abiotic and biotic processes occurring in geothermal environments through a review of (i) the chemistry of silica and the thermodynamic and kinetic aspects of precipitation, (ii) the role of specific components of the microbial cell surface and (iii) the pathways of silica-colloid interaction and aggregation on cell surfaces. Only such a synergistic approach can provide a quantitative model for the reactions that drive microbial silicification and that lead ultimately to sinter formation and fossil microbial preservation.

THE CHEMISTRY OF SILICA

Soluble silica or monomeric orthosilicic acid (H_4SiO_4) is composed of a silicon atom coordinated tetrahedrally to four hydroxyl groups. Amorphous silica is defined as a non-stoichiometric, inorganic polymer made up of a mixture of SiO_2 and H_2O units in various ratios. Monomeric silica remains stable in solution at 25 °C, as long as its concentration is below the equilibrium concentration for amorphous hydrated silica [at 25 °C, approx. 100–125 parts per million (p.p.m.); Iler, 1979]. In most natural waters, the concentration of dissolved silica is low (between 1 and 100 μM; Treguer *et al*., 1995) and, specifically in marine settings, the silica concentrations are regulated by the growth of diatoms and radiolarians. In contrast, in the surface expression of active geothermal

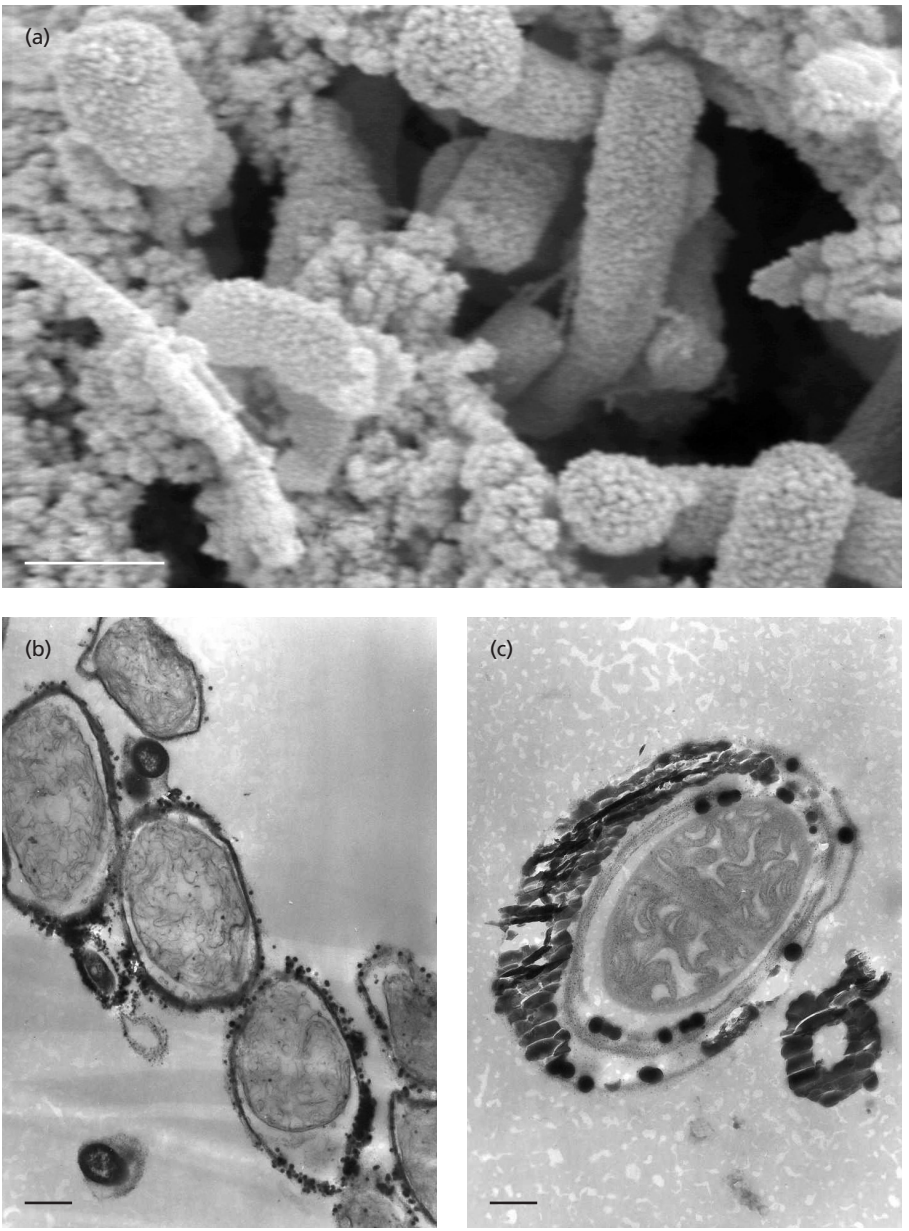

Fig. 1. Silicified microbes from the New Zealand geothermal hot springs. (a) High-resolution field emission gun scanning electron micrograph showing silica nanoparticles attached to microbial cells from the Rotokawa Geothermal Pool; bar, 500 nm. (b) Transmission electron micrograph of silicified micro-organisms from the Wairakei Geothermal Field; bar, 1 μm. (c) Transmission electron micrograph of fully silicified micro-organism from the Wairakei Geothermal Field; bar, 500 nm. Note the small (30–200 nm) silica particles that form aggregates on the surface of the bacterial sheath.

systems, where temperatures are higher (approx. 30–100 °C), the dissolved silica concentration in effluent solutions often exceeds the equilibrium solubility of amorphous silica. Total silica in hot-spring effluents can be as high as 1000 p.p.m. and this represents a level many times higher than saturation, even at 100 °C. Subsurface, geothermal fluids may be undersaturated with respect to amorphous silica but, upon reaching the surface, drastic changes in temperature and other physico-chemical parameters will induce the autocatalytic polycondensation/polymerization of silica monomers, because these changes will induce amorphous silica saturation to be surpassed. Field experimental determination of precipitation rates showed that the ratio of monomeric to polymeric silica in the effluent solution plays an important role in controlling silica-precipitation rates (Carroll *et al*., 1998).

In a purely inorganic system, the polycondensation process follows a series of steps that progress from the polymerization of initial monomers to form dimers, trimers etc. and, finally, to the formation of highly soluble, critical nuclei of approximately 3 nm in size, which correspond to approximately 800–900 silicon atoms and have an approximate molecular mass of around 50 kDa (Iler, 1980; Perry, 2003; Icopini *et al*., 2005). The initial step occurs via the condensation of two silicic acid molecules and the expulsion of water:

$$H_4SiO_4 + H_4SiO_4 \rightarrow (HO)_3Si{-}O{-}Si(OH)_3 + H_2O \qquad \text{(equation 1)}$$

Once the silicic acid molecules condense and Si–O–Si siloxane bonds form, cyclic ring structures will grow and other monomers, dimers etc. will react preferentially with these nuclei via Ostwald ripening. Dove & Rimstidt (1994) showed that the surface free energy of such a particle, σ [erg cm^{-2} (1 erg = 10^{-7} J)], can be linked with the bulk precipitate $\Delta G_{f(\text{bulk solid})}$ and the particle surface area (A, cm^2) to give the free energy of the particle, $\Delta G_{f(\text{particle})}$. This in turn can be expressed as a function of the particle radius (r, cm; assuming spherical morphology) and the molar volume (V_m, cm^3 mol^{-1}) via:

$$\Delta G_{f(\text{particle})} = [-4\pi^3 \Delta G^0 / 2V_m] + [4 \times 10^{10} \pi r^2 \sigma] \qquad \text{(equation 2)}$$

and, from equation 2, an expression for the solubility of a single particle can be derived (Dove & Rimstidt, 1994). Alexander (1975) calculated the surface free energy for amorphous silica in equilibrium with a solution to be approximately 45 erg cm^{-2} (4·5 × 10^{-6} J cm^{-2}). This number increases dramatically with particle size and ordering of the silica phase, reaching a value of 120 erg cm^{-2} (12 × 10^{-6} J cm^{-2}) for quartz (Rimstidt & Cole, 1983), thus confirming that smaller and less ordered particles will dissolve as larger particles grow. Once formed, the critical nuclei will grow to form

either large nanoparticles (from several hundred nanometres up to a micrometre) or will aggregate to form three-dimensional complex structures (Iler, 1979, 1980; Perry, 2003).

Based on the data of Gunnarsson & Arnórsson (2000), the equilibrium amorphous silica solubility at temperatures from 20 to 95 °C lies between 100 and approximately 330 p.p.m. Conventionally, the equation representing the equilibrium between silica and water is written as:

$$SiO_{2(s)} + 2H_2O \rightarrow H_4SiO_{4(aq)} \qquad \text{(equation 3)}$$

with the equilibrium constant K expressed as the activities (a) of the species:

$$K = a(H_4SiO_4)/a(SiO_2) \,.\, a^2(H_2O) \qquad \text{(equation 4)}$$

This reaction is valid for all thermodynamic calculations, but fails to take into account kinetic effects, as well as the variations in the hydration states of silica. For example, the ratio between SiO_2 and H_2O in the aqueous species, as well as in the solid, often differs from the ideal 1 : 2, due to hydrogen-bonded waters of hydration in the stoichiometry. In addition, in equations 3 and 4, aqueous deprotonated and polynuclear species (e.g. $H_2SiO_4^{2-}$, $H_3SiO_4^-$ and $H_6Si_2O_7^{2-}$) are not taken into account although, in some cases, such species may contribute to up to 40 % of the dissolved silica (Aplin, 1987). Equation 3 is particularly important in geothermal systems where the geothermal solutions are supersaturated with respect to amorphous silica, and polymerization and precipitation are thus induced due to changes in physical and hydrodynamic conditions.

From a thermodynamic point of view, the precipitation of amorphous silica is driven by cooling, evaporation, boiling, solution mixing and changes in pH. These factors all strongly affect the saturation level of amorphous silica. For a general precipitation rate, $Rate_{ppt}$, an equation of the type:

$$Rate_{ppt} = -d[nH_4SiO_4]/dt = -A \times k_{ppt}[a(SiO_2) \,.\, a^2(H_2O)] \qquad \text{(equation 5)}$$

can be written, where n is the no. moles H_2SiO_4, A is the interfacial area (in m^2) and k_{ppt} is the pH-dependent precipitation-rate constant (Iler, 1979; Rimstidt & Barnes, 1980; Carroll *et al*., 1998). In solutions that are close to saturation, a nucleation barrier that needs to be surpassed for the first nuclei to form inhibits the precipitation process. Nielsen (1959) modelled the growth of such nuclei and showed that the flux of monomers towards such nuclei is related to the collision rate, the Boltzman constant,

temperature and the free energy of formation of a critical nucleus. Because quartz has a higher surface free energy than amorphous silica and its nucleation is inhibited, it follows that the nucleation and growth of amorphous silica in geothermal systems are more favoured.

In most geothermal systems, the amorphous silica that precipitates is composed of opal-A, a phase that displays varying degrees of ordering of the SiO_4 rings, as well as varying amounts of structural SiO_2 units and degrees of hydration. Opal-A (nominally $SiO_2 . nH_2O$) is a poorly ordered, highly hydrated phase that displays only one weak, broad Bragg diffraction band. Other silica phases observed in geothermal silica-dominated systems are considered good indicators of a diagenetic ageing/altering process. During this ageing, opal-A is transformed into opal-CT, opal-C, moganite, cristobalite, chalcedony and ultimately quartz (Herdianita *et al*., 2000). The main factors influencing this transformation to more stable counterparts are time, re-equilibration with high-temperature or high-pH solutions, dehydroxylation/drying cycles or diagenetic recrystallization. Opal-A can contain between 1 and 13 % water in its structure; this water is present either as network water or as liquid water in interstices bound to either internal silanols or defect sites of surface silanols (Langer & Flörke, 1974; Knauth & Epstein, 1982). During this diagenetic transformation/ripening process, this water is expelled gradually and this is accompanied by a gradual change in *d*-spacing for the main Bragg peak from 4·12 to 4·04 Å (0·412–0·404 nm). This process has been used to derive an indicator of structural ripening, as well as a measure of depth of burial and age. During the ageing and transformation process, water content drops and particle density increases to 2·3 g cm^{-3} (from as low as 1·5 g cm^{-3}). At the same time, porosity (initially between 35 and 60 %) can be reduced by more than half to a value below 30 % [see Herdianita *et al*. (2000) and references therein].

In an effluent solution, the saturation state and thus the precipitation rate of amorphous silica are dependent on a variety of parameters that include thermal gradients, time, changes in pH, concentration of inorganic cations (i.e. Al and trace elements), organics and ionic strength. Furthermore, this rate depends on the presence of nucleation sites/surfaces, as well as hydrodynamic parameters such as evaporation, waves, splash etc. As a result, the precipitated, amorphous silica phases will be highly variable from site to site and the resulting morphology and textures will depend strongly on these precipitation regimes (with resulting morphologies of the precipitated silica varying from nanometre-sized spheroidal particles to flat sheets to bulk silica).

The first precipitated opal-A is usually made up of nanometre-sized spheroids that are later filled in by silica cement to form bulk silica structures. Its formation is a dynamic process and even ‘fresh’ sinter features can appear homogeneous; thus, they

are sometimes difficult to distinguish from aged sinters. This has been a major stumbling block when purely morphological and structurally preserved biosignatures observed in modern sinters have been used to relate and extrapolate to processes in ancient rocks, where subsequent diagenetic or metamorphic processes have homogenized and altered the structures, mineral ordering and composition. Specifically, in ancient rocks, the preservation of biogenic material is hampered by the fact that, in most cases, the only preserved features are the mould or casings surrounding the microbes, whilst the cell walls or sheaths have been lost. However, recent geothermal sinters have revealed that some specific biomarkers (specifically, bacterial and archaeal lipids) can be preserved. This may be the approach to elucidate the preservation of biota in ancient rocks lacking unequivocal morphological indicators (van der Meer *et al*., 2002; Toporski *et al*., 2002; Pancost *et al*., 2005).

CYANOBACTERIAL SURFACE PROPERTIES AND FUNCTION

The structure and composition of cyanobacterial cell walls display a number of characteristics that are atypical of Gram-negative bacteria. They exhibit a thick, highly cross-linked peptidoglycan layer (similar to that of Gram-positive organisms) that makes the cell wall notably stronger (Drews & Weckesser, 1982; Hoiczyk & Hansel, 2000). Additionally, biomolecules commonly found in the cyanobacterial outer membrane, such as atypical fatty acids and carotenoids, are uncommon in other Gram-negative bacteria (Schrader *et al*., 1981; Resch & Gibson, 1983; Jurgens & Mantele, 1991).

As with all bacteria, each component of the cyanobacterial cell envelope plays a specific role. The inner component, the cytoplasmic membrane, behaves as a highly selective barrier, allowing vital nutrients into the cell and excreting waste material out of the cell. The outer component of the Gram-negative cell wall is the outer membrane (an asymmetrical bilayer composed of lipopolysaccharide and phospholipid). This bilayer also acts as a selective barrier, facilitating the transport of low-molecular-mass molecules via proteins known as porins. Housed between the two membranes are the peptidoglycan, which provides rigidity, strength and shape, and the periplasm, which contains functionally important enzymes. Significantly, these components of the cell envelope are highly functional, complex and sensitive. One may then ask how these metabolically vital, and in some cases delicate, layers would respond to encrustation in silica precipitates.

For some Gram-negative bacteria, the outer membrane is the outermost component of the organism and it acts as the interface between the cell and the external environment. In other cases, the organism surrounds itself in an extracellular polysaccharide capsule or sheath. The structure of this extracellular layer can vary considerably, ranging from

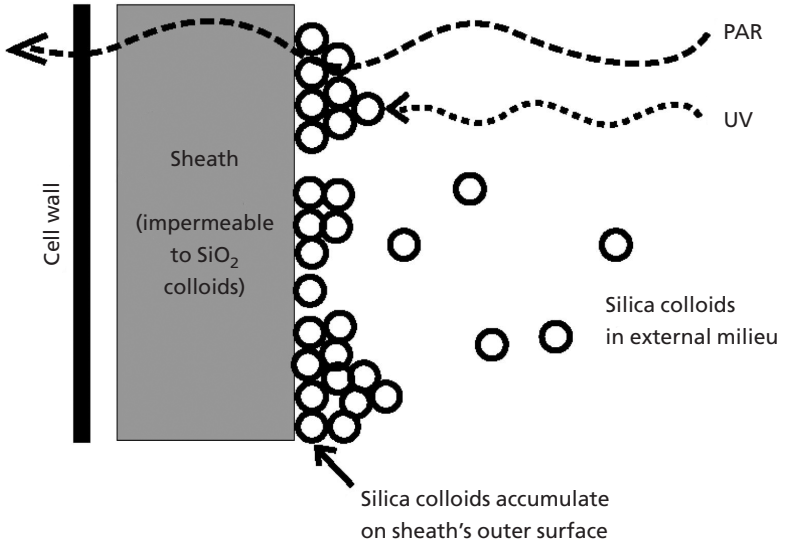

Fig. 2. Summary schematic illustrating extracellular silicification of an ensheathed cyanobacterium. Silica colloids accumulate on the outer surface of the sheath, due to the impermeability of the sheath to 'large' particles. In addition, it is shown how silica inhibits damaging UV light from reaching the cell, whilst the photosynthetically active radiation (PAR) can pass through the silica layer with less attenuation.

diffuse to dense and fibrous. Dense, fibrous polysaccharide layers known as sheaths are particularly common in cyanobacteria (e.g. Rippka *et al*., 1979). Moreover, the cyanobacterial sheath is known to be devoid of metabolically vital components and it is thus likely that the organism can withstand a higher degree of damage to this layer than the rest of the cell envelope. The exact role of the cyanobacterial sheath is not well understood but, as it encloses the more delicate components of the cell envelope, one of its primary objectives may be to help to prevent damage to these components. It has been shown that a coating of extracellular polysaccharide can protect bacteria against dehydration (Dudman, 1977; Scott *et al*., 1996; Hoiczyk, 1998; Tsuneda *et al*., 2003) and predation (Dudman, 1977), or it can aid in adhesion to a solid substrate (Dudman, 1977; Scott *et al*., 1996). More specifically, some cyanobacterial sheaths can contain the UV light-absorbing pigment scytonemin, aiding cyanobacterial resistance to solar radiation (Garcia-Pichel & Castenholz, 1991). Of particular relevance to this chapter is the ability of the cyanobacterial sheath to protect the cell from detrimental biomineralization (Phoenix *et al*., 2000; Benning *et al*., 2004a) and to aid in the aggregation of silica nanoparticles (Benning *et al*., 2004b).

Sheathed cyanobacteria are found in abundance in hot-spring systems, where it has been shown that silica accumulation is restricted to the outer surface of the sheath on

living cyanobacteria (Fig. 1; Phoenix *et al*., 2000; Konhauser *et al*., 2001; Mountain *et al*., 2003). This is likely to occur because the polysaccharide meshwork of the sheath enables it to act as a filter against colloidal silica (Fig. 2). Permeability studies demonstrated that the sheath of *Calothrix* sp. was impermeable to particles of at least 11 nm in diameter, thus preventing the colloids from biomineralizing the sensitive components of the cell wall [Phoenix *et al*. (2000) and references therein].

Interestingly, in the Archaean oceans, which were enriched in silica (Siever, 1992) and inhabited by cyanobacteria, silica biomineralization was likely to have occurred (Cloud, 1965). This is particularly true for the shallow waters, in which intermittently exposed environments were inhabited by stromatolitic communities and evaporation may have controlled silica precipitation. It is possible that the sheath developed/evolved in the early oceans as a response and protection against detrimental silica accumulation on the cell wall. This is supported by several studies, including transmission electron microscope- and synchrotron-based Fourier-transform IR analysis, which have demonstrated that, in response to increased silica exposure, the sheath of cyanobacteria thickens (Phoenix *et al*., 2000; Benning *et al*., 2004a, b). Again, this indicates that the sheath can act as a protective layer against silicification. Naturally, when cyanobacteria are exposed continuously to supersaturated solutions of silica, silicification eventually becomes too extensive and this may be detrimental to the cyanobacteria. Phoenix *et al*. (2000) have shown that even quite thick silica crusts (approx. 5 μm thick for a 10 μm diameter cell) did not appear to be detrimental to the cells. However, whether there is a maximum amount of extracellular silicification that cyanobacteria can withstand has yet to be determined.

One mechanism to overcome extensive silicification may be the release of transient motile phases (hormogonia) (Herdman & Rippka, 1988) from the ends of heavily encrusted filaments and this may provide a pathway for survival. Benning *et al*. (2004a, b) have followed bacterially mediated silica accumulation both *in situ* and *in vivo* via the changes in the IR signature induced by the increase in silica concentration on the organic framework of single bacterial cells. This approach allowed the quantification of the actual and not the apparent bacterial silica-accumulation process, and they showed that the role of the sheath is twofold. Initially, the cells react to exposure to a silica-rich solution by producing more sheath polysaccharide as protection. As this thicker sheath acts as a good ('sticky') substrate for further inorganically precipitated silica-colloid aggregation, silica precipitation is enhanced, with detrimental effects to the cell.

This ability of cyanobacteria to survive and grow continually, despite extensive extracellular silicification in modern hot springs and presumably also in the ancient oceans, may have provided additional advantages to the microbes. This is because

amorphous silica biomineralization has been demonstrated to act as an effective screen against UV radiation (Phoenix *et al*., 2001). These studies have shown that damaging wavelengths of UV-B and particularly UV-C are absorbed strongly by amorphous silica, whilst photosynthetically active radiation (400–700 nm) will pass through the silica with significantly less adsorption (Fig. 2). This process enables cyanobacteria to photosynthesize in environments subjected to elevated UV, a protective mechanism particularly relevant to the Archaean (Phoenix *et al*., 2001), where highly detrimental levels of UV irradiated the Earth's surface (Kasting, 1987). Furthermore, Heijnen *et al*. (1992) have shown that habitation of micro-niches in bentonite clays can protect other bacterial forms from predation by grazing protozoa and, thus, silica encrustation may similarly protect cyanobacteria by making them inaccessible or inedible to protozoa. Biomineralization also plays a key role in the formation of siliceous stromatolitic communities, both modern and ancient, by increasing their structural integrity and thus longevity (Konhauser *et al*., 2001). It has also been speculated that, because the amorphous silica matrix is highly hydrated, it may afford the organisms an additional protection layer against dehydration. Interestingly, these potential advantages are similar to the functions of the sheath, and it thus seems that the sheath and enshrouding silica biominerals may work collectively to protect the organisms within.

CYANOBACTERIAL BIOMINERALIZATION PATHWAYS AND COLLOID AGGREGATION

Benning *et al*. (2004a, b) have followed the processes leading to cyanobacterial silicification by using *in situ* IR microspectroscopy and imaging and have quantified the complex interplay between the cyanobacterial cell components, specifically the sheath, and the polymerizing silica solution. The progression of nucleation, growth and aggregation of nanometre-sized silica particles and their effect on cyanobacterial feedback have been described as a three-stage progression. In the first stage, in response to the presence of polymerizing silica, the cyanobacteria will increase the formation of new polysaccharide polymers, i.e. they will thicken their sheath. Concomitantly, silica will form branched polymers that, upon collapse, will bind to the carbon backbone of the hydrated polysaccharide sheath via hydrogen bridges. This step can be expressed as:

$$[>\mathrm{ROH}] + [>\mathrm{ROH}] \rightarrow 2[>\mathrm{ROH}] \qquad \text{(equation 6a)}$$

and

$$[>\mathrm{ROH}] + [\equiv\mathrm{Si{-}OH}] \rightarrow [\equiv\mathrm{Si{-}OR<}] + \mathrm{H_2O} \qquad \text{(equation 6b)}$$

where >ROH represents the surface-hydroxylated sugar polymer in the sheath and ≡Si–OH is the monomeric silica attached to a surface. Equation 6(b) implies a possible site-specific silica accumulation, with the silica monomers bound via hydrogen bridges

to another OH-containing radical. The sheath polysaccharides are the obvious candidates for this step. Benning *et al*. (2004b) used a kinetic approach to show that this reaction proceeds via a diffusion-limited mechanism (see below), in which polymerizing silica units in the supersaturated aqueous environment begin to coalesce and aggregate on the 'fresh' microbial-sheath surface. This process is enhanced once a silane group is attached to the bacterial sheath via hydrogen bonds and, thus, a further increase in Si load may lead to the formation of a thin, fully hydrated silica network. Subsequently, other silane bonds may form independently of the sheath and this process will become uncoupled from the formation of the silica–carbohydrate hydrogen bonds. This can be expressed as:

$$[\equiv Si{-}OR<] + [\equiv Si{-}OH] \rightarrow [\equiv Si{-}O{-}Si\equiv] + [ROH<] \qquad \text{(equation 7)}$$

At this stage, the formation of additional polysaccharides will no longer compete with the polymerization of silica, a fact supported by the change in IR spectra, which become dominated by the more ionic Si–O–Si bonds. The last stage is the formation of inorganic silane bonds. This process has been shown to be governed by a reaction-limited process that leads to the growth of purely inorganic Si–O–Si bonds via the formation of an oxo bridge (Si–O–Si), whilst one water molecule is expelled. This is similar to the purely inorganic process described in equation 1. When surface attachment is considered, this step can be described via:

$$[\equiv Si{-}OH] + [OH{-}Si\equiv] \rightarrow [\equiv Si{-}O{-}Si\equiv] + H_2O \qquad \text{(equation 8)}$$

In this way, a silica network made of corner-sharing [SiO_4] tetrahedra is obtained when all Si–O groups have reacted and the critical silica nuclei have formed. Their further growth and aggregation will follow and no other connection to the polysaccharide sheath is needed.

In general, for colloid or polymer growth and aggregation, two restrictive regimes have been defined: diffusion-limited aggregation (DLA) and reaction-limited aggregation (RLA) (Everett, 1988; Hunter, 1996; Jamtveit & Meakin, 1999). In the diffusion-limited case, the limiting step is the movement of two polymer units toward each other prior to encounter and formation of a cluster (or aggregate). In such reactions, monomers or oligomers collide and combine instantaneously, producing a relatively porous aggregate. For the formation of critical nuclei of silica, the DLA process has been confirmed experimentally (Lin *et al*., 1990; Martin *et al*., 1990; Pontoni *et al*., 2002). For polysaccharide polymers, however, such data are unavailable, although Rees (1977) showed that glucose polymers – specifically amylose – grow by a DLA process. On the other hand, in RLA, the concentrations of the encountered reactant pairs are main-

tained at equilibrium and thus condensation occurs slower. In addition, a significant repulsive barrier exists, such that the 'sticking probability' upon oligomer–oligomer interaction is small (Everett, 1988; Gedde, 1995). For silica, the RLA process results in a more compact aggregate structure during slow condensation (Martin, 1987; Lin *et al*., 1990).

Based on theoretical calculations for nucleation, crystallization, growth and aggregation of mineral phases and organic polymers, Hulbert (1969) and Gedde (1995) have derived hypothetical constants for the mechanistic constant *n*, which represents a parameter that is related to specific mechanisms and geometric shape of the final mineral particles or polymer. The two types of mechanisms (DLA and RLA) and several different shapes (needles, plates, spheres, fibres, sheaths etc.) have been investigated and values for *n* have been deduced. In general, *n* increases with increasing 'dimensionality' of the resulting particle/polymer and, in heterogeneous systems, a change in mechanism often occurs. Hulbert (1969) and Gedde (1995) have concluded that a particle/polymer of low geometry (one- or two-dimensional; e.g. fibre or sheath), forming via a DLA process, will have values of *n* varying between 0·5 and 2, with the highest values representing two-dimensional growth. On the other hand, if a spherical (three-dimensional) entity grows or aggregates via a DLA mechanism, the value of *n* will vary between 1·5 and 2·5, with the higher numbers indicating a switch to an RLA mechanism. Lastly, if the same three-dimensional spherical entity grows or aggregates via a purely RLA mechanism, values of 3–4 are expected for *n*.

It is well-known that the polysaccharide components of the sheath of *Calothrix* sp. (composed primarily of neutral sugars; Weckesser *et al*., 1988) is usually found in the form of amylose. Amylose is a linear polymer of glucose units joined by repeating covalent C–O bonds, and it normally forms complex aggregates of linear geometry (Rees, 1977). In the cyanobacteria silica system, a low geometric ordering of newly formed polymers was corroborated by Benning *et al*. (2004b), who have derived *n* values for the first step in polysaccharide growth of 0·8–1·1, thus confirming a one-dimensional DLA growth for the carbohydrate polymers. For the second step, which is dominated by the attachment of the formed silica nuclei to the cyanobacterial sheath, Benning *et al*. (2004b) derived a value for *n* of 1·8–2·2, indicating a DLA mechanism for three-dimensional growth. Finally, for the last step, an *n* value of 3·5–3·8 was derived, clearly indicating three-dimensional growth via an RLA mechanism. It needs to be noted, however, that polysaccharide and silica polymers can both form structures of mixed or changing geometry during growth or aggregation and that the data derived by Benning *et al*. (2004b), which were based on a kinetic approach, may not fully describe all steps in this complex process. This is particularly true because, in most polymers and colloid systems, the nucleation and aggregation behaviour in solution is affected

strongly by pH, ionic strength, temperature, organic concentration and type. However, for silica nucleation and growth, the aggregation steps quantified in the laboratory are expected to be similar to processes in modern geothermal hot springs and thus can be used as analogues to model processes in natural environments.

CONCLUSIONS

Cyanobacteria are a major group of phototrophic prokaryotes that play an important role in the textural development of silica sinters in modern geothermal environments. Processes that are analogous to those observed in modern hot springs may also have been active during the fossilization of microbes and the formation of siliceous stromatolites in the Archaean. These, in turn, can provide a proxy for the biogeochemical conditions of the early biosphere. A large number of field observations and experimental laboratory studies, as well as a few molecular-dynamic simulations, have led to the conclusion that, in active geothermal hot springs, cyanobacteria play no active role in the initial silica polymerization. It was shown that covalent bonds between silica and bacterial cell-wall or sheath components are not favoured and that the nucleation of silica from supersaturated aqueous solutions is driven by purely inorganic polycondensation reactions, which are strongly pH-, ionic strength-, temperature- and saturation state-dependent. Nevertheless, many field and experimental microscopic observations showed clear evidence of a link between silica sinter structures/textures and micro-organisms via the deposition of silica nanospheres onto the microbial surfaces. Ultimately, this process promotes the incorporation of micro-organisms into the sinter structure and leads to the preservation of microbial colonies as fossils. However, despite the large variety of studies carried out so far, the question remains as to the exact role of the microbial surface in the processes that lead to silica precipitation. We argue here that the formation of silica sinters is a multi-step process that is governed primarily by inorganically driven polycondensation of silica monomers and the formation of silica nanoparticles. This is followed by the microbially enhanced aggregation of the silica nanospheres into larger assemblages. In the first step, experimental and theoretical evidence indicates that polymerization of silica monomers leads to the formation of branching clusters that eventually collapse to form a spherical particle. Although the parameters and mechanisms controlling this collapse are unclear, some evidence indicates that it may be the dehydroxylation of silanol or silane clusters. When cyanobacteria and their complex surface structures are present, it appears that the polycondensation rates and, thus, silica nanoparticle nucleation rates, are not enhanced. In addition, and more importantly, it is believed that the precipitation of silica does not affect cyanobacterial metabolism or duplication rates. However, cyanobacteria do react by increasing the amount of extracellular polysaccharide that they produce. Once silicification is advanced and thus unavoidable, the thicker polysaccharide sheath will enhance the aggregation of the inorganically nucleated silica

nanoparticles into larger silica assemblages. This process occurs while they are alive, but continued silicification leads to cell death, lysis and finally fossilization.

The surface features seen in active geothermal systems are often regarded as ideal model systems for study, because they can shed light onto processes occurring in the shallow-subsurface portions of the geothermal systems that are linked to deep-seated epithermal ore deposits. In the streams, pools and sinters forming in modern geothermal systems, a vast array of mesophilic and thermophilic organisms thrive at high temperatures and varied pH, as well as high toxic-metal concentrations that are usually detrimental to microbial growth. The knowledge gained from studying such communities and their interaction with the minerals precipitating from the supersaturated solutions can give valuable insights into processes of biomineralization. In addition, our understanding of processes related to the evolution of early life forms in the Archaean and early Proterozoic has been extrapolated from observations (both structural and chemical) of bacterial–mineral interactions in modern hot-spring environments or from morphological observations of Precambrian silicified microfossils and stromatolites.

From field and laboratory observations, reaction pathways for the formation of silica sinters in modern or ancient hot springs have been devised. The abiotic versus biotic components of the silica biomineralization reaction, and thus the role of microorganisms in this process, were defined. These studies have shown that the silicification process follows a series of interlinked but unavoidable steps, starting with the microbes reacting to highly supersaturated silica solutions by increasing their production of exopolymeric material. Simultaneously with this process, but driven inorganically, silica polycondensation is proceeding, with monomers condensing to dimers, trimers etc., leading to the formation of critical silica nanospheres. The newly formed, 'sticky', exopolymeric sugars do not affect this polymerization, but will subsequently enhance the aggregation of the inorganically formed nanospheres on the microbial surface. Finally, this will invariably lead to the full silicification of the organic microbial frameworks and the formation of microfossils that can thus be preserved in modern silica sinter environments and that provide a modern analogue to processes in the ancient past.

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