Non-Peptide, Silicatein α Inspired Silica Condensation Catalyst

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Introduction

A major challenge faced by ceramic chemists today is the production of fully polymerized silica by low temperature (< 50°C) solution routes.[1] Current methods involve high temperature, extreme pH, or both.[2] Biological systems, however, have the ability to produce silica under much more benign conditions.[3] These include near neutral pH and temperatures at or below room temperature. Our goal is to take what has been learned from biology and develop a bioinspired synthetic analog that can be used to produce ceramic materials which are unavailable by current techniques.

The protein Silicatein α has been previously shown[4-6] to catalyze the production of poly(silicate) from tetraethoxysilane (TEOS) monomer. It does this at neutral pH and ambient temperature. The source of Silicatein α for this study was the marine sponge *Tethya aurantia*. This sponge contains silica needles that constitute up to 75% of its dry weight. Occluded in these silica needles are protein filaments that catalyze their synthesis. About 70% of this protein mass is Silicatein α.

Mimicking Silicatein α with synthetic proteins has been shown to be feasible.[7] Proteins were synthesized by a ring opening polymerization method using cyclic amino acid analogs as monomers. Homo- and block- proteins were made and tested for catalytic function in the condensation of TEOS. This work showed that the secondary structure of the protein was not critical to its catalytic function. It also provided evidence as to which amino acids, and thus which functional groups, are critical to the protein's catalytic function. Here, we summarize our work on a purely synthetic, non-peptide copolymer to catalyze the polymerization of silica from a solution of TEOS.

Experimental

Reagents. TEOS, ethanol, and 1.0 M 9-BBN in THF, were purchased from Aldrich and used as receive d. Water was deionized and distilled. Ammonium molybdate was from Fisher. Hydrochloric acid, anhydrous sodium sulfite, *p*-methylphenol sulfate (metol), oxalic acid dihydrate, and sulfuric acid were purchased from ACROS and used as received.

Polymer Synthesis. A block copolymer of poly(butadiene-*b*-2-vinylpyridine) was synthesized using high vacuum techniques described elsewhere.[8] A 20/10 K poly(butadiene-*b*-2-vinylpyridine) block copolymer was synthesized as follows: 250 ml of a 10% benzene in cyclohexane solution degassed and dried over sec-butyllithium/1,1 diphenylethylene was distilled into a high vacuum reaction apparatus. By way of breakseals, 1.0 mmole of sec-butyllithium was added. Next, 5.0 mmoles of 1,2 dipiperidinoethane was added as a promoter in order to give a polymer with high 1,2 microstructure. 5.0 mmoles of LiCl was then added in a 0.24 M solution in THF. To this was added 10 gm of butadiene, previously dried over sec-butyllithium. After reacting for 6 h at room temperature,, 5 gm of 2-vinyl pyridine was added. This was reacted for 30 min followed by quenching with degassed methanol. The reactor was opened and the polymer was precipitated with excess methanol followed by drying in a vacuum oven. GPC and ^{1}H NMR were then obtained for the block copolymer.

Polymer Functionalization. The poly(butadiene) block was hydroborated and the borate converted to hydroxyl functionality following literature procedures.[9] The block copolymer was enriched in 1,2 microstructure due to the 1,2 dipieridinothane used as a promoter. These resulting pendent double bonds were then reacted stoiometrically with 9-BBN.

Molybdic Acid Assay. The molybdic acid assay procedure used involved absorption of the reduced silicomolybate complex.[10,11] 5 mg of the block copolymer was placed in a 2 ml centrifuge tube and dissolved in 0.6 ml of a 1:1 by weight water: ethanol solution. It was left overnight to be sure of complete dissolution. To this was then added 1 ml TEOS. The sample was mixed by vortex and then agitated for 1 h on a shaker plate. After 1 h, the samples were centrifuged for 2 h to force any precipitate to the bottom of the tube. The supernatant was then poured off and replaced by pure ethanol. This was again centrifuged and the supernatant poured off to remove any unreacted TEOS. What remained in the tube was a solid precipitate of the polymer and reacted TEOS. This material was then analyzed by a modification of a molybdic acid assay.

The molybdic acid reagent used for this assay was made according to a literature procedure.[12] Due to the instability of the silicomolybdic acid solutions, they were reduced prior to absorbance measurements. This was accomplished using a metol reduction. The centrifuge tube containing the polymer/TEOS precipitate was rinsed several times with a 1 M NaOH solution to remove all silicate. These rinses were placed in a 50 ml volumetric flask. To this flask was then added 10 ml of the molybdic acid solution. This was allowed to react for approximately 10 min. A yellow color evolved. Next, the reducing solution made from the metol and oxalic acid was added. The solution turned blue, and the absorption at 810 nm in a 1 cm cell was measured after the solution stood for two to three hours. This solution is stable for at least 6 h.

The molybdic acid assay was calibrated by the use of Na_2SiF_6. 0.960 gm were diluted to 1000 ml. This solution thus contains 5 μg of silica per ml of solution. The silica is very stable in this form and can be stored indefinitely.

Results and Discussion

The poly(hydroxylated butadiene-*b*-2-vinylpyridiene) copolymer was checked at each stage of synthesis by GPC. The hydroxylated polymer was soluble in an ethanol: water mixture of 50:50 but was not soluble in either solvent separately. This is in marked contrast to the parent diblock copolymer that was highly hydrophobic and soluble in hydrocarbon and aromatic solvents only. All studies of the polymer, including molybdic acid assay and control reactions were carried out using the 50: 50 ratio of water: ethanol.

In order to mimic the catalytic ability of the Silicatein α protein, we started by choosing monomers that had the functionality shown to be important for the hydrolysis of TEOS in the natural material. Figure 1 illustrates the chemical structures of the monomer we used. The imine in the histidine was mimicked by using a 2-vinyl pyridine monomer. The serine residue was a bit more difficult. This is due to the fact that we wanted to use high vacuum anionic polymerization in order to synthesize well-defined materials. Anionic polymerization is extremely sensitive to reactive protons in the system and thus can not tolerate alcohols functional groups on the monomer. Thus, we incorporated the hydroxyl group in post polymerization functionalization using hydroboration chemistry. Figure 2 shows the structure of the polymer synthesized for this study.

The molybdic acid assay of the reaction product of polymer with TEOS gives us an indication of the extent of TEOS condensation catalyzed in the first hour of reaction.[11] This assay measures the concentration not necessarily of total silica, but of the amount of silica that will react to form the silicomolybdate complex. Only straight chains with very short lengths react. Poly(silica) as short as three or four monomers of silicic acid may be too long to react by this method.[13-15]

Our technique of removing the precipitate by dissolving it in a sodium hydroxide solution largely overcomes this limitation of the assay. The sodium hydroxide dissolves the condensed silicate precipitate by depolymerizing it back to monomeric silicic acid. Our results showed an initial rate of silica formation of 0.11 μmol h^{-1}. This is compared to a rate of less than 0.01 μmol h^{-1} in a control reaction containing no polymer.

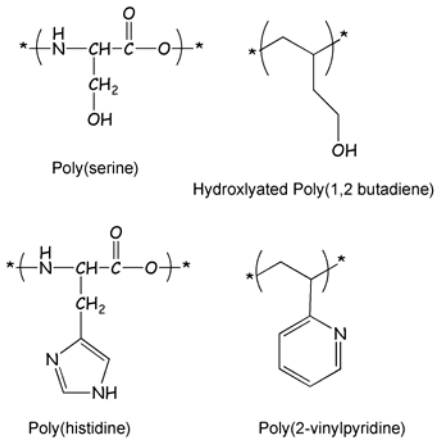

Figure 1. Comparison of amino acid chemical structure with the synthetic mimic. The functionality shown to be vital to catalytic activity of the protein is mimicked by synthetic monomers containing similar functionality.

The results of the molybdic acid assay show very clearly that the condensation of TEOS is significantly faster with the polymer than without it. This quantifies our general observation that upon addition of TEOS to a polymer-containing solution, cloudiness immediately develops. This is in contrast to TEOS being added to a solution containing no polymer. In that case, no change is observed.

Scanning electron microscopy (SEM) and thermal gravimetric analysis (TGA) have been used to characterize the condensed TEOS. TGA has been used with silica systems to give some insight into the composition of inorganic/organic composites.[16-18] It is not entirely clear, however, whether or not we may be losing silicon during our thermal analysis. SEM analysis reveals a smooth material. A control reaction done with no polymer yields a grainy surface. The difference in the two samples is striking and suggests a significant role of the polymer with respect to the mechanism of TEOS condensation.

Our studies to this point indicate here may be a limit to the amount of silica formed. As with the biological systems, this polymer is expected to act as a template for silica formation as well as a catalyst for its formation. The polymer may thus be poisoned by the formation of silica, much as some metal catalysts are poisoned by adsorbed carbon monoxide. The surface area of the catalytic polymer is expected to have a dramatic effect on the amount of silica formed. Using well-defined polymer will give the ability to create self-assembled morphologies to maximize the surface area available to the TEOS or other metal alkoxide precursors.

Conclusions

We have demonstrated the ability of a purely synthetic, non-peptide copolymer to catalyze the condensation of silica by mimicking the functionality of a natural protein. To our knowledge this is the first example of silica produced using a bio-inspired catalytic system of non-peptide macromolecules at neutral pH and ambient temperatures. Molybdic acid assays show the extent of catalysis to be comparable to peptide-based systems. SEM suggests a significant role of the polymer in the mechanism of TEOS condensation while TGA data is currently less clear.

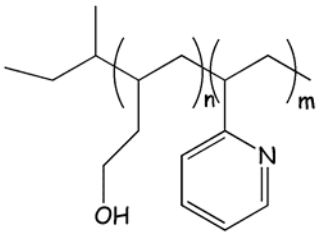

Figure 2. Structure of polymer inspired by Silicatein α. The block copolymer was synthesized by high vacuum anionic polymerization and post-polymerization hydroboration chemistry.

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