Enzyme-mediated sol–gel processing of alkoxysilanes

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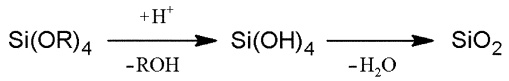
Certain proteolytic enzymes are capable of mediating the processing of tri- and tetra-alkoxysilanes to form monolithic silica *via* the sol–gel process, or silsesquioxane sol–gels under green, solvent-free conditions.

Many marine organisms and plant species have the ability to process silica from their environment to form siliceous spicules, skeletal structures, or crystalline deposits.[1,2] In recent years several groups have reported that this phenomenon can be replicated *in vitro*. Morse and co-workers,[3] Perry and Keeling-Tucker,[4] Kröger and co-workers,[5] and Sumper and co-workers[6] have demonstrated that various biomolecules can direct the *in vitro* polymerization of silicic acid, or silicic acid precursors, to form particulate silica. Several biomimetic approaches to silica formation,[7,8] as well as to the behaviour of silicates in aqueous media, have also been presented.[9]

Enzymes not traditionally associated with silica processing have also been used to perform chemistry at and near silicon. Lipase from the bacterium *Burkholderia cepacia* was shown to accelerate the rates of hydrolysis and condensation of a mixture of methyltrimethoxysilane (MTMS) and tetramethoxysilane (TMOS).[10] Zelisko *et al*. utilized trypsin as a catalyst to cross-link triethoxysilyl-modified silicone polymers.[11] Research reported by Bassindale *et al*. demonstrated that trypsin facilitated the homodimerization of trimethylethoxysilane, and trimethylsilanol; trypsin also performed the ring-opening hydrolysis and condensation of 1,1-dimethyl-1-sila-2-oxacyclohexane.[12]

The sol–gel processing of alkoxysilanes affords a relatively mild method of producing inorganic–organic hybrid materials.[13] Silica sol–gels have been used as biosensors and are suitable for entrapping delicate biomolecules (enzymes and catalytic DNA aptamers) without significant loss of activity in the biomolecule.[14] Typically, these reactions are performed using an alcohol solvent to solubilize both the alkoxysilane and water, and are usually initiated by acid or base catalysis (Scheme 1).[15] However, the initiatives to reduce the use of volatile organic compounds (VOCs) and the associated environmental implications[16] have prompted us to explore routes toward a solvent-free methodology for the synthesis of particulate and monolithic silica.

In this communication we report that α-chymotrypsin and trypsin are able to act as catalysts for the production of silica gels from tetraethoxysilane (TEOS), albeit by two different routes. α-Chymotrypsin was able to generate silica sol–gels from TEOS that were virtually indistinguishable from those produced *via* acid catalysis (see later in Fig. 1). When trypsin was employed, however, a silica monolith was produced but not *via* the sol–gel process.



Scheme 1 The hydrolysis and condensation of a tetraalkoxysilane under acidic conditions.

α-Chymotrypsin- and trypsin-mediated reactions, along with enzyme-free, and inhibited enzyme control reactions were prepared in 4 mL screw-cap vials by dissolving the enzyme (5.0 mg, 210 nmol, 10 mol%) into 160 μL of distilled water (pH 6.4), followed by the addition of tetraethoxysilane (TEOS) (500 μL, 2.2 mmol). The ratio of water-to-silicon was maintained at 4 : 1 for all reactions. The reaction flasks were mixed by inversion for 5 min with subsequent exposure to air for 30 min. Gels were left to age at room temperature. Silica xerogels were prepared by ageing the gels in capped vials at room temperature until spectra were acquired.

Following 48 h of incubation at ambient temperature (*ca*. 22 °C), those reactions mediated by α-chymotrypsin had progressed from a biphasic mixture to a uniform sol that was optically transparent. After ageing for an additional 24 h the sol had transformed into a solid, optically transparent silica monolith. Those reactions mediated by trypsin did not follow a similar route: the formation of a sol was never observed, favouring instead the initial formation of monolithic silica that corresponded only to the volume of water that was included in

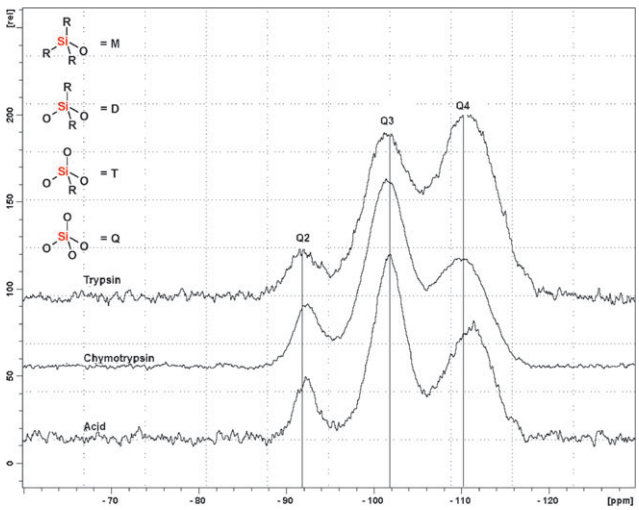


Fig. 1 Solid-state ^{29}Si NMR spectra of silica gels produced under different catalytic conditions.

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the reaction. With time the silica grew so that all TEOS was incorporated into the final gel. Those reactions that did not contain any enzyme did not form sols, sol–gels, or monolithic silica; the reactions remained biphasic at all times.

To ensure that the active site of the enzyme was indeed responsible, and required, for mediating the transformation of TEOS into a silica monolith, α-chymotrypsin and trypsin were inhibited with soybean inhibitor (Worthington Biochemical Corporation, Lakewood, New Jersey). Excess inhibitor and enzyme were combined and allowed to stir at room temperature for 2 h, after which time TEOS was added and the procedure followed as outlined previously. Formation of a silica monolith was never observed following inhibition of the enzyme.

The macromolecular structures of the silica monoliths were compared with one that was prepared by the more traditional route employing acidic conditions. Solid-state ^{29}Si nuclear magnetic resonance (NMR) spectra were acquired on a Bruker AV 600 spectrometer with a 5 mm MAS broadband probe head. A delay of 10.0 s was used and 30–40k scans were collected for each sample. NMR spectra were analyzed using Bruker Topspin software v2.1. The solid-state ^{29}Si NMR spectra of the α-chymotrypsin-, trypsin-, and acid-mediated tetraethoxysilane monoliths showed chemical shifts arising at −91.98, −101.19, and −110.55 ppm as expected for the Q^2, Q^3 and Q^4 regions of a ^{29}Si NMR spectrum corresponding to a silica monolith (Fig. 1). There was no discernable difference between the sol–gels produced under acidic or α-chymotryptic conditions. The ratio of the Q^3 : Q^4 peaks, determined by Gaussian line fitting and deconvolution, gave Q^3 : Q^4 peak ratios of 1.37 : 1 for α-chymotrypsin-mediated sol–gels, and 1.18 : 1 for acid-catalyzed sol–gels. The increased proportion of Q^3 peak observed using α-chymotrypsin as a catalyst indicates a higher degree of functionalization from silanol, residual ethoxide groups, or covalent modification by α-chymotrypsin. However the trypsin-mediated gel had a decreased proportion of Q^3 to Q^4 peak intensities (0.77 : 1) suggesting that condensation was more complete than in the previous examples.

To determine the generality of using proteins as catalysts for the sol–gel processing of alkoxysilanes, we expanded our study to include the carboxypeptidase pepsin, the cysteine proteases bromelain and papain, lipase from *Candida rugosa* (which has the same catalytic triad as the serine proteases),[17] and the transport protein human serum albumin (HSA). Phenyltrimethoxysilane (PhTMOS) was included as an organically-modified alkoxysilane in addition to TEOS in these experiments. Reactions containing PhTMOS were carried out and analyzed in the same manner as described above, but the water-to-silicon ratio was altered to 3 : 1 to compensate for the reduced number of hydrolyzable groups on silicon. Like trypsin, pepsin and bromelain yielded silica when challenged with TEOS, but not *via* the sol–gel process. Papain, lipase, and HSA were unsuccessful in mediating silica formation and only minute amounts of precipitated silica were observed. However, when PhTMOS was used as the substrate, pepsin also mediated the formation of monolithic silica *via* a sol–gel. The data indicate that bromelain, papain, HSA, and lipase do not generate silsesquioxane sols or sol–gels when PhTMOS is used as the silane monomer.

We were interested in how the reaction involving PhTMOS progressed and if all of the expected intermediates were being formed. To probe this question, two solution phase ^{29}Si NMR experiments were conducted: (i) an enzyme-free control reaction to monitor background rates of hydrolysis, and (ii) a trypsin-mediated hydrolysis and condensation of PhTMOS.

Reactions were constructed in the same way as described above, but were performed in a 10 mm glass NMR tube, and were diluted to 1.0 mL with D_2O. In total, 60–61 spectra were acquired for each sample at one-hour intervals.

In the absence of any enzyme there was not a significant amount of hydrolysis after 60 h (Fig. 2, top panel). The predominant peaks were located at −55.5 ppm, and −51.5 ppm corresponding to unreacted PhTMOS and hydrolysis products respectively. In the reaction mediated by trypsin, hydrolysis of the ethoxy moieties had occurred after 1 h, and condensation after 4 h (Fig. 2, bottom panel). Evidence consistent with the condensation of the hydrolysis products began to appear at −61.0 ppm (4 h) with polycondensation products arising at −70.0 ppm (20 h). We are currently conducting experiments that will allow for the complete assignment of all of the peaks associated with the entire reaction coordinate. Unfortunately, we were unable to visualize the T^3 peak expected at approximately −81.0 ppm that

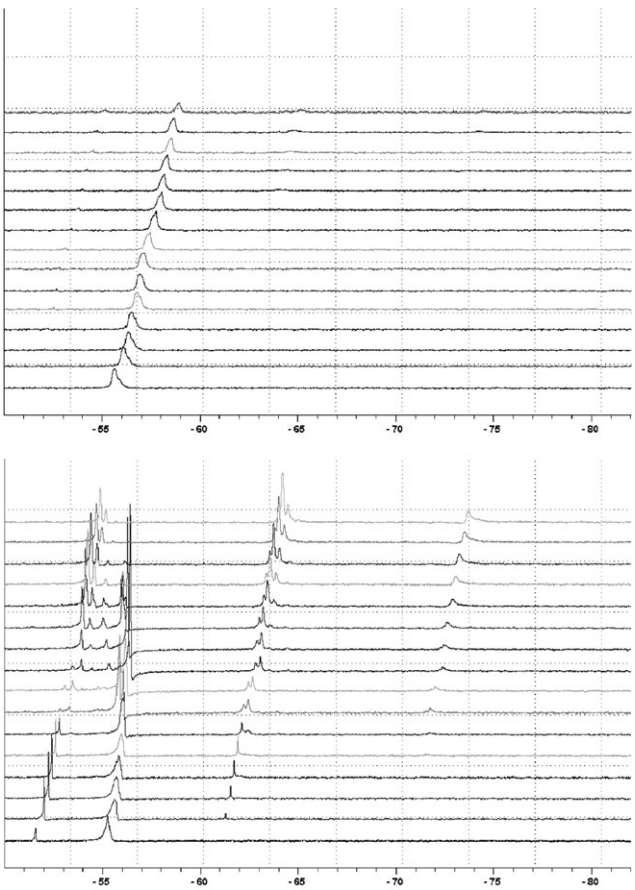

Fig. 2 ^{29}Si NMR spectra of the reaction coordinate for the hydrolysis and condensation of phenyltrimethoxysilane under enzyme-free conditions (top panel), and as mediated by trypsin (bottom panel). The spectra begin at t = 1 h (bottom spectrum) and are representative of the reaction at 4 h intervals up to t = 60 h (top spectrum, top panel) and t = 61 h (top spectrum, bottom panel) for each set of reaction conditions.

would correspond to phenylsilsesquioxane monoliths due to depletion of the D_2O solvent which was evolved as deuterated ethanol, and resulted in loss of the deuterium lock.

This is the first evidence that proteolytic enzymes can be used to mediate the sol–gel processing of alkoxysilanes under solvent-free conditions; typical methods employ alcohols as co-solvents with water.[15] Previous work highlighted the utility of trypsin in catalyzing the formation of disiloxane linkages or the ring-opening hydrolysis of cyclic alkoxysilanes,[12] while silicatein,[3] silaffins,[6] and several polyamines[7] have been used to generate particulate silica, whereas this is the first reported case of enzymes mediating the formation of monolithic silica *via* the sol–gel process. From a biotechnological perspective it is interesting to note that similar enzymes yield different reaction products from common starting materials. This specificity has the potential to impart nanoscale control over silica architectures and opens the door for many applications such as immobilized catalysts or biomedical devices.

Through the judicious combination of enzyme and alkoxysilane species it is possible to affect the formation of particulate, but more importantly, monolithic silica; the latter being synthesized *via* the sol–gel process using 'green', solvent-free conditions. Not only can the enzymes be used to generate silica from tetraalkoxysilanes using sol–gel chemistry, organically-modified silsesquioxanes can be formed using a similar process.

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