Role of biomineralization as an ultraviolet shield: Implications for Archean life

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ABSTRACT

Cyanobacteria, isolated from the Krisuvik hot spring, Iceland, were mineralized in an iron-silica solution and irradiated with high levels of ultraviolet light. Analysis of the rates of photosynthesis, chlorophyll-a content, and phycocyanin autofluorescence revealed that these mineralized bacteria have a marked resistance to UV compared to nonmineralized bacteria. Naturally occurring sinters composed of iron-silica biominerals collected from the Lysúholl hot spring, and made into wafers of 150–250 μm thickness, also provided cyanobacteria with an effective UV screen. Analysis of the UV-absorbing capacity of these wafers showed that they absorbed an order of magnitude more UV than photosynthetically active light (required for photosynthesis). From these results, it is evident that both natural and experimental biomineralization provide bacteria with an effective UV screen through the passive precipitation of iron-enriched silica crusts. The UV-shielding capacity of iron-bearing silicate biominerals may have been important for early life forms. We propose that the biomineralization of Archean bacteria similarly provided protection from the high-intensity UV present at that time, and hence allowed colonization and bacterial diversification of shallow-water environments.

Keywords: Archean, biomineralization, cyanobacteria, silica, ultraviolet.

INTRODUCTION

Many models of atmospheric evolution predict that the Archean atmosphere contained insufficient oxygen to form an effective ozone screen (Berkner and Marshall, 1965; Walker et al., 1983; Kasting, 1987). In the absence of such a screen, ultraviolet radiation may have reached Earth's surface with little attenuation. Estimated levels of UV in the Archean, over the most damaging wavelengths of 240 to 270 nm, fall within the order of 10^{-1} to 10^{-2} $W \cdot m^{-2} \cdot nm^{-1}$ (Rambler and Margulis, 1980; Kasting, 1987; Pierson et al., 1993). These levels are considerably higher than today, when no UV radiation within this range reaches Earth's surface (Bundestag, 1991).

Under such unfavorable conditions, those early life forms would have required some type of effective ultraviolet screen to survive. Microorganisms inhabiting the deep-marine environment would have been shielded from UV by the water column (Margulis et al., 1976; Olson, 1981). However, such shielding would not have been possible in shallow-water conditions intermittently exposed to the atmosphere—for example, those inhabited by microbial mats and stromatolites (Walter, 1983). Other studies have suggested alternative UV-screening mechanisms for shallow-water environments based upon observations in modern biofilms, such as mat-forming habit (Rambler and Margulis, 1980), sheath pigmentation (Garcia-Pichel and Castenholz, 1991), phototaxis (Margulis et al., 1976), and photo repair mechanisms (Walter, 1983). Pierson et al. (1993) suggested that iron-enriched siliceous sediments could provide a viable UV screen. In their experiments with artificial sediments, they demonstrated that amorphous silica, doped with low levels of Fe^{3+} (a strong UV absorber), significantly absorbs harmful UV while still allowing the passage of light (400–700 nm) required for photosynthesis.

In this study, we have furthered Pierson et al.'s (1993) premise by proposing that bacteria, simply by acting as a viable site for mineral nucleation, may have passively precipitated their own ''siliceous sediment type'' UV screen, through the process of biomineralization, which could occur in the silica-enriched shallow-water environments of the Archean. To test this hypothesis, samples of iron-enriched siliceous sinters, collected from the Lysúholl hot spring, and experimentally generated biominerals, formed by cyanobacteria isolated from the Krisuvik hot spring, were irradiated with high levels of UV. We demonstrate that these silica crusts can shield cultures of cyanobacteria from detrimental levels of UV, thereby allowing the cells to survive and grow.

METHODOLOGY

Silicification of Cyanobacteria

Modern-day hot springs provide good analogues for Precambrian oceans because of their similarity in chemistry (high iron and silica concentrations) and microbiology (the presence of phototrophs, presumably both oxygenic and anoxygenic) (Konhauser, 2000). Therefore, in this study, samples of the filamentous cyanobacterium *Calothrix* (strain KC97) isolated from sinters associated with the Krisuvik hot spring, Iceland, were cultured on agar plates, and BG11-n nutrients were supplied (Rippka et al., 1979) until an even and continuous growth across the plates was achieved. These cultures were then mineralized by placing the plates in a mineralizing solution, containing 300 ppm Si and 50 ppm Fe, for 20 days, as described by Phoenix et al. (2000). These concentrations were chosen to mimic saturated hot-spring effluent. Controls (nonmineralizing) were similarly prepared, but without Fe or Si in the solution. Both mineralizing and nonmineralizing solutions also contained 10% BG11-n. During the experiment, the plates were incubated under 18 W cool-white fluorescent lights at an incident irradience of ~700 lux. Samples of culture were collected every 2 to 3 d, fixed in 2% glutaraldehyde, and processed for transmission electron microscopy (TEM) as described by Phoenix et al. (2000). TEM analysis was performed on a Philips CM20 TEM operating at 200 kv, equipped with selected area electron diffraction (SAED) for determination of mineral structure, and an Oxford Instruments energy-dispersive X-ray spectrometer (EDS).

Determination of the UV Shielding Capacity of Silica Biominerals

After 20 d, the agar plates of silicified (incubated in Fe/Si solution) and nonsilicified (incubated in the control solution) *Calothrix* were removed from solution and covered in a layer of cling film to prevent desiccation during irradiation; the cling film has a negligible effect on the incident UV. As the cling film prevented desiccation and the cyanobacteria were substrated to the agar, conditions were suitable for the cyanobacteria to continue to function. These plates were then continuously irradiated over a 16 d period with a Vilbor Lourmat lamp emitting radiation in the middle UV-C (254 nm) band, at 0.35 $W \cdot m^{-2}$. This intensity was chosen to correspond to predicted UV irradiances for the Archean (Kasting,

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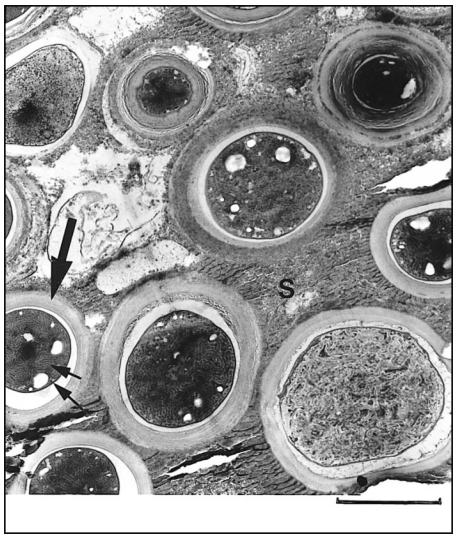

Figure 1. TEM micrograph of *Calothrix* filaments after 20 d of incubation in solution containing iron and silica. Filaments, seen here in cross section, are heavily encrusted in amorphous iron-bearing silica precipitate (S). Iron-bearing silica precipitate is present on thick fibrous sheath (large arrow). Cell wall and cytoplasm (small arrows) are free from precipitate and appear intact. Scale bar = 5 μm.

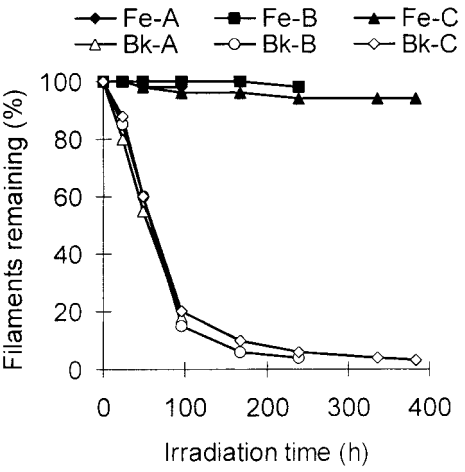

Figure 2. Comparison of resistance to UV irradiation by mineralized (Fe-A, Fe-B, or Fe-C) and nonmineralized (Bk-A, Bk-B, or Bk-C) cells as measured by estimation of viable filaments remaining on plate. A, B, and C represent plates removed at 96, 240, and 384 h, respectively.

1987). During irradiation, the cultures were incubated under 18 W cool-white fluorescent light (700 lux) to allow photosynthesis.

The plates were photographed every 24–48 h to allow visual estimation of the loss of cells from each plate (by visual estimation of the loss of biofilm coverage). To confirm the viability of the remaining cells, agar plates of both the mineralized cyanobacteria and the nonmineralized cyanobacteria were removed at 0, 96, 240, and 384 h for further analysis. First, exactly half of the biofilm (31.81 cm^{-2}) from each plate was harvested, and rates of photosynthesis were analyzed via measuring oxygen production in a Rank oxygen electrode at 1000 lux and 10 000 lux light intensity, using BG11-n liquid media. During oxygen production analysis, rates were carefully monitored to check for decreases due to photoinhibition, a possible problem at 10 000 lux; none were observed within the timeframe of analysis used. Samples from oxygen production analysis were retained for chlorophyll-a determination, performed by extracting the chlorophyll with 5 mL of 90% methanol and measuring absorbance at 665 nm. Both rates of oxygen production and chlorophyll-a concentration were normalized to the surface area of the agar harvested. Prolonged exposure to UV may also cause damage (photobleaching) to the light-harvesting pigment, phycocyanin (Donkor and Hader, 1991). To determine if phycocyanin had been damaged by irradiation, the remaining half of the biofilm from each plate was harvested. The filaments were then analyzed on a Zeiss Axioskop MC100 fluorescent microscope (with short-arc HBO Osram mercury lamp and filter to prevent chlorophyll-a fluorescence), and the percentage of filaments that exhibited phycocyanin autofluorescence were counted.

To confirm that cultures could actively grow in mineralizing solutions under high-UV conditions, filaments were cultured on agar until an even and continuous growth across the plates was achieved. Half the cultured agar was then removed from one side of the plate and replaced with fresh agar. These plates were then placed in beakers of either Fe/Si solution or control solutions as previously described, positioned under the UV lamp, and irradiated with UV-C at 0.35 $W \cdot m^{-2}$. The growth of the inoculum across the fresh agar was then monitored.

To further determine the UV shielding capacity of amorphous, iron-bearing silica biominerals, wafers of 150, 200, and 250 μm thickness were prepared by thin-sectioning sinter collected from the Lysúholl hot spring, Iceland. This material was produced by indigenous cyanobacteria and composed entirely of amorphous silica with 0.7% Fe. *Calothrix* was then cultured on agar plates with BG11-n nutrients until an even spread of filaments across the plates was achieved. Sections of the plates were then covered with the iron-silica wafers, each wafer covering an approximately 1.5 × 3 cm area. Other areas of the plates were left uncovered, and the plates were irradiated for 72 h as previously described. UV damage to the filaments in both the covered and uncovered areas was determined also as previously described.

The transmittance of UV through the wafers was determined by using an International Light UV Actinic Radiometer, measuring effective UV dosage. Readings were taken at 15 cm from the UV lamp with the wafers fixed in position directly upon the radiometer's analyzer. The same procedure was performed by using a Macam Q102 radiometer to measure the transmittance of photosynthetically active light (PAL) (400–700 nm), from an 18 W fluorescent lamp.

RESULTS

Silicification of Bacteria

Examination of the bacterial cultures after 3 d by TEM-EDS revealed initial extracellular iron-silica precipitates forming on the filaments in the Fe/Si solution. Analysis of the precipitates by SAED showed that they were amorphous in structure. As the experiment progressed, the filaments steadily became more encrusted in extracellular precipitate, and by 20 d the entire population was totally encompassed (Fig. 1). The thickness of mineralization around each filament was variable, although commonly between 1 and 5 μm, and mineralization always occurred upon the outer surface of the thick fibrous sheath (no intracellular mineralization occurred). Filaments incubated in the control solution remained free from precipitate throughout the incubation period.

Response to Ultraviolet Irradiation

The nonmineralized filaments showed rapid and significant damage by UV light (Fig. 2); only an estimated 15% of the initial filaments remained on the plate after just 96 h of irradiation. Thereafter, damage to the nonmineralized filaments were less pronounced, probably owing to the persistence of a few small clusters of filaments; the lysed cells on top of these clusters provided some UV protection. In contrast, the mineralized filaments displayed a notable resistance to UV; ~90% of them remained after 384 h of irradiation (Fig. 2).

Measurements of photosynthesis show that prior to irradiation, both mineralized and nonmineralized biofilms exhibited very similar rates of photosynthesis of $\sim 27 \times 10^{-11}$ $mol \cdot cm^{-2} \cdot s^{-1}$ at 10 000 lux light intensity (Fig. 3A). However, after just 96 h of irradiation, the nonmineralized biofilm exhibited a very low oxygen production rate of only 1.2×10^{-11} $mol \cdot cm^{-2} \cdot s^{-1}$ at 10 000 lux, and no detectable oxygen production at 1000 lux. In contrast, the mineralized biofilms again displayed a marked resistance to UV; even after 384 h the mineralized biofilm exhibited a rate of photosynthesis on the order of 50% of the value prior to irradiation. Measurement of

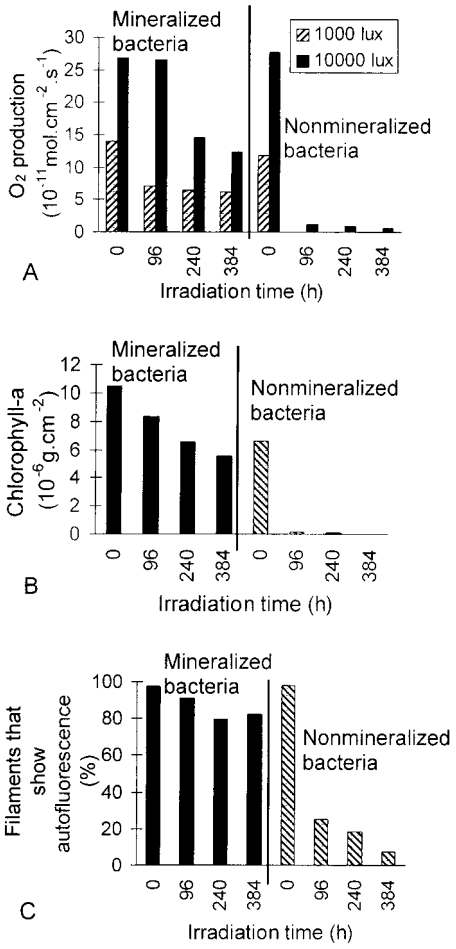

Figure 3. Suite of analyses performed on biofilms removed at 96, 240, and 384 h irradiation. A: Rates of photosynthesis (indicated by oxygen production) normalized to surface area of biofilm, measured at 1000 lux and 10 000 lux light intensity (PAL). B: Chlorophyll-a content. C: Percentage of filaments that exhibit phycocyanin autofluorescence.

chlorophyll-a concentration and phycocyanin autofluorescence corroborate these results. Chlorophyll-a concentration in the mineralized biofilms dropped only slowly, decreasing to half its original value after 384 h of irradiation (Fig. 3B). Conversely, the nonmineralized biofilms suffered rapid chlorophyll-a loss in just 96 h. The nonmineralized filaments also showed a pronounced "bleaching" effect; only 7% of the filaments exhibited phycocyanin autofluorescence after 384 h of irradiation (Fig. 3C). It should be noted here that this decrease in autofluorescence may also have been affected by the loss of phycocyanin from cells during lysis. In contrast, over 80% of mineralized filaments still exhibited phycocyanin fluorescence after 384 h.

Growth rates of filaments in mineralizing and nonmineralizing solutions under high-UV conditions again showed contrasting results. Filaments in the mineralizing solution grew across the agar at a steady rate of 0.2 mm/d, a predictable rate of growth for this naturally slow-growing cyanobacterium in such a low-nutrient solution (10% BG11-n). This result clearly demonstrates that mineralization does not inhibit growth, even under elevated levels of UV radiation. In contrast, the inoculum in the nonmineralizing solution lysed rapidly within 4 d and thus failed to colonize the agar.

The wafers of amorphous, iron-rich silica crust also afforded the bacteria a significant UV shield. The 150-, 200-, and 250-μm-thick wafers provided the bacteria with total UV protection over the first 48 h of irradiation. After 72 h, only 1%–2% of cells had lysed from under those wafers; the losses were due to small (<0.25 mm diameter) holes in the wafers that allowed ultraviolet light to penetrate. Even the thinnest (150 μm) wafer was thick enough to provide near 100% UV protection. In contrast, those areas left uncovered underwent significant UV damage in that almost 70% of the cells lysed in the first 24 h. These results were confirmed by further analysis after 48 h of irradiation (i.e., rate of oxygen production at 10 000 lux: wafer-covered biofilm = 7.0×10^{-11} mol · cm^{-2} · s^{-1}, uncovered = 1.1×10^{-11} mol · cm^{-2} · s^{-1}; phycocyanin autofluorescence: covered = 92.7%, uncovered = 34.8%; chlorophyll-a content: covered = 6.3×10^{-6} g · cm^{-2}, uncovered = 2.4×10^{-6} g · cm^{-2}). Transmittance analysis shows that the wafers exhibit an approximately tenfold increase in UV absorption compared to PAL absorption (Fig. 4). Although it is difficult to interpolate between the zero and 150 μm slice thickness, one may speculate that layers thinner than 150 μm would still produce a significantly higher UV absorption than PAL. The convergence of the data in Figure 4 (wafer set a) at 250 μm thickness reflects the significance at such low transmittance levels of small holes in the sections; the holes allowed as much UV as PAL to pass.

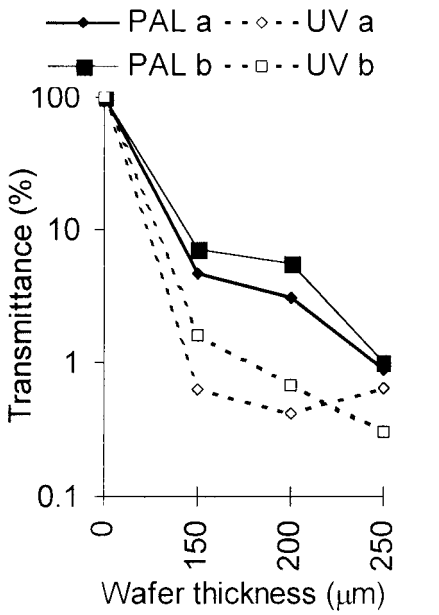

Figure 4. Two data sets (a and b) showing transmittance of UV and PAL through iron-bearing silica biomineral wafers of 150, 200, and 250 μm thickness. Transmittance of UV and PAL through wafers is shown as percentage of original intensity, prior to absorption by wafers.

DISCUSSION

In this study we have shown that cyanobacteria, mineralized in an amorphous silica-iron matrix, exhibited a significant resistance to UV. This finding is confirmed by the conspicuously higher rates of photosynthesis, chlorophyll-a content, and phycocyanin autofluorescence in mineralized biofilms than in nonmineralized biofilms. Additionally, filaments were able to grow and mineralize in silicifying solutions under elevated UV; thus, precipitation of the UV shield did not inhibit the biofilm's ability to recolonize new areas. This likely occurs because as the filamentous cyanobacteria divide, they grow out of the ends of the filament, which is the youngest part of the microorganism, where silicification is most minimal, thus keeping pace with silicification. Those cyanobacteria screened by wafers of natural, amorphous, iron-bearing silica biominerals were also afforded significant UV protection. Amorphous silica also absorbs more UV than photosynthetically active light (PAL) required for photosynthesis (Pierson et al., 1993). Moreover, small concentrations of ferric iron in the silica (100 to 1000 ppm) dramatically increases its UV absorbing capacity, without notably reducing the influx of PAL (Pierson et al., 1993). Although these characteristics have been proved for laboratory-synthesized gels and solutions (Olson and Pierson, 1986; Pierson et al., 1993), the UV and PAL transmittance data collected here confirm that amorphous, iron-bearing silica biominerals also absorb significantly more UV than PAL. This is significant in that the transmittance of PAL is vital to any phototrophs that inhabit a silicified biofilm. Certainly, the fact that the biomineralized filaments in this study exhibited significant rates of photosynthesis substantiates that such biomineralization may not inhibit photosynthesis. This has been noted in a previous study by Phoenix et al. (2000), which showed that cyanobacteria can survive the mineralization process through the formation of an extracellular sheath, which acts as an alternative mineral nucleation site and filter against colloidal silica, preventing detrimental cell-wall and cytoplasmic mineralization. Thus, by providing a mechanism to

survive silicification, silicification may become advantageous to the microorganism.

Chemical analyses of silica biominerals from modern hot springs indicate that the small quantities of iron required to form an effective UV shield are generally present, even when the iron levels in the effluent water are very low (Konhauser and Ferris, 1996). This fact is illustrated by electron-microprobe analysis of silica biominerals collected from Krisuvik, Iceland, which showed that the sinter had iron levels (presumably Fe^{3+}) of ~300 ppm, despite iron levels in the spring water being as low as 0.05 ppm. Similarly, analysis of Precambrian cherts has shown iron levels exceeding 1000 ppm (Tazaki et al., 1992), and they therefore contain enough iron to form an effective UV shield.

The UV shielding capacity of iron-bearing silicate biominerals may have been important to early life forms. Biomineralization would have been a characteristic of Archean microbial life because of the high concentrations of dissolved silica (Siever, 1992) and iron (Morris, 1993) in the ancient oceans. This conclusion is further substantiated by the excellent preservation of microfossils in Precambrian cherts and stromatolites (e.g., Lowe, 1983; Schopf and Packer, 1987), where there is significant evidence that the silica is primary in origin (Sugitani, 1992). It follows that those early life forms could have precipitated their own UV shield in such a solute-rich environment, simply by serving as a template for silicification, thus inducing passive biomineralization (Schultze-Lam et al., 1995; Konhauser and Ferris, 1996). Furthermore, the cyanobacteria would have been able to function and grow, once silicified, allowing colonization and bacterial diversification of shallow-water environments intermittently exposed to the atmosphere.

These early life forms are likely to have utilized many of the screening mechanisms exhibited by modern cyanobacteria, such as phototaxis (Margulis et al., 1976), matting habit (Rambler and Margulis, 1980), sheath pigmentation (Garcia-Pichel and Castenholz, 1991), and photo repair mechanisms (Walter, 1983), and silicification may have worked in conjunction with these to protect the microorganism from harmful UV. Although these mechanisms are highly viable, phototaxis and sheath pigmentation can be damaged by UV (Donkor and Hader, 1991). Also, those cells in the upper layers of a biofilm, which may provide a UV shield, are damaged by irradiation and eventually lyse. Subsequently, they must be continuously replaced by fresh cells from below (Pierson et al., 1993; Pierson, 1994). The extensive sheaths of *Calothrix* failed to effectively prevent cell lysis in this study; any UV-absorbing pigments they contained were ineffective against high levels of UV-C. It follows that the susceptibility of these mechanisms to ultraviolet damage may result in the destruction of the microbial colony. Siliceous biominerals, however, undergo no such damage, and we suggest that they therefore provided a more resilient UV shield.

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