Molecular characterization of cyanobacterial cells during silicification: a synchrotron-based infrared study.

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Introduction.

The reactions occurring at the bacterial/mineral/fluid interface strongly control the processes related to biomineralization. However, the molecular level response of the organic framework of bacterial surfaces (cell walls, membranes, or sheaths) to growth in a mineralizing solution is still poorly understood. Bioassay work has identified the major functional groups associated with cell outer surfaces, while acid-base titration studies have shown how the ionization of those groups varies with solution pH[1-6], yet these techniques allow only bulk determinations. In addition, conventional microscopic techniques used to inspect single biomineralized cells require sample pre-treatment (drying, staining, coating etc), thus impeding the investigation of live organisms and their organic structure.

Here we present results from an infrared spectroscopic study that was carried out to ascertain the changes in vibrational frequencies of the organic functional groups on cyanobacterial cells in the course of biomineralization experiments. We used synchrotron-based infrared micro-spectroscopy (SR-IR)[7,8] to determine *in situ* and *in vivo* the molecular level interaction between cyanobacterial surfaces and silica solutions by monitoring the changes in chemistry of the organic framework of single cells. The active precipitation of silica is an important geological process and the silicification of microorganisms occurs abundantly in many modern hot spring environments[9,12-19]. In addition, these processes may also provide analogues for silicification and fossilization of early Precambrian microbes[21]. Field based studies of modern hot springs suggest that cyanobacteria may act as nucleation sites for silica precipitation, and that they impart a controlling influence over silicification rates and sinter morphology[13-19]. In most hot springs the supply of silica in the effluent solution is continuous (supersaturated with respect to silica), and adsorption / precipitation processes are invariably sustained via a combination of inorganic[10,22-24] and biogenic processes[11,19]. Interestingly, laboratory studies suggest that the initial precipitation process is microbially mediated[25-28] and that this first step appears to lower the activation energy barriers which inhibits inorganic nucleation to a level where inorganic precipitation may occur. This biogenic step thus provides nucleation sites and enables silica precipitation to continue via a mixed inorganic-biogenic process[19,29]. However, due to a lack in understanding of the reactions occurring at the bacterial surfaces, specifically the interaction between silica and the various surface organic functional groups that control the reactivity of a bacterial cell the reactions controlling the silicifcation process are still unclear.

Experimental methods.

The cyanobacteria, *Calothrix* (strain KC97) used as a type strain. The bacteria were cultured in liquid BG11-n media at 28˚C under an average light irradiance of 700 lux. After 4 weeks, the clusters of filaments were dispersed and diluted with 18 MΩ water and aliquots of this suspension were mixed with a solution containing 300 ppm Si (in 10% BG11-n nutrient media). The silica stock solution

(monomeric silica from $Na_2SiO_3.7H_2O$) was neutralized to pH ~7 with 2 M HCl just prior to mixing with the cyanobacterial suspension. Silicification was carried out for time periods between 1 hour and 30 days, during which the silica solution was refreshed every 2 to 3 days. Just prior to the infrared measurement each sample was washed in 18 MΩ water and a dispersed on SnO coated slides. The infrared experiments were carried out on station 13.3 at the Daresbury Laboratory (UK) using a NicPlan Infrared Microscope with a MCT detector. Spectra were collected in reflectance mode, at 256 scans per point, 4 cm^{-1} resolution. A camera enabled optical imaging and recording of the areas investigated. Changes in absorbance peak position for the Si-O, the carbohydrate and the amine I and II bands were used to define the reaction progress.

Results

The silicification of viable cells occurs rapidly in polymerizing silica-supersaturated solutions and under silica-supersaturated conditions, Calothrix cells become heavily encrusted in a matrix several µm thick[25,26]. TEM examination indicates that mineralization is restricted to the outer surface of the extracellular sheaths (Fig. 1).

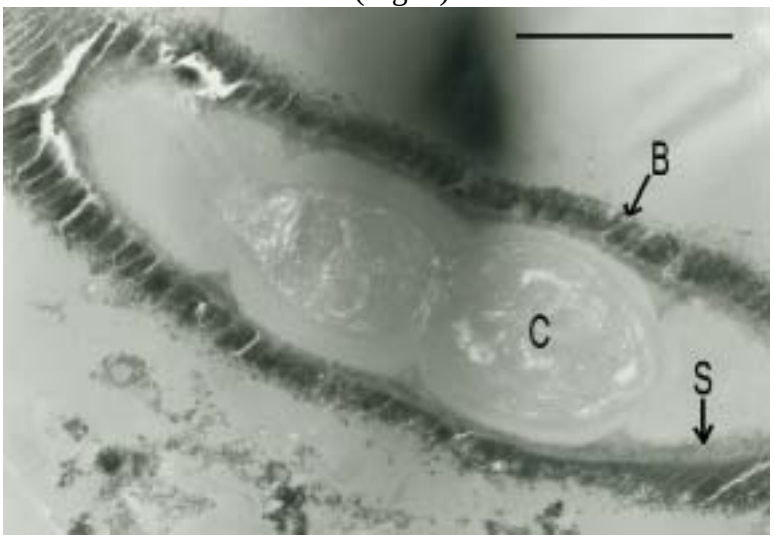
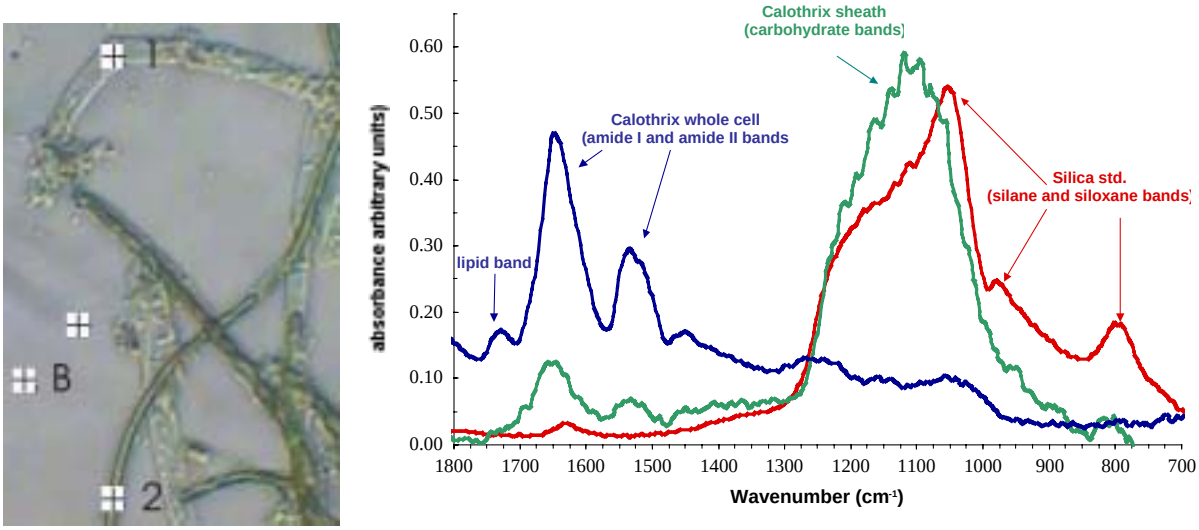


Figure 1: TEM Microphotograph of an unstained laboratory silicified *Calothrix* filament. (B = silica biominedralization; S = sheath; C = cytoplasm. Scale bar: 5 µm.

The infrared spectroscopic measurements show that silica vibrational bands are very distinct and that even thin silica crusts are likely to change the biochemical structure of the cells substantially. Initially, intact cells, mechanically separated sheaths, and a silica standard (Fig. 2a and b) were inspected in order to establish a unambiguous base line for the unsilicified microorganism. As can be seen in Fig. 2b, the intact cells are characterized by the amide I and amide II bands (amide I = ~ 1695 cm^{-1}, C=O stretching, coupled to N-H bending mode, amide II = ~1567 cm^{-1}, C=O bending vibrations, coupled to the N-H stretching mode). In addition, typical is the distinctive lipid band at ~ 1740cm^{-1}. At lower wavenumbers (<1300) weak phospholipid and carbohydrate bands are visible (1240 cm^{-2} and 1100-1000cm^{-1}) and these correspond mostly to the sheath. In contrast, the base line established for the bacterial sheaths is composed mostly of polysaccharide peaks (C-O and C-O-C and P-O-C stretching vibrations) with weak amide bands from remnant protein. Thus the cell spectrum shows, as expected, a combination of protein, lipid and carbohydrate bands while the sheath is mainly polysaccharides.



(a) **(b)**

Figure 2. (a) Infrared image of a separated *Calothrix* sheath (point 1) and of a whole cell (point 2, B = background). The cross is 10x10 µm. (b) Absorbance vs. wavenumber (cm^{-1}) plot with spectra for a whole cell, a bacterial sheaths and a silica standard.

Follow up work has shown that chemically purified sheaths shows pure carbohyrdate bands with less than 3% proteins[30]. For the silica standard the asymmetric Si-O-Si stretching vibrations are found in the region between 1100 and 1000cm^{-1}, and due to the greater ionic character of the Si-O group, this band is much more intense than the corresponding carbohydrate C-O bands[31].

The silicification experiments indicate that the sheath (i.e., the carbohydrates) provides the binding site for silica (Fig. 3); with decreasing silicification time a decrease in the importance of the Si-O and carbohydrate peaks is observed. This may corresponds to a decrease in concentration of silica on the bacterial surface and/or to a decrease in sheath thickness. These two processes are not exclusive[25] however, the appearance of siloxane bands below 850cm^{-1}, strongly suggest that silica binds to the exo-cellular polysaccharide sheath.

Changes in protein and lipid structure upon silicification have also been observed, yet these are more subtle (i.e., a ~ 30 wavenumber shift in the lipid band at 1740cm^{-1}). The change in frequency of these vibrations depends on the nature of the hydrogen bonds involving the C=O and N-H groups, and this in turn is determined by the particular secondary structure adopted by the proteins and lipids[32,33].

Summary

This study ascertained for the first time *in situ* and *in vivo* the relationship between silicification and the organic character of cyanobacterial cells. We (i) have showed that it is feasible to monitor the reactions occurring between organic biomolecules on a bacterial surface and a silica solution over time; (ii) have determined the structure of these biomolecules and (iii) have determined the changes in vibrational frequencies caused by the interaction of silica with the main functional groups on the cell surface.

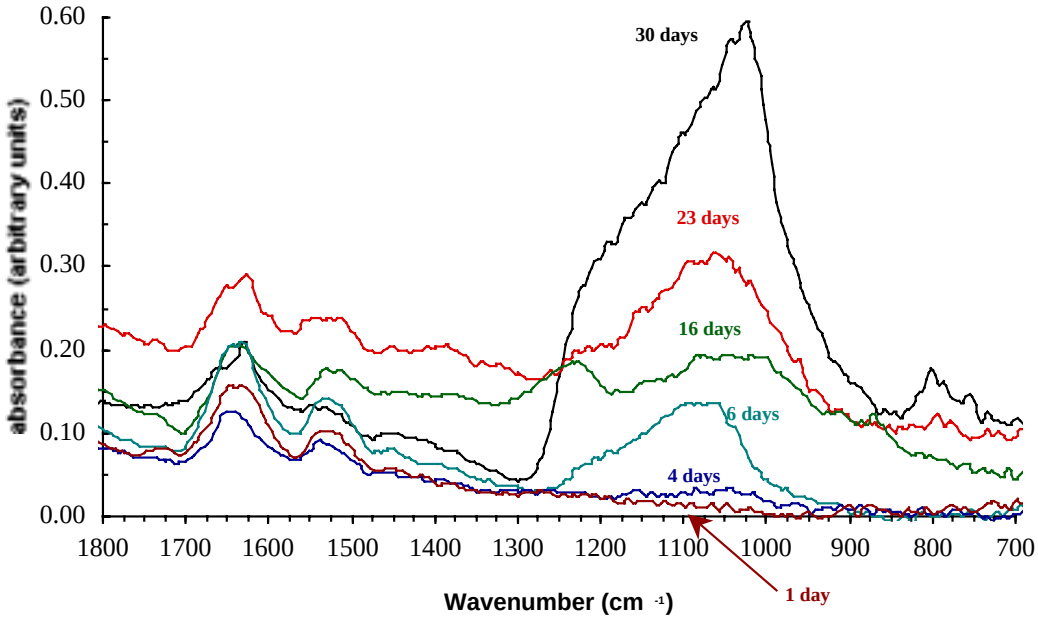


Figure 3. Absorbance spectra for the silicification of Calothrix over a period of 30 days.

The results presented here show that silica actively reacts with various organic molecules on the bacterial surface. In a further step, the changes in peak position and intensity for the various vibrational frequencies (i.e., silica, amide, carboxyl, phosphoryl etc.) will be analyzed of this will lead to a quantitative measure for the silicification processes.

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