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Bifunctional Small Molecules Are Biomimetic Catalysts for Silica Synthesis at Neutral pH

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Abstract: Silicatein is an enzyme isolated from the biosilica produced by the marine demosponge, *Tethya aurantia.* Once isolated from the sponge, silicatein can be used in vitro to catalyze the hydrolysis and direct polycondensation of a wide variety of alkoxide, ionic, and organometallic precursors to the corresponding chalcogens at standard temperature and pressure and neutral pH. On the basis of these results, an array of small molecules that mimic the unique physiochemical environment found in the enzyme active site was investigated for catalytic activity in the formation of silica from silicon alkoxides at neutral pH. The most successful of these biomimetic catalysts (cysteamine) was used to encapsulate firefly luciferase, green and blue fluorescent proteins (GFP, BFP), and *Escherichia coli* cells expressing GFP in silica matrixes. The benign conditions required for the catalysis of synthesis of these silica composites does not impair the activities of the encapsulated enzyme, fluorescent proteins, or live cells as shown by fluorescence measurements. In conjunction with microcontact printing, this biomimetically catalyzed encapsulation method has been used to produce patterned functional arrays of silica nanoparticulate composite materials.

I. Introduction

Silicon, the second most abundant element on Earth, is widely used in the manufacture of numerous siloxane-based materials, including semiconductors, glasses, ceramics, plastics, elastomers, resins, mesoporous molecular sieves and catalysts, and optical fibers and coatings.[1,2] The manufacturing of these materials typically requires high temperatures and pressures or the use of caustic chemicals. In contrast, the biological production of amorphous silica is accomplished under mild physiological conditions and produces remarkably exquisite structures, such as sponge skeletal elements (spicules) and diatom frustules. This contrast in processing conditions and the growing demand for benign and flexible industrial synthetic routes that minimize adverse environmental effects have spurred interest in biologically inspired silicon technology.

Thousands of living species produce ordered silica structures under mild physiological conditions of near-neutral pH and low temperature. The biological syntheses of silica demonstrate that these species have evolved novel mechanisms for the control of siloxane polycondensation. Silicateins from sponge biosilica of the marine demosponge *Tethya aurantia* have been found to catalyze hydrolysis and subsequently direct polycondensation of numerous silica and chalcogenic precursors.[2−12] Thermal denaturation abolishes catalytic activity of the protein, suggesting the indispensability of the three-dimensional structure of the native protein molecule.[4] Additionally, the composition of certain key residues must be conserved to retain full catalytic activity. Site-directed mutagenesis of the cloned DNA encoding silicatein α, the principal subunit comprising about 70% of the mass of the silicatein filaments, revealed that two specific amino acids, serine26 (hydroxy side chain) and histidine165 (imidazole side chain), are required for the catalytic activity of the protein.[5] These residues are critical for the effectiveness of the catalytic active site to function in a number of structurally and functionally related enzymes found in evolutionarily divergent species.[13]

The dependence on two conserved residues suggests a reaction mechanism in which the nucleophilic side chain of the serine residue forms a hydrogen bond with the imidazole

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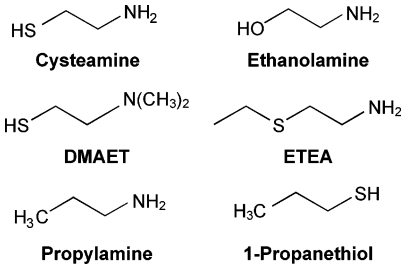
Chart 1. Structures of Small Molecule Catalysts



nitrogen of the histidine residue, at position 165, thereby enhancing the nucleophilicity of the serine oxygen in the putative binding site.[5] This mechanism may be similar to that of the structurally related proteases and other hydrolases, in which the hydrogen bonding acts cooperatively across the active site cleft to increase the nucleophilicity of the catalytically active serine hydroxyl oxygen.[13] As a result of this enhancement, the efficiency of S_N2 type nucleophilic attack is greatly enhanced.[5,14] The reaction of silicatein with alkyloxysilane precursors is postulated to proceed by such a mechanism through a transitory protein−silicon intermediate, which would be additionally favored by the potential for stabilization as a pentavalent silicon species formed via a donor bond from the imidazole nitrogen.[4,5] A pentavalent intermediate similarly is postulated to occur in the nonenzymatic formation of silica from various ethoxy precursors.[14] The intermediate is then subjected to hydrolysis, with the resulting generation and release of the reactive silanol, and regeneration of the serine-histidine hydrogen-bonded pair at the enzyme's active site. This proposed mechanism accounts for the observation of the catalytic hydrolysis of silicon ethoxides by silicatein at neutral pH, in which the generation of the silanol is known to be the rate-limiting step in the subsequent polycondensation to yield silica and the polysilsesquioxanes.[15]

Inspired by the finding of conserved residues in silicatein, block copolypeptides were synthesized in an effort to mimic the catalytic hydrolysis and polycondensation activity promoted by silicatein filaments.[7] The resulting amphiphilic copolypeptides that were produced self-assemble into structured aggregates that hydrolyze tetraethoxysilane (TEOS) at neutral pH and low temperature, while simultaneously directing the formation of ordered silica particles. Systematic substitution of residues used in the construction of these peptides showed that the rate of catalysis was proportional to the strength of nucleophilicity of the nucleophilic side chain, that hydrogen bonding was required for efficient catalysis, and that, among the polypeptides tested, cysteine-lysine block copolypeptides were the most effective.

On the basis of the above results, we set out to investigate small molecules that incorporate the features found essential for silicatein's catalysis of hydrolysis at neutral pH. A group of analogous small organic molecules bearing a nucleophilic group, such as −SH, −OH, or $-SC_2H_5$, and a hydrogen-bonding acceptor group, such as a primary or substituted amine (Chart 1), were evaluated.

One potential application for small molecule catalysts of sol−gel synthesis would be in the encapsulation of biological materials in silica or silisesquioxanes at neutral pH. Encapsulation in silica has been shown to improve long-term stability of cells and enzymes for clinical, sensing, and industrial applications, including, for example, the incorporation of enzymes in flow-through reactors utilizing solid supports.[16−21] Encapsulation allows for facile immobilization, manipulation, and recovery of enzymes in a chemically inert matrix without the need for covalent modification. Typical silica sol−gel synthesis, however, requires strong acid or alkaline hydrolysis of the precursors, which may compromise the integrity, activity, or longevity of enzymes and live cells.[22] We demonstrate here the utility of a small molecule catalyst of sol−gel synthesis at neutral pH, used in conjunction with microcontact printing (μCP), to pattern silica with encapsulated biological materials. In related studies, synthetic peptides based on those found in diatom silica were found capable of nucleating the precipitation of silica from silicic acid to encapsulate proteins.[23]

II. Materials and Methods

A. Chemicals, Materials, and General Procedures. TEOS, tetramethoxysilane (TMOS) (**CAUTION: Vapors can cause blindness; goggles must be worn**), methyltriethoxysilane (MTEOS), ethyltriethoxysilane (ETEOS), and diethyldiethyloxysilane (DEDEOS) were purchased from Gelest (Tullytown, PA). 2-Aminoethanethiol hydrochloride (cysteamine, 98%), ethanolamine hydrochloride (ethanolamine, 98%), 2-(dimethylamino)ethanethiol hydrochloride (DMAET, 95%), 2-(ethylthio)ethylamine hydrochloride (ETEA, 95%), propylamine (99%), 1-propanethiol (99%), and TRIZMA base (tris, = tris-hydroxymethylaminomethane) were purchased from Aldrich (Milwaukee, WI) and used without further purification. Green fluorescent protein (GFP), blue fluorescent protein (BFP), and recombinant bacterial cells expressing GFP were obtained from Clontech Laboratories (Palo Alto, CA), and Quantilum recombinant firefly luciferase (10−15 mg ml^{-1}) was purchased from Promega (Madison, WI). Water was filtered using a Milli-Q purification system and had a resistivity of at least 18 MΩ/cm.

Catalyst solutions at a concentration of 1 M were prepared in 0.025 M Tris-HCl buffer, and the final pH values were adjusted to 7.0, except for 1-propanethiol, which was used without dilution. In biphasic reactions, calculated amounts of 1 M catalyst solution, pure silicon alkoxide, and 0.025 M Tris buffer, pH 7.0, were mixed in 1.5-mL centrifuge tubes, vigorously shaken for 1 min, and then mixed on a tube rotator at a constant speed and room temperature for the times indicated. The resulting silica precipitate was collected by centrifugation, washed with ethanol to remove unreacted silicon alkoxide precursor, and dried at 45 °C for 24 h. Dried silica samples were dissolved in 200 μL of 50% HF solution and diluted with H_2O. Silicon concentration in these dissolved samples then was determined with an IJA IRIS inductively coupled plasma (ICP) spectrometer using a silicon standard solution (1000 ppm single element) from High Purity Standard Co. (Charleston, SC). Reactions were conducted in triplicate; deviations from the averages of the triplicate values reported here were less than 10%.

Solution pH values were measured with an Accument pH meter 925 (Fisher Scientific). For electron microscopy, samples were washed with

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water, dried, and mounted onto adhesive carbon pads attached to cylindrical aluminum stubs and gold/palladium coated to reduce charging. Scanning electron micrographs were recorded on a JEOL 6300 F scanning microscope operating at an accelerating voltage of 5 kV.

B. Biomaterial Encapsulation. Protein encapsulation experiments were performed with 0.2 mL of pure TMOS mixed with 0.2 mL of methanol and 5 mL of 0.025 M Tris-HCl buffer, pH 7.0. After mixing the components by pipetting for 1 min, 0.1 mL of 1 M catalyst solution was added, followed by 5 μL of protein solution, giving a final protein concentration of 15 μg mL^{-1}. After reaction for 30 min, the transparent gel formed was washed thoroughly with Tris-HCl buffer. Control experiments were performed with no catalyst. All manipulations were carried out in ice to slow gelation and minimize denaturation of the enzyme. Activity of the gel-encapsulated luciferase was assayed by addition of a luciferin/ATP reagent. Luminescence generated by the enzymatic reaction was quantified with a Beckman LS 500 TA liquid scintillation counter. Gels encapsulating GFP were isolated at the bottom of 1.5-mL centrifuge tubes and photographed under UV excitation using a Chromato-VUE TM-15 UV transilluminator (UVP, Inc).

C. Microcontact Printing. For microcontact printing of protein entrapped in gel matrixes, a TEM grid (Ted Pella, Reding, CA) was used as a master, and a PDMS stamp was fabricated according to published procedures.[24] The template was covered with a mixture of Sylgard silicon elastomer 184 and Sylgard curing agent (Dow Chemical) and cured for 1 h at 65°C, and the stamp was then removed from the master. Protein encapsulation solution was dropped onto a polished silicon wafer (Virginia Semiconductor), and the stamp was then applied to the surface using light (10−20 psi) pressure.

After curing, micromolded silica encapsulating green fluorescent protein (Clontech Laboratories, Inc.) or recombinant *Escherichia coli* cells expressing GFP (Clontech Laboratories) were deposited on the surface of a silicon wafer, and fluorescent images were obtained with a fluorescence microscope (Zeiss).

III. Results and Discussion

A. Screening for Catalytic Activity. Mutational analyses demonstrated that the catalysis of silica formation from TEOS by silicatein is strictly dependent on the close proximity of a hydrogen bond donor and good hydrogen-bonding nucleophile at the enzyme's active site. When either of these chemical moieties was replaced with an inert ($-CH_3$) functionality, catalytic activity was decreased 10-fold, analogous to results seen with other serine proteases and hydrolases.[5] In the case of silicatein and many other hydrolases, the required functionalities are presented by the histidine (hydrogen bond donor) and serine (good nucleophile) residues.[3,25] Starting with these cues, and the observed activities of the biomimetic block copolypeptides cited above,[7] a short list of small molecules containing both chemical functionalities with the necessary steric tolerances was determined. Additionally, only water stable and soluble molecular species were considered.

A variety of small molecules exhibiting chemical similarity to the active site functionalities of silicatein was investigated. The staggering number of possible small molecule candidates was narrowed to a field of six (Chart 1) based on the proximity of a good hydrogen bond donor and nucleophile. Systematic variation in these functionalities was used to optimize biomimetic catalysis. Chart 1 is by no means an exhaustive list of possible molecules, but rather it represents a broad stroke of varying chemical and stereochemical functionality that roughly mimics the physiochemical environment in the active site of silicatein. This family of bifunctional, biomimetic catalysts possesses two functional groups, a nucleophilic functionality, such as −SH or −OH, and a hydrogen-bonding acceptor group, such as $-NH_2$ or −NH−.

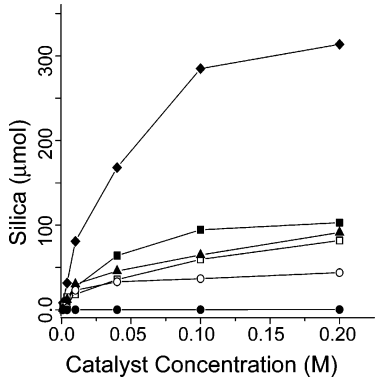

Figure 1. Effect of catalyst concentration on the hydrolysis and polycondensation of TEOS after 72 h with varying concentrations of: (◆) cysteamine, (■) ethanolamine, (▲) ETEA, (□) DMAET, (○) propylamine, or (●) 1-propanethiol.

Molecular modeling simulations based on cathepsin L, the serine protease found most closely homologous to the silicatein structure,[3] suggest a spacing between sulfur and nitrogen in the active site to be 3.2 Å. This is in close agreement with other hydrolases containing the catalytic triad of serine, histidine, and asparagine.[13] The molecular candidates in Chart 1 have a well-defined distance between the chemical functionalities with a single carbon−carbon bond (1.5 Å) coupled with a dihedral angle of 120° providing the geometric constraint. This will yield a common spacing of 2.9 Å between functional groups, which is approaching a typical hydrogen bond distance of 1.3−2 Å. By maintaining this common spacer distance, we were able to eliminate one design variable from the potential list of molecular candidates. Within this constraint, a range of functionalities was investigated.

The yield of precipitated silica was determined as a function of reaction time for each of the candidate catalysts, using the biphasic reaction conditions described in Materials and Methods. Silica formation was quantified using ICP; only silica polymers and particles large enough to be isolated with centrifugation from the bulk solution were considered.

Cysteamine and ethanolamine are the two most obvious analogues of the active site residues, and these show the highest catalytic activity with TEOS at neutral pH as seen in Figure 1. In the absence of the catalysts, TEOS remained stable when mixed with 0.025 M Tris-HCl buffer at pH 7 and rotated at room temperature for several days. Figure 1 reveals that even a low concentration (10 mM) of cysteamine is capable of a 10-fold acceleration of silica formation over the buffer control. As can be seen from the data, large differences in recovered silica occur with small changes in catalyst concentration. As seen in Figure 2, cysteamine is capable of producing approximately 40% more silica per unit time than the next most promising candidate, ethanolamine. ETEA has a secondary thiol that withdraws electron density from the thiol group, thereby decreasing the nucleophilic character of the sulfur, and thus, the yield of silica is correlated with the nucleophilicity of the catalyst. DMAET retains the primary sulfur, but the amine is tertiary, and the hydrogen bond accepting ability thus is greatly reduced, as manifest in the low reactivity relative to that of cysteamine. The monofunctional candidates (propanethiol and propylamine) were

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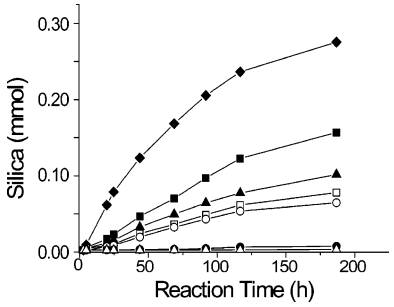

Figure 2. TEOS polycondensation as a function of time in the presence of 50 mM: (◆) cysteamine, (■) ethanolamine, (▲) ETEA, (□) DMAET, (○) propylamine, (●) 1-propanethiol, or (△) buffer only.

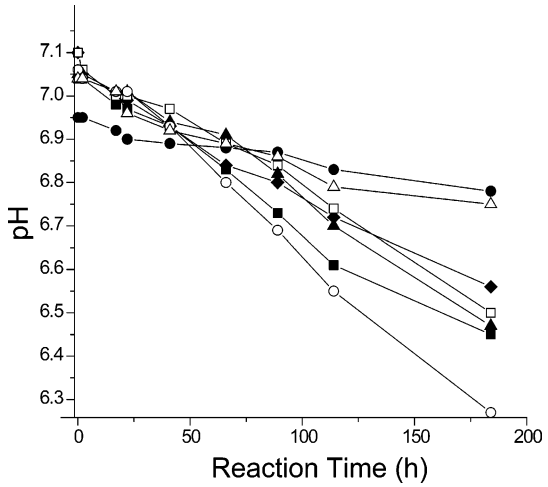

Figure 3. pH evolution as a function of time during TEOS polycondensation in the presence of 50 mM: (◆) cysteamine, (■) ethanolamine, (▲) ETEA, (□) DMAET, (○) propylamine, (●) 1-propanethiol or (△) buffer only.

least active, highlighting the requirement of bifunctionality for efficient catalysis.

The continuous slow decrease of pH of the reaction systems shown in Figure 3 indicates an increase in concentration of hydrolyzed species in the reaction media, while the presence of the buffer maintains moderately stable pH over the course of the experiment. The buffer alone may have had some effect on the hydrolysis of TEOS, as the pH of the reaction mixture with no catalyst added decreased from 7.0 to 6.8. However, no recoverable silica was formed under this condition, indicating that Tris buffer alone does not significantly catalyze the condensation and hydrolysis of the silicon ethoxide. Figure 3 also confirms that the silica forming reaction is not simply due to acid or base hydrolysis. Throughout the entire time course of the experiment the reaction pH remains in a region where spontaneous hydrolysis of silicon alkoxides is at a minimum.[14]

B. Morphological Analysis of Recovered Silica. XRD measurements show that the product of all reactions we observed was amorphous silica powder. SEM micrographs of the silica product from the cysteamine catalyzed reaction are seen in Figure 4. Spherical particles ranging from ~40−100 nm were observed. No higher ordered structures were observed by SEM and small angle XRD (data not shown). Cysteamine, unlike diblock copolypeptides[7] and long chain silafin peptides,[23,28] has little ability to attract and align nuclei, but rather acts only as a catalyst for the hydrolysis of silica precursors. This is apparent in the morphology of the resulting silica, as all particles are spherical, presumably reflecting their formation via homogeneous nucleation (as would occur in the absence of any surface).[15] The size regime observed could prove useful for encapsulation of pH-sensitive biological material.

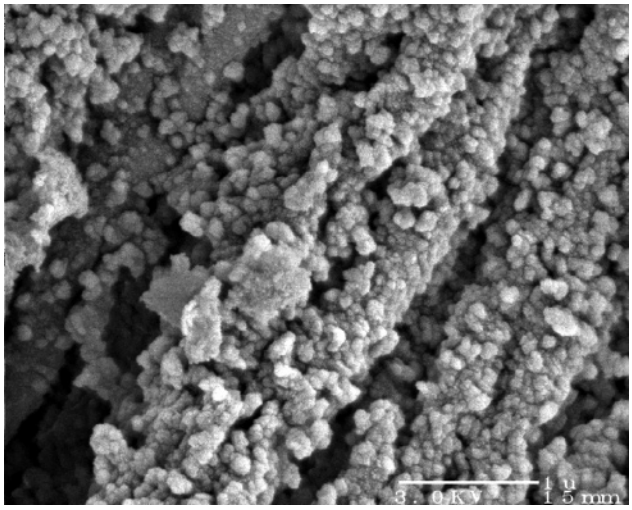

Figure 4. SEM micrographs of silica obtained from polycondensation of TEOS catalyzed by 10 mM cysteamine (scale bar is 1 μm).

Table 1. Cysteamine Concentration Affects Silica and Silsesquioxane Synthesis at Neutral pH and Room Temperature in a Biphasic Reaction

	cysteamine concentration (mM)	time for observable precipitate to form (h)
TEOS	50	5.5
	25	46
ETEOS	50	2.5
	25	70
MTEOS	50	3.5
	25	24
DEDEOS	50	0.25
	25	4.5

C. Cysteamine Reactivity Toward Substituted TEOS Precursors. Three organically substituted analogues of TEOS were investigated as precursors in conjunction with the most efficient catalyst (cysteamine). All three appear more reactive with cysteamine than TEOS, yielding observable precipitates of the corresponding silsesquioxanes in as little as 15 min. As seen in Table 1, the fastest time to observable precipitate formation occurs with DEDEOS, followed by the less substituted molecules. This hierarchy presumably reflects the fact that the silicon ethoxy bond energy decreases with increasing polarity, thereby allowing faster hydrolysis of the more substituted analogues.[14] Furthermore, the electron-donating alkane chains increase the electronegativity of the silicon atom and thereby increase the likelihood of nucleophilic attack. This translates into faster reaction rates for ETEOS, MTEOS, and DEDEOS. Even though TEOS has four ethoxy groups that can react to form silica, this statistical advantage over the silsesquioxane precursors is not enough to overcome the effect of substituent chemistry.

The reactivity of TMOS also was investigated (Figure 5). The results demonstrate that low concentrations of cysteamine can catalyze the rapid formation of silica from TMOS. Even

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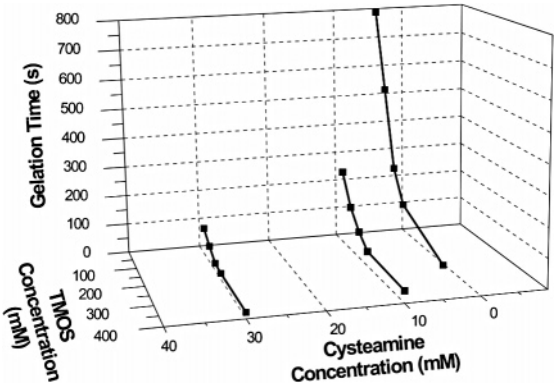

Figure 5. Catalytic activity of cysteamine toward silica synthesis from TMOS is dependent on catalyst and precursor concentration.

though TMOS is much more readily hydrolyzed than TEOS, a dilute (100 mM) solution at pH 7 shows no gelation after 35 h. The bond energies of the Si−O bonds in TMOS and TEOS are 477 and 484 kJ mol^{-1}, respectively. This difference in bond energies and the steric hindrance of ethoxy groups relative to methoxy are likely to account for the observed differences in susceptibility to hydrolysis. Because of the faster rate of reaction of TMOS, this precursor was used for the encapsulation studies described below.

D. Encapsulation of Biomaterials. Encapsulation of biomaterials including enzymes, antibodies, and cells in silica or siloxane gels provides a facile path to immobilization in robust but permeable hosts for a wide range of catalytic, sensor, and controlled release applications.[16,17,19,21] Sol−gel matrixes can be produced in a variety of forms, including optically transparent films, fibers, micro- and nanospheres, and monoliths, providing great flexibility for these applications.[27] When the pH of the reaction media is kept above the zero charge point of silica (pK_a $\approx$ 3),[26] the silica moieties will be negatively charged, leading to the formation of networks of interconnected particles ultimately producing a sol.[27] The advantages of a catalytic method for sol−gel polymerization and encapsulation at neutral pH, over conventional acid/base technologies, include: (1) protection of acid-sensitive, alkali-sensitive, and heat-sensitive molecules and materials, (2) physical entrapment of enzymes and cells without covalent bonding or denaturation, thus preserving their native structures and activities, (3) the ability to rapidly coat, insulate, seal, encapsulate, package, or sheath sensitive biological materials and components in silica, glass, or silsesquioxanes (silicones) with a wide range of tunable physical properties, including stability to heat, chemicals, and photodegradation,[29] (4) environmentally benign synthetic conditions and lower energy and capital costs, and (5) opportunities for co-encapsulation[30] of biologically sensitive molecules to enhance performance and cell viability.

Two catalysts, cysteamine and ethanolamine, were employed in the catalytic formation of sol−gel matrixes for enzyme and bacterial cell encapsulation. TMOS was used as silica precursor instead of TEOS because of a faster gelation rate as seen in Figure 5. Methanol was used as a cosolvent to form a one-phase reaction system rather than ethanol to decrease cell toxicity.

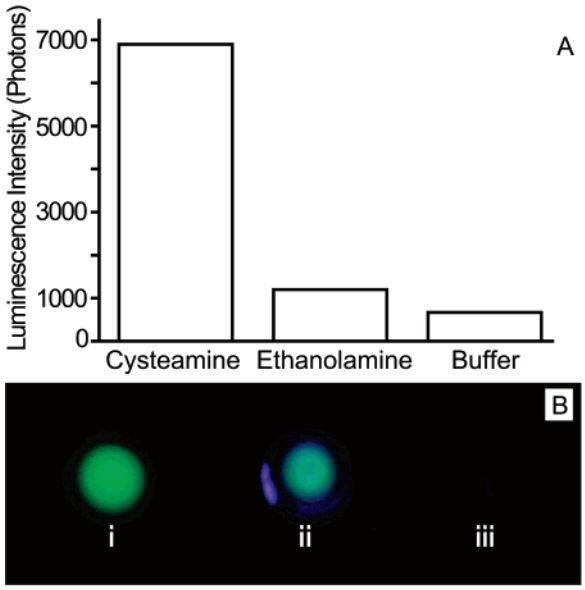

Figure 6. (A) Luminescence intensity of silica encapsulated luciferase after addition of luciferin reagent. (B) Fluorescent images of GFP entrapped within sol−gel matrixes formed with (i) cysteamine, (ii) ethanolamine, and (iii) buffer only.

E. Luciferase and GFP Encapsulation. The firefly luciferase/luciferin reaction is one of the most frequently used bioluminescent reactions for a variety of analytical purposes. It can be used for assays of ATP and any enzyme or metabolite participating in ATP formation or degradation. The pH optimum for firefly luciferase is 7.8; hence, maintenance of a near-neutral pH during encapsulation would be beneficial for both the stability and activity of the enzyme. At this pH, spontaneous hydrolysis of TMOS is slowest[14] (cf. Figure 5); for these reasons, cysteamine and ethanolamine were used to catalyze the encapsulation of active firefly luciferase and GFP within a sol−gel matrix.

Under the conditions we employed, homogeneous gels were formed encapsulating the luciferase. With no catalyst present, no gel was formed within the 48 h reaction time; in contrast, the addition of cysteamine or ethanolamine induced efficient gel formation from TMOS at neutral pH and room temperature. After washing, the gel-encapsulated enzyme was assayed by exposure to luciferin substrate, and the resulting luminescence was quantified by photon counting as seen in Figure 6A. The results indicate that the enzyme remains active after encapsulation and is accessible to the luciferin reagent provided in solution. If we assume that the yield of fluorescence under standardized conditions can be directly correlated with the extent of active protein encapsulation, then the gels formed by catalysis with cysteamine and ethanolamine were found to have retained 87 and 17% of the original luciferase activity, respectively. Assuming the two products have the same porosity, these results are in good agreement with those of Figures 1 and 2, in which the observed catalytic efficiency of cysteamine is significantly greater than that of ethanolamine.

Using the same method as above, we entrapped purified GFP in silica. After sufficient curing (30 min) and washing, fluorescent images were acquired as seen in Figure 6B. These

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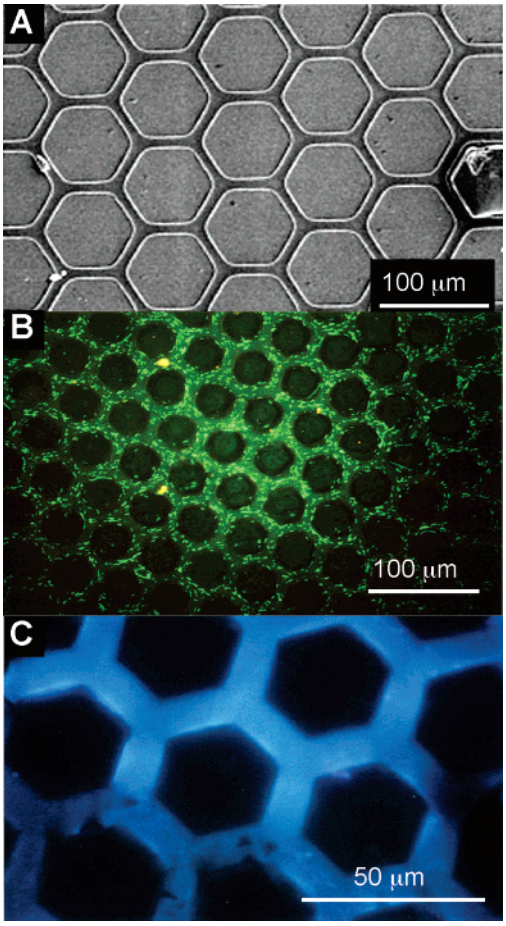

Figure 7. (A) SEM image of the surface of a PDMS stamp used during μCP. Fluorescent images of micromolded silica encapsulating (B) recombinant *E. coli* cells expressing GFP and (C) blue fluorescent protein on a silicon wafer using cysteamine as a catalyst.

results mirror the trends observed in luciferase activity and Figures 1 and 2. Fluorescence quenching by the silica was not observed.

F. Micropatterning BFP and GFP-Expressing Recombinant *E. coli* Cells. On the basis of the preliminary protein encapsulation experiments described above, we immobilized purified BFP and live, recombinant *E. coli* cells expressing GFP in two-dimensionally micropatterned gel matrixes. Patterns were produced by microcontact printing as described in Materials and Methods, using a TEM grid to form the master polymeric stamp (Figure 7A). A suspension of live cells expressing GFP or a solution of purified BFP was mixed with silica precursor, cysteamine, and buffer and dropped onto a silicon wafer. The stamp was quickly applied to the surface, and the mixture was allowed to cure. After removal of the stamp, the silica gel remained on the surface, and the cells or BFP were found to be restricted to the stamped areas as seen in Figure 7B,C. The resulting microarrayed patterns of encapsulated products printed on silicon wafers remained stable and active for 7 days at 4 °C, confirming the stabilizing effect of the silica matrix.[29]

IV. Conclusions

Whereas high molecular weight block copolypeptides based on the silicatein active site have been shown to mimic the catalytic activity of the enzyme, the results reported here are the first to demonstrate that small, rationally selected bifunctional molecules can mimic the catalytic activity of silicatein. Thus, cysteamine, ethanolamine, and their analogues prove to be efficient catalysts of hydrolysis and polycondensation of silicon alkoxide precursors at neutral pH at room temperature. As in the natural enzyme, the nucleophilic attacking group, either −SH or −OH, is shown to be essential for hydrolysis of the silane, while the protonated amine group as well as its hydrogen bonding nature enhance this reaction and facilitate the sequential polycondensation to yield the silica product. Cysteamine, which contains both −SH and $-NH_2$, showed the best catalytic effectiveness of the molecules tested. Analogues lacking either one of these two functionalities exhibit dramatically reduced catalytic activity, in close agreement with the results of alteration of the silicatein active site by genetic engineering. Amorphous silica was the product of all reactions observed, with particle sizes ranging from ∼40−100 nm. The unique catalysts described herein have potential in the area of biomaterial encapsulation and sol−gel processing, as demonstrated by the encapsulation and micropatterning of enzymes, fluorescent proteins, and live whole cells.

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