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Cryogenic silicification of microorganisms in hydrothermal fluids

Mark G. Fox-Powell [a,b], Alan Channing [c], Daniel Applin [d], Ed Cloutis [d], Louisa J. Preston [e], Claire R. Cousins [a,b,*]

[a] School of Earth and Environmental Sciences, University of St Andrews, Irvine Building, North Street, St Andrews, Fife, KY16 9AL, UK
[b] St Andrews Centre for Exoplanet Science, University of St Andrews, UK
[c] School of Earth and Ocean Sciences, Cardiff University, Cardiff, Wales, CF10 3AT, UK
[d] Department of Geography, University of Winnipeg, Winnipeg, MB, R3B 2E9, Canada
[e] Department of Earth and Planetary Science, Birkbeck, University of London, Malet St., Bloomsbury, London, UK

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ABSTRACT

Silica-rich hydrothermal fluids that experience freezing temperatures precipitate cryogenic opal-A (COA) within ice-bound brine channels. We investigated cryogenic silicification as a novel preservation pathway for chemo- and photo-lithotrophic Bacteria and Archaea. We find that the co-partitioning of microbial cells and silica into brine channels causes microorganisms to become fossilised in COA. Rod- and coccoidal-form Bacteria and Archaea produce numerous cell casts on COA particle surfaces, while *Chloroflexus* filaments are preserved inside particle interiors. COA particles precipitated from natural Icelandic hot spring fluids possess similar biomorphic casts, including those containing intact microbial cells. Biomolecules and inorganic metabolic products are also captured by COA precipitation, and are detectable with a combination of visible – shortwave infrared reflectance, FTIR, and Raman spectroscopy. We identify cryogenic silicification as a newly described mechanism by which microbial biosignatures can be preserved within silica-rich hydrothermal environments. This work has implications for the interpretation of biosignatures in relic hydrothermal settings, and for life-detection on Mars and Enceladus, where opaline silica indicative of hydrothermal activity has been detected, and freezing surface conditions predominate.

1. Introduction

Opaline silica (opal-A) has been a common preservation agent for microorganisms throughout the Earth's geological record (Cady and Farmer, 1996; Westall et al., 2015), and is found in many neutral-alkaline hydrothermal systems worldwide. Precipitated by the cooling of aqueous hydrothermal solutions, colloidal silica is widely observed to mineralise microbial cells (Cady and Farmer, 1996; Jones et al., 2001) and mats, and associated organic material (Preston et al., 2008) in modern-day siliceous hot spring environments. Ambient temperature groundwater springs are also capable of precipitating silica and entombing microbes, preserving them as microfossils (Grasby et al., 2009), and relic Archaean hot spring silica sinters may preserve some of the oldest evidence for life on Earth (Djokic et al., 2017). This physical capturing and subsequent silicification of microbial biosignatures makes opaline silica an attractive target material in the search for life beyond Earth, namely on Mars, and icy moons (i.e. Europa and Enceladus) (Westall et al., 2015; Ruff and Farmer, 2016).

Opal-A has been observed on Mars (Milliken et al., 2008; Squyres et al., 2008; Skok et al., 2010; Ruff et al., 2011) in association with volcanic centres (Skok et al., 2010) and hydrovolcanic structures (Rice et al., 2009), while one of the shortlisted landing sites for the NASA Mars 2020 rover includes a relic hydrothermal system bearing opal-A deposits (Squyres et al., 2008; Farley and Williford, 2017). Recent detections of colloidal silica associated with Enceladus (Hsu et al., 2015), and molecular hydrogen within the Enceladan cryovolcanic plumes (Waite et al., 2017) offer the strongest evidence to date for ongoing hydrothermal activity on another planetary body, and suggests that the liquid water sourcing these plumes has conditions suitable for biological methanogenesis (Waite et al., 2017; Taubner et al., 2018). The sub-freezing surface temperatures that have prevailed on Mars for much of its history (Carr and Head, 2010), and characterise present-day conditions on icy moons, mean that silicification mechanisms will differ from those typically observed on Earth. This has direct implications for the preservation of any resident microorganisms and their biosignatures.

* Corresponding author at: School of Earth and Environmental Sciences, University of St Andrews, Irvine Building, North Street, St Andrews, Fife, KY16 9AL, UK.
E-mail address: crc9@st-andrews.ac.uk (C.R. Cousins).

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Under non-freezing conditions, silica-chloride hot springs precipitate siliceous sinters through the cooling of hot (>100 °C) silica-supersaturated hydrothermal fluids, which leads to the nucleation and eventual sedimentation of colloidal silica. When warm hydrothermal fluids are discharged into a freezing environment, the crystallisation of water as ice accelerates the precipitation of colloidal silica, which is forced into brine veins in between ice crystals, along with dissolved solutes (such as Na^+ and Cl^-) (Channing and Butler, 2007). The result is the precipitation of cryogenic opal-A (COA) particles with distinctive morphologies defined by the physical dimensions within the brine veins (Channing and Butler, 2007). Microorganisms are known to partition into brine vein networks in ice, exploiting them as a liquid water microenvironment (Mader et al., 2006). However, the fate of microorganisms when opal-A precipitates within these brine channels has not been previously investigated. Terrestrial environments where both silica and microorganisms can be co-partitioned during ice formation are found in geothermal regions in Iceland and Yellowstone National Park (USA) where the air temperature is seasonally sub-zero °C for >50% of the year (Channing and Butler, 2007; Jones and Renaut, 2010), and in high altitude geothermal systems such as El Tatio (Chile) (Nicolau et al., 2014). Silicified microorganisms associated spatially with COA particles from Iceland have been previously observed (Jones et al., 2001), however without any prior demonstration of silicification under cryogenic conditions, it is not possible to ascertain whether they formed cryogenically or during subsequent mineralisation under non-freezing conditions. Here, we combine an examination of natural COA samples from Iceland with experimental cyrosilicification of microorganisms to investigate the preservation (or lack thereof) for microorganisms and associated spectral biosignatures during freezing of silica-rich hydrothermal fluids.

2. Materials and methods

2.1. Microbial strains and growth conditions

Four previously described microbial strains were selected to experimentally test the process of microbial cryosilicification. The strains were chosen primarily to capture a range of morphological, metabolic and phylogenetic diversity representative of microorganisms found across a diverse range of hydrothermal systems (Skirinisdottir et al., 2000; Hedlund et al., 2013; Akimov et al., 2013), with an emphasis on readily-cultivable strains isolated from high-temperature neutral-alkaline environments. Where possible, strains isolated from hydrothermal systems were used. Strains included (*i*) the thermophilic sulfate-reducing bacterium *Thermodesulfovibrio islandicus* DSMZ-12570 (Sonne-Hansen and Ahring, 1999), (*ii*) the thermophilic methanogenic archaeon *Methanoculleus thermophilus* DSMZ-2373 (Rivard and Smith, 1982), (*iii*) the filamentous anoxygenic phototroph *Chloroflexus aurantiacus* DSMZ-635 (Pierson and Castenholz, 1974), and (iv) the photoferrotrophic bacterium *Rhodopseudomonas palustris* TIE-1 (Jiao et al., 2005). *R. palustris* grows readily with either Fe^{2+} or acetate as an electron donor for phototrophy, thus batches cultivated on both substrates were used in experiments to assess differences in silicification and resulting biosignatures for these different metabolic pathways. Growth media and conditions were all as described previously (Sonne-Hansen and Ahring, 1999; Rivard and Smith, 1982; Pierson and Castenholz, 1974; Jiao et al., 2005), with the exception of *C. aurantiacus*, which was cultivated under natural daylight in a clear-fronted incubator rather than under the recommended 1000 lx. This resulted in slower growth but no discernible difference to cellular morphology.

2.2. Experimental silicification

Sodium metasilicate ($Na_2SiO_3.5H_2O$) was dissolved in deionized water to create a synthetic "hydrothermal" fluid at 500 ppm Si, similar to that used for previous cryogenic opal-A experiments (450 ppm Si; Channing and Butler, 2007), and also to concentrations found in hot spring fluids at Strokkur (476 ppm dissolved Si; Konhauser and Ferris, 1996) and in Yellowstone National Park (312–654 ppm) (Fournier, 1989). This solution was buffered to pH 7.7 using HCl, and sterilised via 0.22 µm filtering. Cultures of microorganisms were harvested by centrifugation (10,000× g for 15 min), washed once in phosphate-buffered saline (130 mM NaCl, 1 mM NaH_2PO_4, 9 mM Na_2HPO_4, pH 7.7), and once in sterile 500 ppm silica solution. Washed cells were resuspended in sterile 500 ppm silica solution that had been preheated to 55 °C. An aliquot of sterile 500 ppm silica solution served as an experimental blank. All experiments were frozen at −20 °C for 24 h, after which they were thawed and prepared for analysis.

2.3. Field sampling

Fresh, naturally-formed cryogenic silica gels were collected from frozen hydrothermal fluid on the Strokkur outflow apron, Geysir geothermal area, Iceland (64.312542°N; 20.300367°W) in January 2007 following the protocol in Channing and Butler (Channing and Butler, 2007). The Strokkur spring was the focus of sampling as the Geysir spring has become much less active and is no longer continuously discharging fluid. Strokkur hydrothermal fluid geochemistry was comparable to the experimental set-up, with alkaline (pH 8.5) fluids previously characterised by 476 ppm dissolved Si (Konhauser and Ferris, 1996). COA particles were extracted from these fresh gels as with the experimental samples, with further volumes melted and evaporated within sterile Petri dishes for visible – shortwave infrared (Vis-SWIR), FTIR, and Raman spectroscopic analysis. Natural hydrothermal fluids and their indigenous microbial communities were also collected for laboratory cyrosilicification. These were collected aseptically from the Strokkur outflow in sterile bottles in February 2017, where the dissolved Si content was 149 ppm (see below); considerably lower than previously measured (Konhauser and Ferris, 1996). These fluids were then frozen at −20 °C in the laboratory, and the resulting COA precipitates extracted as described above.

2.4. Determination of dissolved silica concentration

Dissolved silica content of a fluid sample from the Strokkur discharge apron was determined by Inductively Coupled Plasma Optical Emission Spectroscopy (ICP-OES) at the Open University using a Prodigy7 (Teledyne–Leeman) OES system at 250.960 nm. Samples were diluted by a factor of 20 prior to analysis.

2.5. Optical and fluorescence microscopy

For optical microscopy, aliquots of thawed experimental samples and thawed natural fluids were transferred to glass slides and allowed to fully evaporate, before being gently rinsed with 0.22 µm-filtered MilliQ water to remove salt crystals. These were imaged using a Keyence VHX 2000 digital microscope. For fluorescence microscopy, aliquots of defrosted sample were stained with 1× SYBR Gold (Invitrogen), incubated at room temperature in the dark for 15 minutes, and mounted on 25 mm diameter, 0.22 µm pore size black polycarbonate filters. Samples were excited at 490 nm and imaged at 590 nm using an Amscope T600 series epifluorescence microscope.