Energetic clues to pathways to biomineralization: Precursors, clusters, and nanoparticles

Alexandra Navrotsky*

Thermochemistry Facility and Nanomaterials in the Environment, Agriculture and Technology Organized Research Unit, University of California, 1 Shields Avenue, Davis, CA 95616

Contributed by Alexandra Navrotsky, July 2, 2004

Nanoparticle and nanocluster precursors may play a major role in biomineralization. The small differences in enthalpy and free energy among metastable nanoscale phases offer controlled thermodynamic and mechanistic pathways. Clusters and nanoparticles offer concentration and controlled transport of reactants. Control of polymorphism, surface energy, and surface charge on nanoparticles can lead to morphological control and appropriate growth rates of biominerals. Rather than conventional nucleation and growth, assembly of nanoparticles may provide alternative mechanisms for crystal growth. The Ostwald step rule, based on a thermodynamic view of nucleation and growth, is supported by the observation that more metastable phases tend to have lower surface energies. Examples from nonbiological systems, stressing the interplay of thermodynamic and kinetic factors, illustrate features potentially important to biomineralization.

Biomineralization, the production of inorganic phases (oxides, sulfides, silica, carbonates, and phosphates) by living organisms, produces metabolic energy and/or mechanical support for a variety of organisms from unicellular to mammalian. Although the starting point for the concentration and transformation of components in an aqueous medium to form crystals is generally assumed to be an aqueous solution containing dissolved ions, there is increasing evidence that clusters, nanoscale amorphous precipitates, and other more complex precursors in the aqueous phase may play an important role in crystallization (1–3). The purpose of this article is to summarize some of the features known about these complex nanoscopic pathways to crystallization in nonliving systems, discuss implications for biomineralization, suggest possible ways in which organisms can control the phases formed and their morphology using nanoscale intermediates, and propose specific features to look for in biomineralizing systems as evidence for such pathways. Emphasis is on biologically controlled rather than biologically induced mineralization, because the former involves much more delicate control of polymorphism, crystal morphology, and composite shape. It is argued that such control can be achieved by mechanisms involving nanoparticle precursors and their complex interactions with biomolecules. Because crystallization involves both thermodynamic and kinetic controls, both energetics and mechanisms are discussed, with emphasis on their interplay.

Energetics and the Control of Polymorphism

Many of the compositions that form as biominerals can exist in several different structural modifications: calcite, aragonite, and vaterite for CaCO3; wurtzite and sphalerite for ZnS; numerous iron and manganese oxides and oxyhydroxides; quartz, cristobalite, tridymite, and many open zeolitic structures for SiO2 as well as in poorly crystalline, amorphous, and hydrated forms. Although one crystalline polymorph is thermodynamically the most stable under a given set of conditions (temperature, pressure, oxygen, and water fugacity), others are often only slightly metastable by only a few kilojoules per mole (4–12) (see Figs. 1 and 2) and can be accessed by specific synthetic paths. This metastability at room temperature is governed mainly by a $\Delta H$ term of 1–10 kJ/mol, with the $T\Delta S$ term forming a correction of 10–20% [larger when dehydration is involved (8–13)]. The important point is that these $\Delta H$ and $\Delta G$ terms are small enough such that the order of thermodynamic stability can be inverted by a number of factors, including particle size. Figs. 3–5 show the crossover in energetic stability as a function of surface area for alumina, titania, and zirconia as measured by high-temperature oxide-melt solution calorimetry of phases having different surface areas (7–9). The slope of the relation between enthalpy and surface area gives the surface enthalpy (enthalpy per unit surface area); a steeper curve or line implies a higher surface enthalpy. The transformation and surface enthalpies are shown in Table 1. Although such crossovers were hypothesized much earlier (14, 15), the calorimetric data provide direct confirmation of a crossover in thermodynamic stability as well as in kinetically controlled occurrence of phases.

Calorimetry measures the surface enthalpy. To a good approximation, the terms “surface enthalpy” and “surface energy” can be used interchangeably, because at 1 atm (1 atm = 101.3 kPa), the effect of any small volume differences, $T\Delta V$, is expected to be small. The stability relations are determined of course by the free-energy change, $\Delta G$, rather than the enthalpy, $\Delta H$. Recent measurements by adiabatic calorimetry of the heat capacity of nanophase CoO (L. Wang, V. Kiem, A.N., R. Stevens, B. F. Woodfield, and J. Boeiro-Goates, unpublished work) suggest that the surface entropy is small (<2 J/K·mol⁻¹), making its contribution, $T\Delta S$, to the free-energy minor. Assuming that this is generally true, the pattern of stability follows the surface enthalpy (or energy). Thus, a strategy for making a given polymorph in the laboratory, and one that may be used by organisms as well, is to control the size of the initial crystal (typically by controlling concentration of reactants, ionic strength, and organic and inorganic additives) to precipitate the desired polymorph with a relatively uniform size distribution within the size range in which that polymorph is stable and then to aggregate and coarsen the particles without phase transformation. In ceramic processing, coarsening is often accom-
plished by controlled heating; nature finds other aggregation and growth mechanisms at biological temperatures.

Within a given material (e.g., alumina, titania, or zirconia), the order of decreasing surface enthalpy follows the order of increasing metastability, which is often the order of decreasing density (7–9). Less dense phases are expected to have a lower packing density of atoms on their surfaces and therefore a lower density of uncompensated charge. The less close-packed surface structures also may be able to relax more readily by rotating polyhedra or distorting bond lengths and angles. Both of these factors could lead to lower surface energy for more open structures. Even some more dense metastable polymorphs, especially those having a higher coordination number for cations, such as high-pressure ZrO2 polymorphs, may exhibit longer and weaker cation–oxygen bonds and more distorted cation–oxygen polyhedra (16), allowing more surface relaxation.

Thus, the correlation between increasing metastability and decreasing surface energy may represent quite general behavior. For hydroxyapatite, for example, hydrated surface layers intermediate between hydroxyapatite and octocalcium phosphate have been found (17). Such additional surface relaxation and compositional change clearly complicates any simple picture of surface enthalpy.

Furthermore, such structural flexibility and nonstoichiometry may affect both the rates and distribution for ion exchange. Such surface coefficient processes are of course critical for bone growth and remodeling.

The amorphous phases studied by solution calorimetry (zirconia, silica) have significantly lower surface enthalpies than their dense crystalline counterparts (9, 10). Thus, amorphous phases may be thermodynamically as well as kinetically preferred under constraint of small particle size. Amorphous phases also may be more hydrated than well crystalized materials, as is seen for silica (18–20) and calcium phosphates (17). The role of amorphous precursors in biominalization is just beginning to be appreciated (17, 21, 22), whereas such amorphous or highly disordered intermediates are well known in sol-gel synthesis of ceramics (19, 20, 23, 24).

For biominalers there are several noticeable gaps in surface-energy data. The surface energies of calcite, aragonite, and vaterite are not known from direct experimental measurement. There seems to be little data for the surface energy of any of the varieties of hydroxyapatite and related phosphates, although Suzuki et al. (25) recently determined the surface tension of chlorapatite. There is still some uncertainty about the surface energy of silica (26, 27). A current working hypothesis, supported by available data, is that silica glass, amorphous silica prepared near room temperature, and zeolitic and mesoporous silicas all have a similar and rather low surface energy of \( \sim 0.1 \) J/m\(^2\) (27), and this value applies to both external surfaces and those on the inside of pores and channels (27, 28).

Fig. 2. Energetics of silica and calcium carbonate polymorphs (48, 49).

Fig. 3. Enthalpy of formation from oxides \( \Delta H_{\text{f,oxides}} \) (coarse \( \alpha-Al_2O_3 \) or coarse \( 1/2 \alpha-Al_2O_3 + 1/2 H_2O \)) as a function of surface area for boehmite (AlOOH), \( \gamma-Al_2O_3 \), and corundum (\( \alpha-Al_2O_3 \)) (7, 45).

Fig. 4. Enthalpy of titania polymorphs as a function of surface area (8).

Fig. 5. Enthalpy of zirconia polymorphs as a function of surface area (L. Wang, V. Kiem, A.N., R. Stevens, B. F. Woodfield, and J. Boerio-Goates, unpublished work). w.r.t., with respect to.
Table 1. Surface enthalpies and transformation enthalpies relative to bulk stable polymorph for several oxides

<table>
<thead>
<tr>
<th>Oxide</th>
<th>Surface enthalpy, ( \Delta H_{\text{s}} )</th>
<th>Transformation enthalpy, ( \Delta H_{\text{tr}} )</th>
</tr>
</thead>
<tbody>
<tr>
<td>( \alpha-Al_2O_3 )</td>
<td>2.6 \pm 0.2 J/m²</td>
<td>0 kJ/mol</td>
</tr>
<tr>
<td>( \gamma-Al_2O_3 )</td>
<td>1.7 \pm 0.1</td>
<td>13.4 \pm 2.0 kJ/mol</td>
</tr>
<tr>
<td>AlOOH (boehmrite)</td>
<td>0.5 \pm 0.1</td>
<td>-17 \pm 1 kJ/mol</td>
</tr>
<tr>
<td>TiO₂ (rutile)</td>
<td>2.2 \pm 0.2</td>
<td>0 kJ/mol</td>
</tr>
<tr>
<td>TiO₂ (brookite)</td>
<td>1.0 \pm 0.2</td>
<td>0.7 \pm 0.4 kJ/mol</td>
</tr>
<tr>
<td>TiO₂ (anatase)</td>
<td>0.4 \pm 0.1</td>
<td>2.6 \pm 0.4 kJ/mol</td>
</tr>
<tr>
<td>ZrO₂ (monoclinic)</td>
<td>6.5 \pm 0.2</td>
<td>0 kJ/mol</td>
</tr>
<tr>
<td>ZrO₂ (tetragonal)</td>
<td>2.1 \pm 0.05</td>
<td>9.5 \pm 0.4 kJ/mol</td>
</tr>
<tr>
<td>ZrO₂ (amorphous)</td>
<td>0.5 \pm 0.05</td>
<td>34 \pm 4 kJ/mol</td>
</tr>
<tr>
<td>Zeolitic silicas</td>
<td>0.09 \pm 0.01</td>
<td>8 - 15 kJ/mol</td>
</tr>
<tr>
<td>Amorphous silica</td>
<td>0.1</td>
<td>9 kJ/mol</td>
</tr>
</tbody>
</table>

See refs. 7, 8, 10, 27, and 45.

*Enthalpy relative to stable bulk anhydrous polymorph plus H₂O (liquid) if applicable.

A corollary is that the energy of a zeolite changes very little after diminution of particle size, because the internal surface area dominates the external, even for particles as small as 30 nm (28). Similar trends may apply to porous amorphous silica materials in which neither the pores nor the frameworks are periodic.

**The Ostwald Step Rule and Metastable Phases**

The observations discussed above offer insight into the Ostwald step rule, a conjecture often supported by observation that a crystallizing system first will form a sequence of available metastable phases before finally forming the stable phase. Such behavior is observed in crystallization sequences in systems as diverse as aqueous solutions and silicate melts. For example, disordered dolomite (high-magnesium calcite), a demonstrably metastable phase (29), forms before dolomite in a variety of environments. Similarly, a metastable stuffed-silica derivative forms from a magnesium aluminosilicate glass before cordierite crystallization (30).

Why should such behavior occur? The common understanding, or rationalization, is that the metastable phases are somehow structurally more similar to their precursors in solution, melt, or glass and are able to nucleate more readily. One can recast this conjecture in terms of the competition of surface energy and polymorphism. If there is a general correlation between increasing metastability and decreasing surface energy, as discussed above, then crossovers in thermodynamic stability at the nanoscale may be a common occurrence. That is, not only will the nucleus of the metastable phase have a lower activation barrier (and smaller critical size), but also there may be a lower free energy for the growing crystal of the metastable polymorph relative to the phase stable in the bulk over a relatively wide size range (see Fig. 6 for a schematic representation). Once such metastable crystals grow, a separate set of nucleation events (and/or a dissolution–reprecipitation process) is required to form the next more stable phase.

The Ostwald step rule and its interpretation involve an intertwined collection of thermodynamic and kinetic arguments. The thermodynamic argument is that the system visits a series of metastable phases on its way down to the most stable phase, decreasing its free energy by a series of small steps rather than in one big leap. That this stepwise progression is kinetically favored relies on the assumption that structural rearrangements involving smaller changes in free energy occur faster, perhaps having smaller activation energies, and that these rearrangements are rate-determining. For reactions involving molecules in the gas phase or in solution, this idea is reminiscent of “linear free-energy relations,” which relate the kinetic and thermodynamic parameters of a group of analogous reactions. For example, see ref. 31 for a discussion relating mineral dissolution rates and the reactivities of aqueous complexes. Intuitively, this idea seems reasonable and familiar; the more different the initial and final state, the higher in energy the transition state.

For reactions that form a condensed phase (crystalline, amorphous, or liquid), classical nucleation theory describes the barrier for a growing particle as created by the competition of a positive surface free energy (proportional to the surface area by the square of the particle radius) and a negative bulk free-energy change (proportional to the volume by the cube of the particle radius). The maximum in free energy as a function of radius defines the “critical radius,” that particle size for which, on average, particles will continue growing rather than shrink. The observation made in this article, that the surface energy becomes smaller as the phase becomes more metastable, thus offers an immediate rationalization of the Ostwald step rule; the smaller surface-energy term leads to a lower barrier. Indeed, because the nucleation barrier height is proportional to the square of the surface energy, a small change in surface energy can produce large differences in barrier height.

However, this argument poses at least two difficulties. The first problem, inherent to classical nucleation and growth theory, is that the argument discussed above is essentially thermodynamic rather than kinetic in nature. That is, one traces the states of lowest free energy of the growing particle and assumes that they define the kinetically favored path. The details of attachment/detachment of the surface-reacting species (atom, ion, molecule, cluster, or nanoparticle) are not considered. This issue leads to the second conceptual difficulty, namely that a growing nanoparticle is not simply a sphere with a sharp and uniform bounding surface. Rather, the growing particle has a variety of attachment sites and local surface environments, and the solution or matrix near it may itself be different from the bulk. The larger the attaching/detaching clusters and the more complex the structure of the growing particle, the less satisfying are the classical, continuum-based concepts of nucleation and growth.

For ceramics crystallizing from melts or glasses at high temperature, the attachment/detachment of ions indeed may be fast and not rate-determining. In aqueous solutions crystallizing “simple” solids by attachment/detachment of small molecules or ions, this hypothesis also may be true. In such cases, the thermodynamic arguments leading to both classical nucleation and growth theory and to the Ostwald step rule may be adequate and useful. For the oriented attachment of larger clusters or nanoparticles in the 2- to 5-nm range, as in the formation of zeolites (3) and TiO₂ (1) and in silica biomineralization (21), the situation may be far more com-
complicated, and attachment/detachment processes could be rate-determining. It remains to be seen whether the concepts of nucleation and growth are still useful or whether new fundamental approaches other than purely empirical fitted rate laws need to be developed.

The CaCO₃ polymorphs are very interesting in this context. Vaterite is less dense than calcite, whereas aragonite is more dense, yet both occur as metastable phases at ambient conditions, and all three can be produced by biominalization. It would be very instructive to know their surface energies. Furthermore, it would be crucial to know to what extent the surface energies are affected by impurities, particularly Mg, Ba, and Sr. These alkaline earths affect carbonate polymorphism and surface chemistry. Mg, of course, is very important biologically, with proper Ca/Mg balance being crucial to electrolyte balance, cardiac and neurological function, membrane transport, and photosynthesis. Obviously, surfaces of different orientations will have different surface energies and different affinities for adsorbed ions and molecules. The important point is that the three polymorphs of CaCO₃ are close enough in free energy such that surface energy and/or impurity effects can cause crossovers in stability, and therefore which phase crystallizes at the nanoscale can be controlled by thermodynamic as well as kinetic factors.

**Hydrated Surfaces, Gels, and Hydrous Phases**

The living world is wet; thus, anhydrous biomineral surfaces do not exist. Clusters and nanoparticles in contact with biological fluids have their surfaces bonded to H₂O, H⁺, or OH⁻ as well as to organic molecules. The surfaces of growing minerals are charged positively or negatively depending on whether the pH is lower or higher than the point of zero charge. Although physiological pH, or that of sea water, is not far from neutral, the local pH at the surface of a growing biomineral may be quite different and is difficult to determine. There is relatively little experimental work on the energetics of hydration of mineral surfaces and of nanoparticles, and there are no direct measurements of mineral–water interfacial energies. For alumina, there seems to be a distribution of hydration energies, with γ-Al₂O₃, the metastable bulk phase with lower surface energy, having overall less exothermic hydration energetics than α-Al₂O₃, the stable bulk polymorph with higher surface energy (7). The surface sites of highest energy hold on to adsorbed water most strongly, are the first to be hydrated, and are the last to be dehydrated. Thus, a biological strategy for exposing specific surface sites might be to decrease hydration by decreasing the water content of the solution layer near the interface or by preferentially adsorbing and then removing organic molecules.

When precipitated from aqueous solution at room temperature by change of pH or hydrolysis of organometallics, the oxides of Al, Fe, Ti, Zr, Mn, and Si each form highly hydrated amorphous precipitates. When these precipitates incorporate the aqueous phase to form a coherent gelatinous mass, they are called gels. The crystallization, coarsening, and phase transformation of these materials by further processing (aging, heating, or solvent removal) forms the basis of sol-gel processing of ceramics (19, 20, 24). The changes in structure and the removal of water are intimately linked. Typically, the initial precipitation step, forming a poorly crystalline solid from truly dissolved ionic species, is strongly exothermic (−100 to −300 kJ/mol metal), the crystallization of the hydrous gel to a hydrous crystalline phase is only modestly exothermic (−2 to −30 kJ/mol metal), and the dehydroxylation to form anhydrous oxide plus gaseous water is endothermic in enthalpy and driven on heating by the entropy of vaporization (18, 19). To make dehydroxylation possible by biological processes near ambient temperature, the water must be transferred to a state that lowers the free energy of the system, or active transport, with the expenditure of ATP energy, must be involved. Thus, in cases in which hydration is strong, such as in apatite, the final product remains hydrated, whereas in cases of weak hydration, such as calcite, the final product is virtually anhydrous. Hydrated amorphous carbonate precursors, which are not usually encountered in materials processing, are reported in biominalization (32–34). Similarly, apatite formation in new bone goes through a series of nanophase precursors (17).

**Clusters, Sols, Nanoparticles, and Nonclassical Crystal Growth**

Although natural waters and biological fluids are relatively dilute in mineral-forming cations, there is increasing evidence that clusters and small polymeric species are important (2, 18, 19, 21, 23, 26). Although the equilibria among polymeric silica species in aqueous solution have been studied extensively (23, 26), unanswered questions remain. Quantitative modeling of speciation and activity over extended ranges of pH, ionic strength, and temperature remains crude. The effects of organic additives such as ethanol or acetate are incompletely known, and the thermodynamic interactions of dissolved silicate species with large organic molecules, such as the proteins and saccharides involved in the biological transformations of silicon, are largely uncharted.

An optically clear solution of dissolved silica (a silica sol) contains a few monomeric species (ranging from H₂SiO₃⁻ to SiO₄⁴⁻ depending on pH), a significant number of dimers, trimers, and other small polymeric species, and a nonnegligible number of clusters or polymeric fragments large enough to be considered nanoparticles (having diameters in the 1- to 4-nm range and containing dozens to hundreds of atoms) (3, 18–20, 26). Aluminum-containing solutions at near-neutral pH contain extensive clusters, often containing a central tetrahedral Al₂O₃ group bonded to 12 other aluminum ions in octahedral coordination, with water and hydroxyl species holding the cluster together (2, 35, 36). Larger clusters (e.g., containing 30 Al ions) have been observed also (36). The structure and bonding in these clusters already contain many of the features characteristic of the crystalline aluminum oxhydroxides. Iron and chromium also form polymeric clusters (36). Cations of large charge, such as uranium, molybdenum, and tungsten, also exhibit complex clustering in aqueous solution (36). For uranyl species, carbonic groups play a major role in holding the clusters together, and the transition from a dissolved cluster to a poorly crystalline precipitate is very gradual, probably involving the coalescence of such clusters rather than the nucleation of a new phase (36, 37).

It is important to remember that the chemistry of silicates, phosphates, and carbonates differs substantially, both in aqueous solution and in minerals. The nanoscale clusters formed for silicates are most covalent and strongly bound, phosphates are intermediate, and carbonates are most ionic. The more weakly bonded ionic clusters are generally more reactive in terms of both thermodynamics and kinetics. Amorphous calcium phosphate, for example, has been described as consisting of building blocks, referred to as Posner’s clusters (17, 38), containing both ionic and covalent bonding.

Under aqueous conditions, dominated by dissolved clusters or nanoparticles, the formation of a “critical nucleus” of a new phase loses its simple meaning. Even if one bears in mind that there is a distribution of sizes of nuclei and that the “critical size” only tells, on average, which ones will grow or shrink, the real situation is even more complex. Rather than atom-by-atom assembly, initially uphill in free energy, the first stages of forming a crystal may be the coalescence of two fairly large clusters, the elimination of some of the water, protons, and hydroxyls at their interface, and the alignment of their structures to be crystallographically coherent (1, 2). It is not clear whether any of these steps involve an activation energy in the classical sense, although they may.

For the synthesis of siliceous zeolites, important to petroleum cracking and other technology, the art of using organic templates or structure-directing agents has blossomed over the last three decades (39). An organic molecule, typically an alkylammonium ion, but more recently a specially designed complex nitrogen-containing organic molecule (39), is added to the reaction mixture under fairly alkaline conditions, and the reaction pro-
ceeds hydrothermally at temperatures between 60 and 150°C. Although the higher temperature and alkalinity distinguish these reactions from biomineralization, there are several lessons to be learned. First, the structure-directing agent selects the zeolite polymorph that forms, but the phase formed also may depend on the temperature, ionic strength, and time of heating. Because one structure-directing agent may form several different zeolites and the same zeolite may be formed by several structure-directing agents, the structure direction is not a unique lock-and-key templating process. Second, many different zeolitic silica frameworks are almost the same in energy and comparable to amorphous silica (10). They are also very similar in entropy (13). Thus, the role of the structure-directing agent is largely kinetic, selecting a given structure from among many thermodynamically almost equivalent possibilities. Third, the interaction of the organic structure-directing agent with the zeolite framework is also relatively weak energetically (exothermic by $<10 \text{kJ/mol SiO}_2$) (40), which provides additional evidence that the role of the structure-directing agent is mainly kinetic rather than thermodynamic.

The energetics of a specific SiO$_2$ zeolite, MFI framework type, from an initially clear solution containing silica and a tetramethylammonium salt as structure-directing agent has been studied (3). The initial hydrolysis of the tetraethylorthosilicate [(C$_2$H$_5$O)$_4$Si] silica source in the presence of tetramethylammonium proceeds rapidly, producing a clear ethanol-water solution containing mainly 3-nm particles having some of the structural arrangement of the zeolite-to-be. In situ calorimetry, combined with mass balance studies, pH measurements, and $^{29}$Si NMR studies, has shown the following sequence of steps after heating. In a small temperature range, dependent on heating rate and initial reactant concentrations, most of the zeolite formation occurs over several hours. Initially, the reaction is modestly exothermic, releasing $\approx 2$ kJ/mol SiO$_2$ zeolite formed. In this exothermic stage, the formation of zeolite and the release of heat is linear with time, and the pH of the solution remains constant. When the reaction is 50–70% complete in terms of mass of silica converted, it slows down, the enthalpy becomes endothermic, and the pH rises. The reaction slows to imperceptible speed before all the nanoparticles are converted to crystalline MFI.

The results have been interpreted as follows (3). The initial exothermic stage with linear rate law and constant pH involves the oriented assembly of the 3-nm organic-containing nanoparticles onto the growing crystal. Because the reaction occurs at pH 9 or higher, both the crystal and the nanoparticles are negatively charged. Because the pH stays constant, this stage of synthesis must involve the localization of these hydroxyls onto a surface that is decreasing in area. Finally, the buildup of surface charge becomes prohibitive, the particles and growing crystal repel each other too strongly, and the reaction slows. Further reaction can occur only when accompanied by the release of OH$^-$ into solution, raising the pH. This release of hydroxyl is endothermic in energy but driven by entropy, thus the switch to a net endothermic reaction enthalpy. This sequence of steps is shown schematically in Fig. 7.

These observations have several implications for biomineralization, even if organisms do not grow silica zeolites under highly basic conditions. Rather, organisms grow silica at physiological pH, and there is increasing evidence that amorphous silica nanospheres and/or layers are the source of silica, agglomerating and somehow changing structure to form the final mineral (21). Whether this structural transformation occurs by surface reaction or by dissolution-recrystallization (both enzyme-mediated) is less clear. In many ways, such processes are analogous to zeolite growth. An organic structure-directing agent may change the speciation of silica in aqueous solution by converting the distribution of oligomers to a more uniform and useful set of nanoparticle precursors for assembly into the growing solid. The rate of crystal growth can be controlled by varying the surface charge on the growing mineral. It is easy to envision that this control of surface charge could occur by the adsorption of specific organic molecules generated by the organism. Such control could be very spatially specific, causing some places to grow much faster than others and resulting in the complex geometric forms characteristic of biominerals. Even if the growing aggregate is an agglomeration of nanospheres of amorphous silica, one can envision the selective biological release of a surface-active protein at specified locations, controlling the surface charge and growth rate spatially. Different organic molecules could be involved in forming the nanoparticles and in controlling surface charge. Thus, the set of mechanistic crystal-growth pathways discovered in the laboratory for zeolites and other materials over the past two decades may have natural analogues perfected by millions of years of evolution. Indeed, if the crystallites in the assembling solid remain small (i.e., the last steps of making a coherent crystal are hindered), it is possible that biogenic silica contains, heretofore undetected, some of the building blocks of organic-containing structure-directed zeolites. In any event, this analogy suggests some features to be sought in biological systems actively producing silica or, for that matter, carbonate or phosphate.

Mesoporous silica materials, with amorphous silica frameworks but a periodic arrangement of pores in the 1–10-nm size range, have been synthesized by coassembly with surfactants (42). These materials are energetically similar to the larger-pore zeolite structures. Because the mesoporous materials are built by using larger molecules than for zeolite synthesis, one might ask whether natural lipids, sugars, and small proteins can also self-assemble with silica. The sizes of the pore and the silica wall are determined by the character of the surfactant micelle. Probably many failed syntheses have contained comparable porosity on the nanoscale but lacking periodicity. Do organisms self-assemble analogous mesoporous periodic, semiperiodic, or aperiodic structures? The synthesis of amorphous silica spicules in marine sponges, mediated in vivo, and also in laboratory studies, by protein filaments (43), may be an example of such assembly.

**Significance of Metastability and Precursor Phases for Biologically Controlled Mineralization**

Biologically controlled mineralization produces spatially controlled crystallization, in terms of both the development of crystal faces and their orientation and where the crystals grow, producing spicules, tests, shells, or bones of defined shape. To do this, the organism has
to control both nucleation and growth and to be able to stop growth when a desired dimension is reached. Information is being amassed to control both nucleation and growth and to be able to stop growth when needed. Growth rate also may be controlled by changing the charge on the surface, as experiments in zeolite crystal growth (see above) suggest. Such change can occur by the adsorption of organic molecules. The attachment of a nanoparticle with its attached protein may provide the right mass balance and reactivity for the incorporation of the protein into the growing biocomposite, which may be harder to achieve if each protein chaperoned only one or a few ions at a time to the growing surface.

Of course the term “energy landscape” is general and pervasive in biology. It applies to many scenarios in which a large number of possible states or conformations, close in free energy and grouped in one or many “funnels” of related states, are accessible. Finding, controlling, and using such landscapes is a ubiquitous strategy of biologists and probably of biology itself. Such concepts, especially applied to shape control in biominalization, are reviewed in a recent book by Mann (44). These more general concepts place the specific nanoscopic-level discussion in this article in a broader context.

In conclusion, nanoparticle precursors offer a rich and controllable source of material for biominalization.

I thank J. DeYoreo, P. Dove, and C. Drouet for thoughtful discussion and review; and DeYoreo and Dove for organizing the Mineralogical Society of America Short Course on Biominalization in 2003, which inspired me to write this article. This synthesis of various concepts and observations is based on several different studies supported by the National Science Foundation and the Department of Energy.


Navrotsky