METHODS, COMPOSITIONS, AND BIOMIMETIC CATALYSTS FOR IN VITRO SYNTHESIS OF SILICA, POLYSILSEQUioxANE, POLYSILoxANE, AND POLYMETALLO-oxANes

Inventors: Daniel E. Morse, Santa Barbara, CA (US); Galen D. Stucky, Goleta, CA (US); Timothy D. Doming, Summerland, CA (US); Jennifer Cha, Goleta, CA (US); Katsuhiko Shimizu, Tochigi (JP); Yan Zhou, Goleta, CA (US)

Assignee: The Regents of the University of California, Oakland, CA (US)

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Primary Examiner—Margaret G. Moore
Attorney, Agent, or Firm—Fulbright & Jaworski

ABSTRACT
Methods, compositions, and biomimetic catalysts, such as silicatein and block copolypeptides, used to catalyze and spatially direct the polycondensation of silicon alkoxides, metal alkoxides, and their organic conjugates to make silica, polysiloxanes, polymetallo-oxanes, and mixed poly(silicon/metallo)oxane materials under environmentally benign conditions.

46 Claims, 5 Drawing Sheets
FIG. 2
FIG. 3
$R = -(\text{CH}_2)_4\text{NHC(O)OCH}_2\text{C}_6\text{H}_5$

$[\text{Ni}] = \text{INITIATOR ENDCROUP}$

$R' = -(\text{CH}_2)_4\text{NH}_3+\text{BR}$

$Z = -\text{C(O)OCH}_2\text{C}_6\text{H}_5$

**FIG. 4**
METHODS, COMPOSITIONS, AND BIOMIMETIC CATALYSTS FOR IN VITRO SYNTHESIS OF SILICA, POLYSILSEQUIOXANE, POLYSILXOANE, AND POLYMETALLOXANES

CROSS REFERENCE TO RELATED APPLICATIONS

This application claims the benefit of Provisional Patent Application No. 60/112,944, filed Dec. 18, 1998.

STATEMENT REGARDING FEDERALLY FUNDED RESEARCH OR DEVELOPMENT

This invention was made with Government support under Grant No. N0014-93-10584, awarded by the Office of Naval Research; Grant No. DAAH-04-96-1-0443 awarded by the Army Research Office; Grant No. NA36RG0537, awarded by the National Oceanic and Atmospheric Administration; and Grant Nos. DMR32716 and DMR34396, awarded by the National Science Foundation. The Government has certain rights in this invention.

BACKGROUND OF THE INVENTION


By contrast, the biological production of amorphous silica, the simplest siloxane (\(\text{SiO}_2\)), is accomplished under mild physiological conditions, producing a remarkable diversity of exquisitely structured shells, spines, fibers, and granules in many prokaryotes, diatoms, sponges, molluscs and higher plants [Simpson, T. L. and Volcani, B. E. (1981) *Silicon and Siliceous Structures in Biological Systems*, Springer-Verlag; and Voronkov, M. G., Zelchan, G. I. and Lukevits, E. J. (1997) *Silicon and Life* (2nd edn), Zinatne Publishing, Vilnius, Lithuania]. These biologically produced silicas exhibit a genetically controlled precision of nanoscale architecture that, in many cases, exceeds the capabilities of present-day human engineering. Furthermore, the biological production of siloxanes occurs on an enormous scale globally, yielding gigatons per year of silica deposits on the floor of the ocean. DIatomaceous earth (composed of the nanoporous skeletons of diatoms) is mined in great quantities from the vast primordial deposits of this biogenic silica. Biotechnical approaches are now starting to unlock the molecular mechanisms of polysiloxane synthesis under physiological conditions, offering the prospect of new, environmentally benign routes to the synthesis and structural control of these important materials. Taking advantage of marine organisms that produce large relative masses of biogenic silica, molecular biologists have begun to isolate the genes and proteins controlling silica biosynthesis and nanofabrication.

Hildebrand and colleagues made a significant breakthrough by cloning and characterizing the cDNA encoding the first silicic-acid [\(\text{Si(OH)}_4\)] transporter to be unequivocally identified [Hildebrand, M., Volcani, B. E., Gassman, W., & Schroeder, J. I. (1997) *Nature* 385, 688–689]. They showed, by analysis of the encoded protein and by injection of the mRNA (synthesized in vitro from the cloned cDNA) into Xenopus eggs, that the transporter protein forms a sodium-dependent transmembrane ion channel that mediates the transport of silicic acid. The action of this protein can account for the uptake of the silicic acid precursor for the white pool of silicic acid in oceanic and fresh water, and similar transporters may pump silicic acid (or its conjugates) into the lumen of the silica-deposition vesicle (silicalamella), in which polycondensation (polymerization) is known to occur.


In contrast to anthropogenic and geological syntheses of these materials that require extremes of temperature, pressure or pH, living systems produce a remarkable diversity of nanostuctured silicates at ambient temperatures and pressures and at near-neutral pH. Laboratory methods have been unable to replicate these results and rely instead on extreme pHs and/or surfactants to condense silica precursors into specific morphologies or patterned structures. These conditions are undesirable for environmental reasons and therefore methods to direct silica assembly under conditions similar to those used in nature (i.e. biometrically) are desired.

SUMMARY OF THE INVENTION

The present invention overcomes the drawbacks of prior efforts to condense silica precursors into specific morpholo-
gies or patterned structures, and provides heretofore unattainable materials having very desirable and widely useful properties. These materials are prepared at ambient temperatures and pressures and at near-neutral pH.

The method of the present invention for in vitro polymerization of silica and silicone polymer networks, includes the steps of (1) combining a catalyst and a substrate, wherein the substrate is selected from the group consisting of silicone alkoxide, metal alkoxide, and organic conjugates of the foregoing; and (2) polymerizing the substrate to form silica, polysiloxanes, polymetallo-oxanes, or mixed polysilicon/metallo-oxane materials at about neutral pH. Preferably the substrate is a silicone alkoxide having the general formula $R$-Si($O-\text{Et})_n$ wherein $E$ is ethyl and $R$ is methyl, phenyl, or ethoxy. Moreover, the polymerized materials preferably include apolyisobisquinoxane having the general formula (RSIO$_3$)$_n$ wherein $n$ is an integer greater than 1.

The present invention also provides compositions for use in polymerizing silica and silicone polymer networks, which include a silicone alkoxide substrate; and a catalyst that assembles, hydrolyzes, and condenses the substrate at about neutral pH. A catalyst according to the present invention is generally a protein or polypeptide. Preferred protein or polypeptide catalysts include silicatein filaments, silicatein subunits, cysteine homopolymers, and cysteine-containing block copolypesthes. A preferred silicatein is a protein comprising an amino acid sequence at least 70% identical to the amino acid sequence of silicatein $\alpha$, i.e., SEQ ID NO:1. Alternatively the catalyst is a recombinant protein encoded by a nucleotide sequence at least 70% identical to the coding regions of SEQ ID NO:2, which is the cDNA sequence of the silicatein $\alpha$ gene. Yet another group of catalysts of the present invention, which unexpectedly mimic the polymerizing and scaffolding activities of silicateins, are cysteine-containing block copolypesthes. The most preferred ones are diblock copolypesthes of poly[(L-Cysteine)$_{a,b}$-L-Lysine$_{c,d}$], poly[(L-Cysteine)$_{a,b}$-L-Lysine$_{c,d}$], poly[(L-Cysteine)$_{a,b}$-L-Lysine$_{c,d}$], and poly[(L-Cysteine)$_{a,b}$-L-Lysine$_{c,d}$].

Silicified structures can be synthesized according to the method of the present invention. These structures assume a shape directed by the scaffolding activity of the catalyst. Such silicified structures can include shapes, such as filaments, spheres, elongated globules, and columns.

**BRIEF DESCRIPTION OF THE DRAWINGS**

These and other features, aspects, and advantages of the present invention will become better understood with regard to the following description, appended claims, and accompanying drawings, where:

**FIG. 1** shows scanning electron micrographs of the products of reactions between silicone alkoxides and silicatein or cellulose filaments. (A) silicatein filaments prior to reaction. (B) silicatein filaments after 12 h reaction with TEOS (1.0 ml; 4.5 mmole) plus Tris-HCl buffer. (C) air-dried silicatein filaments incubated with TEOS as in (B) but with no additional water. (D) silicatein filaments after 8 h reaction with phenyltriethoxysilane (1.0 ml; 4.1 mmole) plus Tris-HCl buffer. (E) cellulose fiber. (F) cellulose fiber after 12 h reaction with TEOS as in (B).

**FIG. 2** shows $^2$Si magic-angle spinning (MAS) NMR spectra of silica and silsesquioxane products on silicatein filaments [Schwab, D. W. & Shore, R. E. (1971) *Biol. Bull.* 140, 125-136]. Samples were prepared as described for FIG. 1B and D. (A) a single-pulse MAS spectrum of the reaction product of silicatein filaments and TEOS. (B and C) Cross-polarization MAS spectra of the reaction products of silicatein filaments and phenyltriethoxysilane (B) and TEOS (C) respectively.

**FIG. 3** shows a proposed reaction mechanism of silicon ethoxide condensation catalyzed by silicatein $\alpha$, based on the well-characterized mechanism of catalysis by the serine-histidine and cysteine-histidine active-site proteases [Morse, D. E. (1999) *Organosilicon Chemistry IV: from Molecules to Materials*, eds. Auner, N. and Weis, J. (Wiley-VCH, New York), 1999; pp. 5-16; incorporated herein by reference]. As stated therein, “Our final objective is then to use the information obtained from the studies of the mutationally altered proteins to design synthetic peptide based catalysts to test the validity of our conclusions, and to guide the design of synthetic non-peptide-based catalysts and microstructure-directing scaffolds that are both less expensive and more robust than the natural and genetically engineered proteins.” $R$ is phenyl- or methyl- for the siliconetriethoxide substrates, and $R=CH$, CH$_3$-O-(Et)$_2$ for the ve.

In the present invention, the hydroperoxy group between the imidazole nitrogen of the conserved histidine and the hydroxyl of the active-site serine is proposed to increase the nucleophility of the serine oxygen, potentiating its attack on the silicon atom of the substrate; nucleophilic attack on the Si places ethanol, forming a covalent protein-Si intermediate (potentially stabilized as the pentavalent Si adduct via donor bond formation with the imidazole N); addition of water completes hydrolysis of the first alkoxide bond, condensation initiated by nucleophilic attack of the released Si-O on the silicon of the second substrate molecule then forms the disiloxane product.

**FIG. 4** shows the stepwise polymerization of monomers, N$_2$-carboxybenzyl-L-lysine NCA followed by S-carboxybenzyl-L-cysteine NCA gave the protected polymer that was then deprotected using equimolar amounts of trichlororacetic acid and 33% HBr in acetic acid to give 6. $\text{H}C=\text{N(COD)}=2,2'-$bipyridyl$\text{NiCl}_2(1,5$-cyclooctadiene). The protected copolymer was analyzed using size-exclusion chromatography in DMF at 60°C to verify the molecular weight. Polymer composition was verified by $^1$H NMR analysis of the deprotected copolymer in TFA-d.$^4$

**FIG. 5** shows different ordered silica shapes obtained using block copolypesthes 6. In a typical procedure, TEOS (2.0 mL) was added to 500 $\mu$L of a solution of 0.5 mg/mL in 30 mM Tris-HCl buffer, pH 6.8, and the resulting biphasic mixture was agitated vigorously and then allowed to stand for several hours with no stirring, whereupon some of the TEOS had emulsified into the aqueous phase. After 24 h, the resulting silica precipitate was collected from the aqueous phase, washed with 95% ethanol and air dried.

**DETAILED DESCRIPTION OF THE PREFERRED EMBODIMENT**

This invention provides a simple and general procedure for the in vitro syntheses of silica, polysiloxanes, polymetallo-oxanes, and mixed polysilicon/metallo-oxane materials under environmentally benign conditions.

Proteins, such as silicatein filaments, subunits, and recombinant subunit $\alpha$; synthetic polypeptides, including block
copolypeptides or cysteine homopeptides; and other polymers can be used as catalysts, which spatially direct the polymerization of silicon alkoxides, metal alkoxides, and their organic conjugates.

Potential applications for the reaction products include, but are not limited to, resin toughening, electronic and optoelectronic devices, packaging, insulators, fire-resistant materials, construction materials, plastics, metalloplastic composites, adhesives, water-resistant sealants, and filtration membranes.

Representative examples include: the synthesis of silica, poly-methyl-silsesquioxane and poly-phenyl-silsesquioxane catalyzed and spatially directed by silicatein filaments, subunits, and subunit a produced in bacteria from a recombinant DNA template. Additional examples include the catalysis and spatial control of silica and polysilsesquioxane synthesis by block copolypeptides and other polymers.

Catalysts

The catalysts used in the present invention include proteins and synthetic polypeptides that mimic the in vivo activity of proteins that control silicification in marine organisms. For example, the marine sponge, Tethya aurantium, produces copious silica spicules (1−2 mm length×30 µm diameter) that constitute 75% of the dry weight of the organism. These spicules each contain a central axial filament of protein (1−2 mm length×2 µm diameter) consisting of three very similar subunits we have named silicateins (for silica proteins) [Shimizu, K., Cha, J., Stucky, G. D., & Morse, D. E. (1998) Proc. Natl. Acad. Sci. U.S.A. 95, 6234−6238; incorporated herein by reference in its entirety]. The α, β, and γ subunits are quite similar in amino acid composition and pl, with apparent molecular masses of 29, 28, and 27 kDa. Densitometric analysis reveal these subunits to be present in relative proportions of approximately α:β:γ = 12:6:1.

Silicatein filaments and their constituent subunits comprising the axial cores of silica spicules in a marine sponge chemically and spatially direct the polymerization of silica and silicone polymer networks from the corresponding alkoxide substrates in vitro, under conditions in which such syntheses otherwise require either an acid or base catalyst. Characterization of silicatein α (the subunit comprising nearly 70% of the mass of the filaments) and its cloned cDNA revealed that silicatein α is homologous to members of the cathepsin L subfamily of the papain family of proteolytic enzymes. The amino acid sequence of the silicatein α subunit is disclosed herein as SEQ ID NO:1, and the cDNA encoding this sequence is SEQ ID NO:2.

Preferred versions of the present invention utilize catalysts that are members of the silicatein family. As will be appreciated by those of skill in the art, members of the silicatein family may be subject to evolutionary and man-made variations. Accordingly, silicateins include: (1) amino acid sequences that are, at least 70% identical, preferably at least 80% identical, more preferably at least 90% identical, and most preferably at least 95% identical to SEQ ID NO:1; (2) the functional equivalents of these proteins which retain catalytic and/or scaffolding activities; and (3) biologically active derivatives, including silicatein-derived fragments. The percent identity of the amino acid sequences of silicateins are as determined by FASTA or BLAST using default opening and gap penalties and a default scoring matrix (available at the National Center for Biotechnology Information website, http://www.ncbi.nlm.nih.gov/). For simplicity, the term “silicatein” is used to describe both the native or wild type silicateins and those silicateins with sequences altered by the hand of man (engineered silicateins).

More particularly, the protein catalysts include silicateins derived from the spicules of a marine sponge. A macroscopic silicatein purified from spicules that contains assembled subunits is an “silicatein filament,” whereas an isolated silicatein protein molecule is referred to as a silicatein subunit. Examples of silicatein subunits include subunits α, β, and γ as described by Shimizu et al. (1998), supra.

The term “wild type” refers to those silicateins that have an amino acid sequence as found in the natural environment. This term therefore refers to the sequence characteristics, irrespective of whether the actual molecule is purified from natural sources, synthesized in vitro, or obtained following recombinant expression of a silicatein-encoding DNA molecule in a host cell.

The terms “mutant, variant or engineered” silicatein refer to those silicateins the amino acid sequence of which have been altered with respect to the sequence of the silicatein found in nature. This term thus describes silicateins that have been altered by the hand of man, irrespective of the manner of making the modification, e.g., whether recombinant DNA techniques or protein chemical modifications are employed.

“Native” silicateins are those that have been purified from their natural sources, such as from spicules of a marine sponge. Native silicateins will also generally have wild type sequences.

“Recombinant” silicateins are those molecules produced following expression of a silicatein recombinant DNA molecule, or gene, in a prokaryotic or eukaryotic host cell, or even following translation of an RNA molecule in an in vitro translation system. “Synthetic” silicateins are those silicateins produced using synthetic chemistry, most usually in the form of automated peptide synthesis. Both recombinant and synthetic silicateins may have either wild type or mutant sequences, as designed.

Structural and Functional Domains of Silicatein Catalysts: Analysis of the amino acid sequence revealed that silicatein α is made biosynthetically as a “pre-pro-protein”, with two peptide fragment successively removed from the amino-terminal end by proteolytic enzymes that cut the protein as it is secreted into the membrane-enclosed silica deposition vesicle (SDV) and then folded into its final 3-dimensional conformation. The “signal peptide” that facilitates recognition and secretion into the SDV is cleaved by a specific “signal peptidase”. After folding the protein within the SDV, the remaining N-terminal “propeptide” is then removed to release the mature silicatein. The sequences of the amino acids that specify the sites of these two cleavages are homologous to those found in the precursors of other members of the papain family as well.

Comparison of the silicatein α and cathepsin L sequences [Shimizu et al. (1998), supra] also reveals that the six cysteine residues that form intramolecular disulfides in cathepsin L are fully conserved in the silicatein, suggesting that the 3-dimensional structures of the two proteins are quite similar. Two of the three residues (His and Asn) of the “catalytic triad” of the cathepsin active site also are conserved in silicatein α, but the third active-site residue in cathepsin, Cys, is replaced in the silicatein by Ser, preventing this protein from being an effective protease [Shimizu et al. (1998), supra]. At this position, the structure of silicatein α resembles that of the other major class of proteases, the serine proteases, typified by trypsin and

Hecky et al. postulated that the hydroxyl-rich proteins of the silicified diatom wall might condense with silicic acid monomers, thus serving as a template to organize the growth of the silica [Hecky et al. (1973), supra]. Thermodynamic calculations have been presented in support of that suggestion [Lobel et al. (1996), supra]. Such a mechanism may also contribute to the results reported here. While the lack of activity of the hydroxyl-rich cellulose and silk polymers indicates that the simple density of hydroxyls is not alone sufficient for polymerization of the silicon alkoxides, the condensation of such groups in the silicatein molecule may be important for the template-like scaffolding activity. Indeed, several runs of contiguous hydroxyls are found in silicatein α [Shimizu et al. (1998), supra], which might be important in orienting the siloxane groups of either the substrate or product.

Mechanism of Action: Homology of silicatein α to the well-known enzyme, cathepsin L, points to a possible reaction mechanism that is supported by recent site-directed mutagenesis experiments. The condensation of silicon alkoxides promoted by the silicateins and the cleavage of peptides catalyzed by the proteases both must proceed through an obligatory hydrolysis reaction, and both are known to be accelerated by general acid-base catalysis, suggesting that the mechanism of action of silicatein α in this process may be fundamentally related to that of its homologous enzyme counterparts. The requirement for the specific serine, and histidine residues of silicatein α for catalysis of the siloxane polymerization suggests that the mechanism of silicatein-mediated catalysis of siloxane polymerization from the alkoxide substrates may be closely parallel to that of the well-characterized Ser-His and Cys-His active site proteases [Lehninger, A., Nelson, D., & Cox, M., eds. (1993) in Principles of Biochemistry (Worth Publishers, New York), pp. 223–227].

FIG. 3 illustrates such a mechanism, whereby the silicatein actually functions as a hydrolase with these substrates, converging the silicic alkoxides to their corresponding silanols, which are known to condense rapidly and spontaneously to form polysiloxanes. This mechanism may help explain the observed acceleration of silicon alkoxide condensation promoted by silicatein α and the silicatein filaments in vitro, since it is known that the rate-limiting step in this condensation is the initial hydrolysis of the alkoxide required to generate the reactive Si-O species, and that the rate of spontaneous hydrolysis is lowest at neutral pH [Her, R. K. (1979) in The Chemistry of Silica: Solubility, Polymerization, Colloid and Surface Properties, and Biochemistry (John Wiley & Sons, New York), pp. 98–99].

Methods of Making Silicateins: The preparation of wild type, mutant, native, recombinant and synthetic silicateins will be straightforward to those of skill in the art in light of the present disclosure. Native silicatein filaments and sub-units can be prepared as described in greater detail Shimizu et al. (1998), supra, and in the Example 1 below. Alternatively, a recombinant silicatein can be prepared by expressing the silicating encoding segments of a silicatein gene (see, e.g., SEQ ID NO:2), including wild type and mutant genes, in a recombinant host cell and collecting the expressed protein. Preferably the silicatein encoding segments are at least about 70% identical to the coding sequences of SEQ ID NO:2, more preferably at least 80% identical, and most preferably at least 90% identical. The percent identity of the nucleotide sequences of silicateins are as determined by FASTA or BLAST using default opening and gap penalties and a default scoring matrix (available at the National Center for Biotechnology Information website; http://www.ncbi.nlm.nih.gov/). The host cells can be bacterial, yeast, insect, mammalian or other transformed animal cells. The coding segments can be in the form of naked DNA, or housed within any one of a variety of expression vectors, such as recombinant plasmids or viruses, which have been modified to contain and express the encoded silicatein protein. More particularly, recombinant silicatein subunit α can be expressed as a fusion protein as described in Zhou et al. (1999), supra.

Synthetic silicatein peptides can be made using automated methods for peptide synthesis. Techniques for the operation of automated peptide synthesizers is standard practice in the art and such services are available commercially, as described further in a subsequent example.

Synthetic polypeptide catalysts: Synthetic polypeptides, more particularly, cysteine-lysine block copolypeptides unexpectedly mimic the properties of silicatein. These synthetic copolymers emulate silicatein by self-assembling into superstructures that can hydrolyze silicon alkoxides, while simultaneously directing the formation of the silica into ordered morphologies.

Since TEOS is stable when mixed with water at neutral pH, successful biomimetic silica synthesis from this precursor requires an agent that displays hydrolytic activity simultaneously with structure-directing properties. Site-directed mutagenesis of the cloned DNA coding for silicatein α revealed that interacting histidine and serine residues were required for the hydrolytic activity of this protein. For this reason, simple homopolypeptides of amino acids bearing polar functional groups were evaluated for their ability to mimic the properties of silicatein in the polycondensation of silicon alkoxides. However, homopolymers of L-lysine, L-histidine, D/L-serine, L-threonine, and L-glutamic acid failed to catalyze TEOS hydrolysis and condensation. In contrast, oligomers of L-cysteine efficiently produce silica from TEOS in pH 7 buffer (see Table 2 in Example II, below), when handled under an inert nitrogen atmosphere to prevent oxidation. Accordingly, preferred synthetic polypeptide catalysts include one or more nucleophilic sulfhydryl groups, which can initiate hydrolysis of the silicon alkoxide. Preferred L-cysteine homopolymers are less than about 3000 Da, since higher chain lengths are insoluble. Moreover, when these oligomers are used under air, oxidation of the sulfhydryl groups to disulfide results in insoluble aggregates that are much less active in silica formation.

Diblock copolypeptides that contain covalently linked domains (blocks) of water soluble and insoluble polypeptides are better able to mimic the hydrolytic activity of silicatein. Dissimilarity in the block segments provides the chains with an amphiphilic character, similar to that of surfactants, which results in self-assembly of the chains in aqueous solution. The architecture and design of the block copolypeptides also provide simple means to solubilize water-insoluble domains, e.g., hydroxytically active poly-L-cysteine. Such block copolypeptides allow the directed cooperative assembly, hydrolysis, and condensation of TEOS to form specific silica structures.
Preferably, the solubilizing block copolyepitide components are cationic polyelectrolytes, such as poly-L-lysine, which are known to be water soluble at pH 7. As water insoluble domains, poly-L-cysteine and poly-L-serine are preferred, both for their potential silica-forming hydrolytic activity as well as their ability to aggregate in water by either hydrogen or covalent bonding via β-sheet formation or disulfide linkages. Other less preferred insoluble domains include polar residues that are less nucleophilic than cysteine (poly-L-glutamine and poly-L-lysine) or slightly hydrophobic (poly-L-alanine).

The block copolypeptides that were synthesized and studied are given in Table 2 (see Example II, below). They were prepared from suitably protected amino acid-N-carboxyaminhydride (NCA) monomers by using the initiator 2,2-bispyridyl/Na(1,5-cyclooctadiene). This synthetic protocol has been shown to give block copolypeptides of narrow molecular weight distributions and with controlled molecular weights (see FIG. 4). However poly-L-histidine was not used in these studies because of difficulty in protecting the side-chain to form a suitable NCA monomer.

Cationic block copolymers, showed more activity in silica formation than the corresponding anionic copolymer. In fact, poly-L-glutamate completely inhibited the ability of the polycysteine block to form silica, which supports the hypothesis that polycations are important for interacting with negatively charged silicate precursors. All of the lysine containing copolymers display some activity in silica formation, and the rate of silica production increased steadily as the domain bound to the poly-L-lysine block became more nucleophilic. Since polymer 1, which contains no nucleophile component, was able to produce silica, it appears that poly-L-lysine itself, when constrained in a self-assembling block copolymer, possesses a low activity toward the hydrolysis and condensation of TEOS. However, cysteine and lysine containing copolymers are the only ones tested thus far that are able to control the shape of the silica during its formation, with the cysteine-containing polymers being most active. Accordingly, the synthetic cysteine-lysine diblock combination is most preferred.

Compositions and Methods of Use

The present invention provides methods for in vitro polymerization of silica and silicone polymer networks. The first step of the method is to combine a catalyst and a substrate, wherein the substrate is selected from the group consisting of silicon alkoxide, metal alkoxide, and organic conjugates of the foregoing. Accordingly, compositions for use in the polymerization method include a substrate and a catalyst, described in further detail above, which assemblies, hydrolyzes, and condenses the substrate at about neutral pH. Preferably, the substrate is a silicon alkoxide, such as silicon tetrahydroxide and organically modified silicon triethoxides. More preferably, the substrates are of the general formula R—Si—(O—Et)n, wherein Et is ethyl and R is methyl, phenyl, or ethoxy. These substrates are preferred because of their stability at neutral pH, and the similarity of their chemical reactivity to that of the substrates of proteases.

The next step of the method is to polymerize the substrate to form silica, polysiloxanes, polymetallo-oxanes, or mixed polysilicon/metallo-oxane materials at about neutral pH. When organically substituted silicon trioxides are provided as substrates, the silicatein catalyze their condensation to form the corresponding polysiloxoquinoxes (RSiOx2)n, wherein R is an alkyl or phenyl group. For example, reaction of the macroscopic silicatein filaments with phenyl- or methyl-triethoxysilane promotes rapid polymerization with scaffolding of the resulting silsesquioxane polymer network on the silicatein filament (see FIG. 1C). The in vitro synthesis of silicon and silsesquioxanes by the catalysts of the present invention at neutral pH illustrates how this mechanism may be harnessed for the development of environmentally benign new routes to the synthesis of patterned silicon-based materials.

Moreover, the shapes of the silicone containing polymers produced by the method of the present invention can be varied, depending on the catalyst. For example, when macroscopic silicatein filaments are used as catalysts, the silicone or silsesquioxanes product is formed over the surface of the filament, following the contours of the underlying macroscopic topology (Sec. e.g., FIGS. IA, IB). Thus, the silicatein filaments exhibit both "scaffolding" (macroscopic structure-directing) and catalytic activities in directing the condensation of the alkoxides to form polysiloxanes in vitro.

The synthesis of polymeric networks of phenyl- and methyl-silsesquioxanes coating the surface of the silicatein filaments at neutral pH in vitro shows that this mechanism may be utilized for shaping silicone based materials. However, silica formed by using oligo-L-cysteine is an amorphous powder with no defined macroscopic shape. Thus, simple homopolymers of amino acids, which lack the structural complexity and multifunctionality found in proteins, are unable to reproduce the shape-controlling ability of silicatein. Surprisingly, block copolypeptides of cysteine and lysine can be used to mimic biological silica synthesis, wherein hydrolysis and condensation of an inorganic phase as well as structural templating are all controlled by a single synthetic material at pH 7. Since assembly of cysteine containing block copolypeptides is influenced by oxidation of the cysteine sulphydryl groups, different silica structures can be produced from a single copolymer exposed to different oxidizing conditions. For example, transparent, hard silica spheres or elongated globules are formed by using the fully reduced cysteine-lysine copolymers, while oxidation of the same cysteine-lysine copolymer leads to formation of well-defined columns of amorphous silica.

EXAMPLES

These additional embodiments may be better understood with reference to the accompanying examples, which are intended for purposes of illustration only and should not in any sense be construed as limiting the scope of the invention as defined in the claims appended hereto.

Example 1

Silicatein Filaments and Subunits from a Marine Sponge Direct the Polymerization of Silica and Silicones in Vitro

Materials and Methods

Isolation of Silicatein Filaments and Analysis of Reactions With Silicon Alkoxides. Insoluble silicatein filaments were extracted from the acid- and hypochlorite-cleaned silica spicules of Tethya aurantium by dissolving the silica in buffered HF (1M HF, 5M NH4F) as described previously [Shimizu et al. (1998), supra]. The HF was removed by dialysis against pure water (Milli-Q) and the filaments collected by filtration. Reactions of the insoluble filaments [either air-dried or suspended (at 0.5 mg/ml) in Tris-HCl buffer (0.0 M, 25 mM, pH 6.8)] with TEOS (1.0 M, 4.5 mmole) were performed with gentle shaking at room tem-
perature for 12 h. The silicatein filaments were added in aqueous Tris buffer for all reactions except that illustrated in FIG. 1C, in which the air-dried filaments were reacted with pure TEOS. The reaction also was performed with phenyl-
triethoxysilane (FIG. 1D) in place of TEOS, using 1.0 ml (4.1 mmoles) of phenyltriethoxysilane. For all samples the insoluble materials were collected by centrifugation, air-
dried, gold sputter-coated and imaged by scanning electron microscopy with a JEOL JSM 6300F equipped with a cold cathode field-emission source operated at a beam energy of 3.5 kV (FIG. 1).

NMR Analyses. NMR spectra were acquired on a CMX-
500 Chemagnetics spectrometer operating at 11.7 Tesla and a 29Si frequency of 99.06 MHz referenced to TMS [Smaih, M., Jermouni, T., & Marignan, J. (1995) Chem. Mater. 7, 2293–2299]. The single-pulse spectrum was acquired for 22 h with an 8.35 μs single pulse and a recycle delay of 300s while spinning at 3.5 kHz. Cross-polarization MAS spectra were acquired for 4 h with a contact time of 4 ms, a pulse width of 6 gs, and a recycle delay of 2 s while spinning at 6 kHz.

Silicatein Subunits, and Analysis of Reactions with Sili-
cone Alkoxides. Silicatein subunits (Table 1A) were solubi-
lized from the purified filaments [Shimizu et al. (1998), supra] by treatment with 10 mM NaOH for 5 min, and the soluble subunits then dialyzed extensively at 4°C against Tris-HCl buffer (25 mM pH 6.8). Silicatein α (Table 1B) was expressed from a recombinant DNA template in E. coli, purified and reconstituted by standard procedures [Zhou et al. (1999), supra] and dialyzed as above. Denatured proteins were boiled for 15 min. The proteins then were utilized immediately for the following assay: TEOS (1 ml; 4.5 mmoles) was added to 0.6 ml protein (0.26 or 0.5 mg/ml in Tris buffer as specified). The mixtures were thoroughly resuspended by pipetting and the reactions allowed to con-
tinue for 15–60 min at 20 °C. The samples then were centrifuged to collect the silica products; the pellets were washed a minimum of 3 times with ethanol to remove unreacted TEOS, collected by centrifugation and then either hydrolyzed with 1M NaOH for 10 min or suspended only with water to quantify residual adsorbed TEOS. The samples then were diluted and the released siliceous acid quantified using a modification of the calorimetric molybdate assay [Strickland, J. D. H. & Parsons, T. R. (1972) in A Practical Handbook of Seawater Analysis (2nd ed.) Fish Res. Bd. Can. Bull.] with the reagent blank of Brzezinski & Nelson [Brzezinski, M. A. & Nelson, D. M. (1986) Mar. Chem. 19, 139–151] yielding a detection limit of 50 nM Si(OH)4.

<table>
<thead>
<tr>
<th>Protein</th>
<th>Polymerized Si (mmoles)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Native</td>
<td>214.0 ± 2.0</td>
</tr>
<tr>
<td>Denatured</td>
<td>24.5 ± 2.0</td>
</tr>
<tr>
<td>Bovine serum albumin</td>
<td>42.1 ± 6.7</td>
</tr>
<tr>
<td>Papain</td>
<td>22.0 ± 1.0</td>
</tr>
<tr>
<td>Trypsin</td>
<td>16.5 ± 2.6</td>
</tr>
<tr>
<td>(None)</td>
<td>10.2 ± 1.3</td>
</tr>
<tr>
<td>B.</td>
<td>10.8 ± 1.9</td>
</tr>
</tbody>
</table>

TABLE 1

Silicatein subunits catalyze polymerization of silica

Proteins in 0.6 ml Tris-HCl buffer (25 mM, pH 6.8) were incubated with 1 ml (4.5 mmoles) TEOS and the polymerized silica quantitated after centrifugation and hydrolysis as described in Materials and Methods. (A) Proteins at 0.3 mg; reaction for 15 min; (B) protein at 0.06 mg; reaction for 60 min.

Results

The silicatein filaments can be dissociated to their constituent subunits, α, β and γ [Shimizu et al. (1998), supra]. These subunits accelerate the in vitro polymerization of silica (SiO2), from the monomeric TEOS at neutral pH (Table 1A). Electron microscopy confirms the formation of a dendritic silica precipitate (not shown). Little polymeriza-
tion is seen in the absence of these proteins; it is known that under these conditions, polymerization of silica from TEOS normally requires either an acid or base catalyst. The activity of the silicatein subunits is abolished by thermal denaturation, demonstrating a dependence on the native 3-dimensional conformation of the subunit proteins. Denatura-
tion with the detergent, SDS (sodium dodecyl sulfate), also abolishes activity (results not shown). Specificity of the observed effect is indicated by the finding that the condensa-
tion of TEOS under these conditions is significantly slower when trypsin, papain, or bovine serum albumin are substituted for the silicatein.

Silicatein α comprises ca. 70% of the mass of the silic-
atein filaments in Jethya aurantiha [Shimizu et al. (1998), supra]. This subunit, when expressed in bacteria from a recombinant DNA template and subsequently purified and reconstituted, proves to be sufficient to accelerate the poly-
erization of silica from TEOS at neutral pH (Table 1B). In this case also, thermal denaturation abolishes reactivity with the silicon alkoxide. These findings are significant because the complete amino acid sequence of the a subunit reveals a high similarity to members of a well-characterized enzyme superfami-

The intact silicatein filaments also are active, promoting the condensation of silicon alkoxides and organically modi-
50 fied silicon alkoxides to form the corresponding polymer-
ized silica or silsesquioxanes (RSiO1.5), (silicones in which R=an organic sidechain) at neutral pH (FIGS. 1 and 2). The microscopic filaments serve as scaffolds to organize the deposition of the resulting silica and silsesquioxanes (FIG. 1).

Organization of the resulting silica is more clearly seen when the condensation of TEOS is performed in the absence of added water (other than the water of protein hydration), restricting the dendritic growth of the silica by limiting hydrolysis of the precursor to create a silica substructure that follows the longitudinal axis of the protein filament (FIG. 1C). In the absence of the filaments, no polymerization of TEOS was observed at neutral pH during the course of the experiments, consistent with the known requirement for acid or base catalysis. The activity of the silicatein filaments is abolished by thermal denaturation, indicating a dependence on the native conformation of the constituent proteins. Neither silk (not shown) nor cellulose (FIGS. 1E, F) fibers
exhibit any activity with TEOs under the same conditions, demonstrating that polymeric fibers with high surface densities of hydroxyl groups are not sufficient to accelerate or organize silica polymerization from TEOs at neutral pH. The acceleration of polymerization and structure-directing activities of the silicatein filaments are also evident with organically substituted trichlorosilane precursors with the general structure R—Si—(OEt)₃, where R=phenyl, methyl, etc. When phenyltrimethoxysilane is provided as substrate, a polymerized product is formed on the filament surface. 

The synthesis of polymeric networks of phenyl- and methyl-silesquioxanes by the silicatein filaments at neutral pH in vitro suggests that this mechanism may be harnessed for the development of environmentally benign new routes to the synthesis of patterned silicon-based materials. Solid-state ²⁵Si NMR was used to analyze the extent of polymerization of the silesquioxanes on the protein filaments. Analysis of the product formed from TEOs (FIGS. 2A, C) revealed three inhomogeneously broadened peaks corresponding to Q⁰ (590 ppm), Q¹ (510 ppm) and Q² (510 ppm) silesquioxane species, indicative of a disordered, incompletely polymerized opal-like silica network characteristic of the silica found in biological materials. In contrast to these results, cross-polarization ²⁵Si/NMR analysis of the product formed from the phenyltrimethoxysilane precursor revealed no Q² species (FIG. 2B). This result is consistent with the silesquioxane structure of the polymerized product, as would be predicted from polymerization of the precursor which contains only three functional groups available for the formation of silesquioxane linkages. The phenylsilesquioxane exhibits a T² resonance at -79 ppm (shifted downfield by 20 ppm due to the phenyl substituent) [Smiali et al. (1995), supra] and possible T² and T⁰ resonances.

Example II

Block Copolyamide Mediated Biomimetic Synthesis of Ordered Silica Structures

Block copolyamides were screened for their ability to react with TEOs to form silica. Referring to Table 2 (below), Yield=total isolated yield of deprotected copolymer, SiO₂ Rate=initial rate of silica formation (mmol/min) by block copolyamide at a concentration of 5 mg/mL in 50 mM Tris-HCl buffer, pH 6.8 and an initial TEOs concentration of 3.4 M. The silica precipitate was collected by centrifugation, washed with 95% ethanol and solubilized in 0.2 M NaOH at 37°C. The amount of silica was then determined using the spectrophotometric molybdate assay (24,25). N₂=silica preparation was carried out under an oxygen-free nitrogen atmosphere; Air=silica preparation was carried out in air. In the absence of polymer, buffer alone was used as the control. Shape=morphology of silica particles; N=non-ordered; S=spheres; E=elongated globules; C=columns. NA=not applicable.

<table>
<thead>
<tr>
<th>Entry</th>
<th>Composition</th>
<th>Yield (%)</th>
<th>SiO₂ Rate (mmol/min)</th>
<th>Shape</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>poly(L-Alanine)₆₇₈₇-b-L-Lysine₆₇₈₇</td>
<td>89</td>
<td>9.06 (2)</td>
<td>NA</td>
</tr>
</tbody>
</table>

Since TEOs is stable when mixed with water at neutral pH, a successful biomimetic silica synthesis from this precursor requires an agent that displays hydrolytic activity simultaneously with structure-directing properties. Site-directed mutagenesis of the cloned DNA coding for silicatein revealed that interacting histidine and serine residues were required for the hydrolytic activity of this protein. Based on this precedent, simple homopolyamides of amino acids bearing polar functional groups were evaluated for their ability to mimic the properties of silicatein in the polycondensation of silicon alkoxides. When the homopolymers of L-lysine, L-histidine, DAL-serine, L-threonine, and L-glutamic acid were separately dissolved in aqueous pH 7 buffer and mixed with TEOs, it was found that none of these polymers was able to produce silica at ambient temperature over a 24 hour period. Furthermore, mixtures of these homopolymers also failed to catalyze TEOs hydrolysis and condensation. In contrast, we found that oligomers of L-cysteine (ca. 3000 Da, used since higher chain lengths were insoluble) efficiently produce silica from TEOs in pH 7 buffer (Table 2), when handled under an inert nitrogen atmosphere to prevent oxidation. This result was presumably due to the nucleophilicity of the sulphydryl group, which may enable it to initiate hydrolysis of the silicon alkoxide. When these oligomers were used under air, oxidation of the sulphydryl groups to disulfides resulted in insoluble aggregates that were less much active in silica formation (Table 2). However, the silica formed by using oligo-L-cysteine was an amorphous powder with no defined macroscopic shape. From these results we concluded that simple homopolymers of amino acids, which lack the structural complexity and polyfunctionalitly found in proteins, are unable to reproduce the shape-controlling ability of silicatein.

In an effort to better mimic this protein, we synthesized diblock copolyamides that contained covalently linked domains (blocks) of water soluble and insoluble polypeptides. Dissimilarity in the block segments imparted the chains with an amphiphilic character, similar to that of surfactants, which resulted in self-assembly of the chains in aqueous solution. The architecture and design of the block copolyamides also provided a means to solubilize water-insoluble domains, e.g. hydrolytically active poly-L-cysteine. For these reasons, block copolyamides were
expected to allow the directed cooperative assembly, hydrolysis, and condensation of TEOS to form specific silica structures. The solubilizing block copolypeptide components were either cationic or anionic polyelectrolytes, such as poly-L-lysine and poly-L-glutamate, which are known to be water soluble at pH 7. As water insoluble domains, poly-L-cysteine and poly-L-serine were chosen both for their potential silica-forming hydrolytic activity as well as their ability to aggregate in, water by either hydrogen or covalent bonding via \( \beta \)-sheet formation or disulfide linkages. Other insoluble domains chosen included polar residues that were less nucleophilic than cysteine (poly-L-glutamine and poly-L-tyrosine) or slightly hydrophobic (poly-L-lysine). Poly-L-histidine was not used in these studies because of difficulty in protecting the side-chain to form a suitable NCA monomer. The block copolypeptides that were synthesized and studied are given in Table 2. They were prepared from suitably protected amino acid-N-carboxyanhydride (NCA) monomers by using the initiator 2,2-bipyridylIn(1,5-cyclooctadiene). This synthetic protocol has been shown to give block copolypeptides of narrow molecular weight distributions and with controlled molecular weights (FIG. 4) [Deming, T. J. (1997) Nature 390, 386–389].

The cationic block copolymer, showed more activity in silica formation than the corresponding anionic copolymer. In fact, poly-L-glutamate completely inhibited the ability of the polyelectrolyte block to form silica, which supports the hypothesis that polycations are important for interacting with negatively charged silicate precursors. All of the lysine containing copolymer displayed some activity in silica formation, and the rate of silica production increased steadily as the domain bound to the polystyrene block became more nucleophilic. Since polymer 1, which contains no nucleophilic component, was able to produce silica, it appeared that poly-L-lysine itself, when constrained in a self-assembling block copolymer, possessed a low activity toward the hydrolysis and condensation of TEOS. However, the cyssteine and serine containing copolymer were the only ones that were able to control the shape of the silica during its formation, with the cysteine-containing polymers being most active. For these reasons, further studies were focused on the cysteine-lysine block copolymer combination.

In initial experiments, polymer 6 was deprotected and handled under a nitrogen atmosphere and thus was used in its reduced form when reacted with TEOS. Dynamic light scattering measurements of 6 in aqueous solution (1.6 mg/mL) showed that this polymer self-assembled into large aggregates approximately 600 nm in diameter. This colloidal polymer solution, when mixed with TEOS, formed a two-phase system in which some TEOS was emulsified into the aqueous phase. After 24 h, the formation of transparent, composite silica spheres (diameter, ca. 100 nm) was observed (FIG. 5). 29Si MAS NMR measurements confirmed the existence of highly condensed silica (35% Q3 and 65% Q4 species). When calcined at 500°C, the spheres remained both intact and transparent without a decrease in apparent size, although TGA analysis revealed a 10% weight loss of organic material. BET nitrogen sorption measurements showed that the spheres were mesoporous; with a broad distribution of pore sizes and a surface area of 436 m²/g (20). Using the block copolypeptide 6 to prepare these hard, transparent, mesoporous silica spheres represents the first example in which hydrolysis and condensation of an inorganic phase as well as structural templating were all controlled by a single synthetic material at pH 7, thus mimicking biological silica synthesis. To the best of our knowledge, other surfactant or polymer based systems developed for shape-selective silica synthesis typically require use of a catalyst and extreme pH conditions (10, 21, 22).

Copolymers similar to 6, but with different block lengths, were also synthesized to determine the role of copolymer composition on silica-forming ability. A polymer with a shorter cysteine domain, 7, was similar to 6 in being able to produce silica spheres. However, when the length of the cysteine domain was increased (8), the formation of more elongated silica particles was observed. Increasing the size of the lysine domain gave a polymer, 9, which behaved in the same way as the smaller, but similar composition, 7. This indicates that the copolymer chain length has little effect on resulting silica shape. It should be noted that mixtures of L-lysine and L-cysteine homopolymers, in proportions similar to those found in the block copolypeptides 6–9, only gave completely disordered silica powders from TEOS.

An additional feature of the cysteine residues in 6 was their ability to form covalent disulfide bonds as inter- and intra-chain crosslinks upon oxidation of the sulfhydryl groups. After deprotection of the copolymer in air, the formation of such disulfide crosslinks in oxidized 6 was evident from the high viscosity exhibited by this sample upon exposure to water. The gel dissolved rapidly upon addition of a reducing agent such as \( \beta \)-mercaptoethanol, indicating the presence of disulfide crosslinks. Dynamic light scattering measurements of oxidized solutions of 6 showed that the block copolymer aggregates had increased in size (ca. 1300 nm dia.) relative to unoxidized samples (ca. 600 nm). Surprisingly, when oxidized 6 was mixed with TEOS, the rate of silica formation was found to change, although 70% of the sulfhydryl groups had been converted to disulfide linkages (Table 1). In addition, ordered columns of silica were observed instead of spheres, showing that oxidation of the poly-L-cysteine domains was sufficient to completely modify the resulting topology of the silica (FIG. 2). With copolymers of different composition (Table 2), it could be shown that a minimum fraction of cysteine (ca. 15 mol %) was required to produce the columnar shaped silica composites. These results illustrate the importance of the self-assembled block copolymer architecture in the formation of silica shapes. The synthetic capability to directly control silica shape, hydrolysis and condensation rate via adjustment of block copolypeptide composition demonstrated herein presents a new route to the environmentally benign, biomimetic synthesis of inorganic materials.

From the foregoing description, one skilled in the art can readily ascertain the essential characteristics of the invention and, without departing from the spirit and scope thereof, can adapt the invention to various usages and conditions. Changes in form and substitution of equivalents are contemplated as circumstances may suggest or render expedient, and although specific terms have been employed herein, they are intended in a descriptive sense and not for purposes of limitation.
SEQ ID NO: 1
LENGTH: 330
TYPE: PRT
ORGANISM: Tetysa aurantia

SEQUENCE: 1

Met Tyr Leu Gly Thr Leu Val Val Cys Val Leu Gla Ala Ala Ile
1      5       10      15

Gly Glu Pro Met Pro Gln Tyr Glu Phe Lys Glu Glu Trp Gln Leu Trp
20     25      30

Lys Lys Glu His Asp Lys Ser Tyr Ser Thr Asn Leu Glu Glu Leu Glu
35     40      45

Lys His Leu Val Trp Leu Ser Asn Tyr Leu Tyr Ile Glu Leu His Asn
50     55      60

Asn Ala Asp Thr Phe Gly Phe Thr Leu Ala Met Asn His Leu Gla
65     70      75      80

Asp Met Thr Asp His Glu Tyr Glu Arg Tyr Leu Thr Tyr Thr Asn
85     90

Ser Lys Ser Gly Asn Tyr Thr Lys Val Phe Lys Arg Glu Arg Pro Thr Met
100    105     110

Asn Thr Pro Glu Thr Val Asp Thr Arg Thr Lys Leu Ala Val Thr Gly
115    120     125

Ile Lys Ser Gln Gly Asp Cys Gly Ala Ser Thr Ala Phe Ser Ala Met
130    135     140

Gly Ala Leu Glu Gly Ile Asn Ala Leu Ala Thr Gly Leu Thr Tyr
145    150     155     160

Leu Ser Glu Gln Asn Ile Asp Cys Ser Val Pro Tyr Gly Asn His
165    170     175

Gly Cys Lys Gly Gly Asn Met Tyr Val Ala Phe Leu Tyr Val Val Ala
180    185     190

Asn Glu Gly Val Asp Asp Gly Gly Ser Tyr Pro Phe Arg Gly Lys Glu
195    200     205

Ser Ser Cys Thr Tyr Gln Glu Glu Tyr Arg Gly Ala Ser Met Ser Gly
210    215     220

Ser Val Gln Ile Asn Ser Gly Ser Glu Ser Asp Leu Glu Ala Ala Val
225    230     235     240

Asn Ala Val Gly Pro Val Ala Val Ala Ile Asp Gly Glu Ser Asn Ala
245    250     255

Phe Arg Phe Tyr Tyr Ser Gly Val Tyr Asp Ser Ser Arg Cys Ser Ser
260    265     270

Ser Ser Leu Asn His Ala Met Val Ile Thr Gly Tyr Gly Ile Ser Asn
275    280     285

Asn Glu Gly Tyr Trp Leu Ala Lys Asn Ser Trp Gly Glu Aen Trp Gly
290    295     300

Gly Leu Gly Tyr Val Lys Met Ala Arg Asn Lys Tyr Asn Glu Gly Cys Gly
305    310     315     320

Ile Ala Ser Asp Ala Ser Tyr Pro Thr Leu
325    330

SEQ ID NO: 2
LENGTH: 1360
TYPE: DNA
What is claimed is:

1. A method for forming a dioxane, oligo-oxane, or polyoxane product, comprising condensing an alkoxide substrate with another alkoxide material at neutral or near neutral pH using a catalyst comprising a molecule having a nucleophilic group that displaces alkanol from said alkoxide substrate facilitating solvolysis to initiate structure-directed condensation with said another alkoxide.

2. The method of claim 1 wherein said structure-directed condensation is by nucleophilic attack.

3. The method of claim 1 wherein said nucleophilic group forms a transitory intermediate in facilitating solvolysis.

4. The method of claim 3 wherein said transitory intermediate is covalent.

5. The method of claim 1 comprising a group that interacts with said nucleophilic group to increase its nucleophilicity.

6. The method of claim 5 wherein said interaction is by hydrogen bonding.

7. The method of claim 1 wherein either or both of said alkoxides or alkoxide-like material is selected from the group consisting of silicon or other metalloid alkoxides, and organic conjugates of the foregoing, to form the corresponding silica, silesquioxanes, polymetalloid-oxanes, polymetallo-oxanes, or the corresponding organic conjugates of the foregoing.

8. The method of claim 1 wherein said molecule is a protein.

9. The method of claim 1 wherein said molecule is an enzyme.

10. The method of claim 9 wherein said enzyme is a silicatein.

11. The method of claim 9 wherein said enzyme is a protease.

12. The method of claim 9 wherein said enzyme is a peptidase.

13. The method of claim 9 wherein said enzyme is a hydrolase.

14. The method of claim 13 wherein said hydrolase is selected from the group consisting essentially of amidase, esterase and lipase.

15. The method of claim 9 wherein said enzyme is a catalytic triad enzyme.

16. The method of claim 1 wherein said molecule is a peptide.

17. The method of claim 16 wherein said peptide contains lysine or poly-lysine.
18. The method of claim 16 wherein said peptide contains serine or poly-serine.
19. The method of claim 16 wherein said peptide contains a tyrosine.
20. The method of claim 16 wherein said peptide contains a histidine.
21. The method of claim 16 wherein said peptide contains cysteine, oligo-cysteine or poly-cysteine.
22. The method of claim 16 wherein said peptide contains a nucleophilic catalytic side-chain.
23. The method of claim 22 wherein said nucleophilic catalytic side-chain is contributed by serine, cysteine, histidine or tyrosine.
24. The method of claim 16 wherein said peptide contains a hydrogen-bonding amine.
25. The method of claim 1 wherein said molecule is a non-peptide-based polymer that operates by a mechanism of catalysis similar to that utilized by silicateins.
26. The method of claim 25 wherein said non-peptide-based polymer contains a hydrogen-bonding amine and/or a nucleophilic group.
27. The method of claim 1 wherein either or both of said alkoxides is a silicon alkoxide.
28. The method of claim 27 wherein said silicon alkoxide is tetraethoxysilicate.
29. The method of claim 1 wherein either or both of said alkoxides is an organosilicon alkoxide.
30. The method of claim 29 wherein said organosilicon alkoxide is methyl-triethoxysilane, or phenyl-triethoxysilane.
31. The method of claim 1 wherein either or both of said alkoxides is a metallo alkoxide.
32. The method of claim 1 wherein either or both of said alkoxides is an organometallo-alkoxide.
33. The method of claim 1 wherein either or both of said alkoxides is a metalloid alkoxide.
34. The method of claim 1 wherein either or both of said alkoxides is an organometalld alkoxide.
35. The method of claim 27 wherein said product is a silsesquioxane.
36. The method of claim 29 wherein said product is a polyorganosiloxane.
37. The method of claim 31 wherein said product is a polymetallo-oxane.
38. The method of claim 32 wherein said product is a polyorganometallo-oxane.
39. The method of claim 34 wherein said product is a polyorganometalld-oxane.
40. The method of claim 1 in which said molecule is self-assembling whereby said structure-directed condensation is provided by a spatial array of structure-directing determinants contained on or within the self-assembling molecule.
41. The method of claim 40 in which said spatial array of structure-directing determinants acts in conjunction with the surfaces of any solid support to which said molecule is attached or in which said molecule is confined.
42. The method of claim 40 wherein said molecule is selected from the group consisting essentially of silicatein, protein, enzyme, peptide, and non-peptide-based polymers, and/or any aggregate, filament, or other assembly thereof.
43. The method of claim 1 in which said nucleophilic group is provided by a hydroxyl or sulphydryl group.
44. A method for forming a dioxane, oligo-oxane, or polyoxane product, comprising condensing an alkoxide substrate with another alkoxide material at neutral or near neutral pH using a catalyst comprising a self-assembling molecule having a nucleophilic group that displaces alkanol from said alkoxide substrate by forming a transitory covalent intermediate facilitating solvolysis to initiate structure-directed condensation with said another alkoxide with structure-directing control of product formation resulting from a spatial array of structure-directing determinants contained on or within the self-assembling molecule acting in conjunction with the surfaces of any solid support to which said molecule is attached or in which said molecule is confined; said molecule being selected from the group consisting essentially of silicatein, protein, enzyme, peptide, and non-peptide-based polymers, that operates by a mechanism of catalysis similar to that utilized by silicateins; and either or both of said alkoxides being selected from the group consisting of silicon or other metalloid alkoxides, metal alkoxides, and organic conjugates of the foregoing, to form the corresponding silica, silsesquioxanes, polymealtoxanes, polymetallooxanes, or the corresponding organic conjugates of the foregoing;
wherein said product is a silsesquioxane, a polyorganosiloxane, a polymetallo-oxane, a polyorganometalld-oxane, or a polyorganometalld-oxane.
45. The method of claim 44 comprising a group that interacts by hydrogen bonding with said nucleophilic group to increase its nucleophilicity.
46. The method of claim 44 in which said nucleophilic group is provided by a hydroxyl or sulphydryl group.

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