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Crystalline silica prepared at room temperature from aqueous solution in the presence of intrasilica bioextracts

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Silica, exhibiting crystalline texture has been prepared from aqueous solution at pH *ca.* 7 in the presence of intrasilica biomolecules extracted from the primitive plant *Equisetum telmateia*; some of the silica shows interplanar lattice spacings of 3.5 Å and is present in lath-like objects up to *ca.* 20–30 nm wide and more than 100 nm in length; an electron diffraction pattern obtained from this material also shows interplanar spacings of 3.46, 2.13 and 1.70 Å suggesting that the material might be quartz.

In the field of biomineralization where biological organisms are able to regulate the formation of both crystalline and amorphous composite mineral phases there is much interest in understanding how mineralization occurs.[1] The process requires the concentration of selected elements, the nucleation, growth and moderation of growth of the mineral phase in predefined locations at specific times during the lifetime of a particular organism. Biominerals are usually composite phases containing organic components such as membranes and/or proteins and carbohydrates together with the mineral phase. The relationship between the two is thought to be important in the regulation of mineral composition, crystallographic phase and morphology.[2] The organic phase is largely found external to the mineral phase but low levels (*ca.* 0.03% by weight) of acidic proteinaceous biopolymers can also be found intercalated within the mineral phase, perhaps at crystal domain boundaries.[3] The intercalated biopolymers may have fundamental roles to play in nucleation and/or crystal growth and their presence has also been shown to have dramatic effects on the mechanical properties of crystalline biominerals.[4] For amorphous biominerals such as silica, biopolymers are also found in intramineral locations,[5–7] however, their role in nucleation, particle growth and aggregation remains unclear.

We are interested in extending our understanding of how biosilicas with specific form[8,9] are laid down and additionally we are interested in the preparation of novel silica phases using knowledge gained from the study of biological systems. To this end we have extracted protein-containing biomolecules from intrasilica locations in the branches of *Equisetum telmateia*[5] and used them in the study of silica precipitation at circumneutral pH.† The biopolymer extract used in this study was released by solubilization of the siliceous phase with buffered solutions of HF following treatment of plant materials with a mixture of concentrated nitric and sulfuric acids. The amino acid composition of this extract was rich in serine, glutamine/glutamic acid and glycine and had associated with it a carbohydrate component enriched in glucose and xylose. The 'model' system used to study particulate silica precipitation utilized a catecholato complex of silicon, $K_2[Si(C_6H_4O_2)_3]\cdot xH_2O$ as the source of soluble silicon.[10] At *ca.* physiological pH the complex partially decomposes to yield orthosilicic acid which immediately undergoes polycondensation reactions to relieve any resultant supersaturation.

50 mM solutions of potassium silicon catecholate were used with/without the biopolymer extract at 1% w/w, the pH of the solution was lowered to *ca.* 7.0 by the addition of a predetermined quantity of HCl and the concentrations of orthosilicic acid measured as a function of time by a colorimetric molybdenum blue method.[11] Kinetic analysis of the solution data was carried out in accordance with our previous studies.[12]‡

The effect of the biosilica extracts on both the early oligomerization reactions (*e.g.* the formation of dimers and trimers of orthosilicic acid) and on particle growth by precipitation/dissolution reactions was studied. Electron microscopy studies of the precipitates gave information on the effect of solution additives on nucleation, particle growth and aggregation.

Statistical analysis (students t-test and Mann Whitney U-test)[13] of the kinetic data‡ showed that the addition of 1% w/w of the *Equisetum telmateia* intrasilica extract to the silica oligomerization medium results in a *ca.* 22% increase in the rate of trimer formation (the second reaction in the oligomerization process). The rate constant for the addition of a monomer to an oligomer larger than a trimer is statistically no different for samples including the proteinaceous extract but the rate of removal of monomers from oligomers is reduced by *ca.* 33%. The effect of these changes is to increase the rate at which orthosilicic acid is removed from solution and to reduce the amount of silicon left in solution at the end of the experiment. Fig. 1 shows the increase in the levels of oligomerized silica at all sampling points. Electron microscopy of the precipitated silica shows evidence for aggregates built up from small particles *ca.* 1–2 nm in diameter, much smaller than is expected for the 'blank' system (data not shown, see ref. 10 for an example). Other structures present include ribbon-like (lath-like) objects and curved loops which often show characteristic fringes of *ca.* 3.50 Å. Energy dispersive X-ray analysis of all such areas indicates that the material contains silicon and oxygen.† The ribbon-like structures and loops, Fig. 2(a)–(c), are found in all samples from 1 h after initiation of the precipitation reaction. Samples taken at 48 h after the start of the experiment occasionally exhibit order over distances > 600 nm in length and *ca.* 20–30 nm in width. The images obtained suggest a 'soft' or perhaps 'layered' material (for an example, see ref. 14) but the electron diffraction patterns obtained from some areas with *d*-spacings of 3.46, 2.13 and 1.70 Å are compatible with quartz [PDF 5-490], Fig. 2(d). X-Ray diffraction data could not be obtained from silica collected by centrifugation 7 days after initiation of the reaction (even after an extended scan over 8 h) indicating that the silica sample prepared in the presence of the

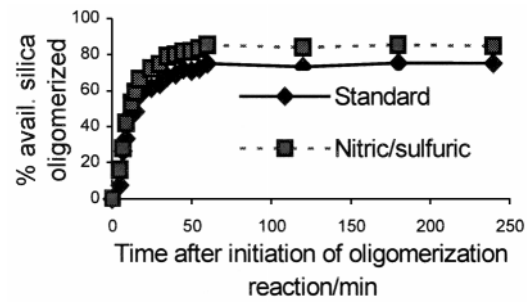

Fig. 1 Plot of % of oligomerized silica (not detectable by the molybdate method) *vs.* time.

biopolymer extract contains only low levels of crystalline material. No additional information was obtained from diffraction studies at low values of 2θ suggesting that the siliceous materials are not mesoporous, lamellar phases.

The presence of the protein-containing extract in the oligomerizing mixture clearly had some effect on nucleation with the formation of (a) smaller particles of silica (the majority of fundamental particles are smaller than 2 nm in diameter as opposed to particles up to 4 nm in diameter for the blank system) and, (b) silica with a crystalline appearance. The presence of the latter material from the earliest analysis point (as assessed by electron microscopy) suggests that this material is formed very early in the reaction profile and is not the result of structural rearrangement with time.

Biopolymers can be removed from the silica precipitated in the 'model' reaction system utilizing the same methods used for their initial production. The levels of proline are decreased and the levels of glycine are *ca*. doubled in the proteinaceous material extracted from the 'model' silica precipitated materials. The amino acid composition of the biopolymer extracts is rich in amino acids known to form β-sheet or β-turn secondary structures. For such structures, the spacing between successive layers is a minimum of 3.5 Å when only glycine is involved but may be 5.7 Å for chains rich in alanine as is found in the silk protein fibroin.[15] It is possible that the observed silica structures are either crystalline silica formed *de novo* from aqueous solution, or more likely, the observed structures are generated by epitaxial matching of the organic and inorganic matrices with the silica structure continuing to develop from the initial biopolymer-controlled nucleation event. It is evident that in the biological environment additional controls must be exerted during the process of silica precipitation in order to prevent the formation of crystalline phases as they are much more difficult to mould into the macroscopic structures produced by living organisms.

Further work will involve identification of the biopolymer component(s) (preliminary studies have shown that the extracts contain both high molecular weight proteins and low molecular weight glycoproteins) which are most effective in the spontaneous generation of crystalline silica structures from supersaturated solutions at room temperature, neutral pH and in the absence of multicharged cations, conditions which would not be expected to yield crystalline silica.[11,16]

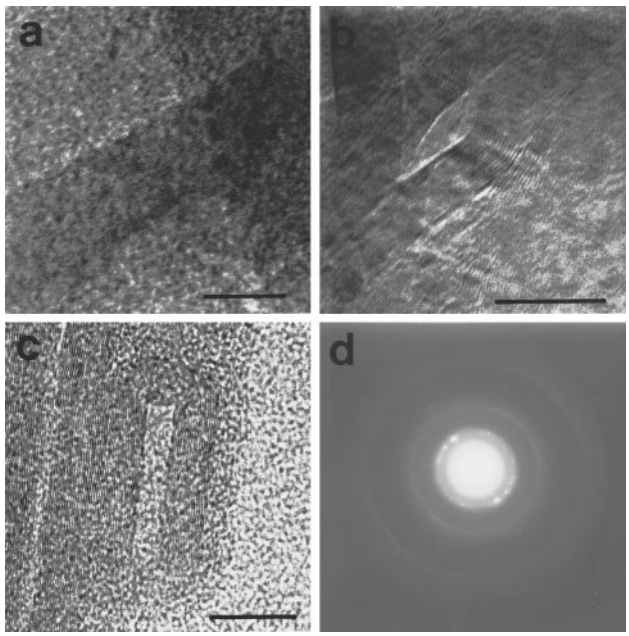

Fig. 2 (a)–(c) Transmission electron microscopy data for unusual silica structures precipitated in the presence of 1% w/w biopolymer extracts. All images shown are from microscope grids prepared by the 'dipping' method. Scale bars represent 10 nm; (d) electron diffraction pattern from (b). Pattern was recorded using a camera length of 100 cm.

Notes and references

† Precipitation experiments were conducted using biopolymer extracts from two separate extractions. The amino acid compositions were measured using an Applied Biosystems 420a amino acid analyser operated by A. C. Willis of the MRC Immunochemistry Unit, Oxford University. For the kinetic measurements, four sample runs were completed for each experiment on the same day using a temperature controlled reaction vessel set at 23 ± 0.1 °C. Samples for electron microscopy studies were taken from parallel experiments by dipping carbon coated formvar covered copper electron microscope grids into the reaction vessel at 1, 4, 24, 48 h and 7 days after the experiment was initiated and allowing the grids to air dry. Samples for electron microscopy were investigated using a JEOL 2010 analytical electron microscope (Link ISIS system) fitted with a LaB_6 filament operating at 200 keV. Magnifications of 200 000 × were necessary to see the lattice fringing present and areas of interest were subjected to energy dispersive X-ray analysis to identify the elements with atomic number ⩾6 present in the sample. A minimum of 10 analyses for each sample area showed that the precipitated material contained Si and O together with traces of K and Cl (N.B. the grids were not washed after sampling by dipping). Crystalline structures as presented in this paper have not been observed by transmission electron microscopy in any of our other model precipitation experiments performed in the presence of a range of singly and multiply charged metal ions, carbohydrates and proteins such as bovine serum albumin, zein, concanavalin A and cytochrome c. Silica samples were also analysed by powder X-ray diffraction; Siemens D500 diffractometer operating in the range 2θ = 1–80° by Professor Mark Weller and Dr Adam Whitehead of Southampton University. The Visual Services Department at The Nottingham Trent University are thanked for printing of the electron micrographs for publication. BBSRC and Crosfield Chemicals are thanked for their funding.

Amino acid composition of bioextract used in the precipitation experiment (mol%); Asx; 8.9, Glx; 15.0, His; 3.5, Lys; 4.94, Arg, 1.54, Ser; 14.24, Thr; 3.91, Tyr; 1.30, Gly; 16.67, Pro; 9.60, Ala; 8.42, Val; 4.07, Leu; 4.3, Ile; 2.64, Phe; 0.98 Amino acid composition of biopolymers extracted from 'model' system precipitated silica (mol%); Asx; 7.56, Glx; 12.3, His; 5.7, Lys; 2.73, Arg, 3.4, Ser; 12.92, Thr; 4.41, Tyr; 2.35, Gly; 30.46, Pro; 4.06, Ala; 6.95, Val; 2.94, Leu; 4.23, Ile; nd, Phe; nd. nd = not detected.

‡ Rate constants:[13] blank system; $k_3 = 4.91 \times 10^{-6}$ mmol^{-2} dm^6 s^{-1}, $k_+ = 5.67 \times 10^{-4}$ s^{-1}, $k_- = 1.19 \times 10^{-5}$ s^{-1}. With 1% biomolecule extracts; $k_3 = 6.29 \times 10^{-6}$ mmol^{-2} dm^6 s^{-1}, $k_+ = 4.73 \times 10^{-4}$ s^{-1}, $k_- = 7.97 \times 10^{-6}$ s^{-1}.

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