Biomimetic Mineralization: Mesoporous Structures

Biological mineral synthesis, in contrast to conventional mineral processing techniques, generates materials of very highly controlled size, habit, texture, composition, and structure. Studies of biomineralization processes since the 1970s have yielded much information on how nature achieves such exquisite control over mineral formation. These biomineralization processes occur in a diversity of species and tissues, including bone mineral, marine shells, tooth enamel, and marine algae shells. Several excellent reviews of biomineralization have been published (Mann 1988, 1993, 1995, Vogel and Boyan-Salyers 1976, Lowenstam and Weiner 1989). One of the most significant findings of these studies is that in virtually all cases of biomineralization, macromolecular structures composed of lipids, proteins, and/or polysaccharides are intimately associated with mineral phases and serve vital roles in their crystallization.

1. Mineralization in Lipid Mesophases: A Common Biological Strategy

One ubiquitous biological strategy employed during the formation of mineral phases is the exploitation of lipid mesophases to control the location, size, composition, and shape of mineral crystals. Mesophases are materials which have domain length scales of the order of a few nanometers to a few hundred nanometers. Lipids are amphiphilic molecules composed of one or more hydrocarbon (hydrophobic) tails and a polar organic (hydrophilic) head group. In aqueous solution, lipids exhibit a strong tendency to self-assemble into a variety of mesophase structures, including micelles, reverse micelles, hexagonal and inverse hexagonal phases, and bilayers (Fig. 1). Phospholipids are amphiphilic molecules which spontaneously aggregate in aqueous solution into ordered lyotropic liquid crystalline phases (mesophases), the form of which depends upon intrinsic factors such as the nature of the lipid headgroup, the length and degree of unsaturation of the acyl chains, and extrinsic factors such as temperature, pH, concentration, and the presence of solutes and other lipids. A common and biologically important lipid mesophase is the lamellar bilayer. Lipid bilayers are the major building blocks of biological membranes which, together with membrane proteins and cholesterol, control cell shape and many cell functions such as the storage of compounds, ion transport, cell fusion, and metabolism.

Phospholipids, often in the form of bilayer vesicles, are commonly employed in natural biomineralization processes to delineate reaction compartments in which mineral formation takes place (Lowenstam and Weiner 1989). Although phospholipid mesophasemediated mineralization occurs in vastly different species and tissues, the roles that the phospholipid assemblies play in these processes can be generalized as follows.

(i) Construction of an enclosed, organized reaction environment, often through the use of bilayer vesicles.
(ii) Control of the physicochemical conditions inside the reaction environment via transmembrane channels, transporters, or selective ion permeability.
(iii) Control of nucleation kinetics. The organic surface serves as a molecular blueprint for the site-directed formation of the inorganic phase, by providing an interface at which the electrostatic, stereochemical, and geometric interactions are conducive to oriented nucleation and growth of the mineral (Mann 1988).
(iv) Production of complex crystal shapes by altering the shape of the lipid matrix during crystal growth.

An exquisite example of the role that phospholipid mesophases play in biomineralization is the formation of coccoliths in the alga *Emiliania huxleyi* (Young et al. 1992). Coccoliths are scales that consist of 30–40 calcite \( \text{CaCO}_3 \) crystals arranged within an oval-shaped structure, and several coccoliths are used by the alga as an exoskeleton to surround and protect itself. The first stage of organized assembly of these structures is the production of a series of phospholipid membrane vesicles that act as compartments for the subsequent calcite crystallization. Other encapsulation structures such as proteins or polysaccharide networks are also used in biomineralization processes such as...
this. The second stage of crystallization occurs by the influx of calcium and carbonate ions. Then, in the third stage, oriented nucleation of calcite crystals occurs within the enclosed vesicles. This is aided by structural, chiral, and chemical complementarity between the organic matrix and the nucleating mineral phase. In the final stage of mineralization, the desired shape of the mineral crystal is achieved by control of the vesicle compartment shape via active cellular cytoskeletal elements. Such dynamic control of crystal shape by the phospholipid mesophase leads to the highly elaborate structures found in coccoliths and other biominerals such as diatom shells and acantharian skeletons.

Vertebrates also initiate mineral formation within lipid vesicles and lipopeptide aggregates. Phospholipid vesicles called matrix vesicles are found in certain actively mineralizing tissues of the human body (growth plate cartilage and tooth dentine) and are the initial sites of calcification in these tissues (Anderson 1995). Matrix vesicles are unilamellar bilayer vesicles, 100–200 nm in diameter, which are formed by budding from hypertrophic chondrocytes and odontoblasts. The membranes of these vesicles are enriched in acidic (i.e., negatively charged at physiologic pH) phospholipids, ion channels, enzymes, and transport proteins. At the time of release from the cell membrane, matrix vesicles do not contain mineralized crystals. However, as the vesicle becomes embedded within the extracellular matrix secreted by the cell, the interior becomes enriched with calcium and phospholipids, in part by the action of calcium-binding phospholipids and proteins, phospholipase enzymes, and calcium ion channels.

When the concentrations of Ca\(^{2+}\) and PO\(_4^{3-}\) reach supersaturation levels in the vesicle compartment, amorphous calcium phosphate then precipitates on the interior surface of the vesicle, converts to an intermediate octacalcium phosphate, and finally into hydroxyapatite. Nucleation at the inner surface of the vesicle membrane appears to be assisted by the chemical and structural complementarity of the inner phospholipid-protein assembly of the matrix vesicle and the mineral phase. Upon further growth, the hydroxyapatite crystals then penetrate the vesicle membrane. Once the membrane has been perforated by the crystal, further mineralization is governed by the solution conditions of the extracellular fluid. By this mechanism, matrix vesicles effectively control the initial nucleation kinetics and crystal structure of the biomineral.

Magnetosomes are another interesting example of phospholipid and organic matrix-mediated mineralization, and their mechanism of formation shares several features in common with matrix vesicle and coccolith formation (Mann et al. 1984, 1990, Schuler and Frankel 1999). Magnetosomes are single crystals of magnetite (Fe\(_3\)O\(_4\)) or greigite (Fe\(_3\)S\(_4\)) encapsulated in a bilayer vesicle. Magnetosomes are highly crystalline, and their sizes and crystal habits are particularly uniform within individual bacterial species. Long chains of connected magnetosomes with a common magnetic orientation are often seen in the cytoplasm of magnetotactic bacteria. These organisms are believed to have evolved to use these structures, which possess a permanent magnetic dipole, to navigate with respect to the earth’s magnetic field. As with coccoliths and matrix vesicles, magnetosomes are formed by the precipitation of mineral within pre-formed lipid vesicles, which exert a high level of control over crystallization by virtue of the encapsulation and templating performed by the membrane surrounding the particle. Molecular recognition at the inner vesicle surface is likely to play a role in control of magnetite crystal orientation.

2. Biomimetic Materials Synthesis

In an effort to develop new inorganic materials with complex architectural features in the nanometer range, numerous research groups have sought to exploit mesostructured organic assemblies as templates for materials synthesis. This general approach to materials synthesis uses the ability of amphiphilic organic molecules to self-organize in aqueous media into complex three-dimensional supramolecular aggregates, which subsequently serve to direct the formation of inorganic solids from soluble precursors. Such organic templating approaches to materials synthesis are inspired by the strategies that living organisms use to construct materials. A theme common to many template syntheses is that the mesostructured inorganic solids resulting from this approach have a three-dimensional architectural organization and length scale (1–100 nm) that reflects the organic assembly from which they were derived. Synthetic and natural amphiphilic organic molecules such as surfactants and phospholipids are attractive candidates for fabricating complex synthetic microstructures whose ultimate architecture is controlled by the nature of the molecular aggregates. Many published studies have demonstrated the enormous potential for using molecular self-assembly to control the formation and structure of inorganic materials.

2.1 Biomimetic Mineralization Within Vesicle Compartments

Some of the first in vitro experiments that attempted to synthetically generate inorganic phases with the controlled features of biominerals sought to exploit the compartmentalization function of lipids to form inorganic particles. Using the aqueous inner compartment of liposomes as micro- or nanoscale reaction volumes, several laboratory research efforts have sought to
duplicate the way in which nature is able to segregate particular ions or reactive species in controlled environments. An early synthetic approach to mimic compartmental biomineralization came from Mann and coworkers in their experiments involving the precipitation of silver oxide inside phosphatidylcholine vesicles (Mann and Williams 1983). In that work, liposomes with entrapped silver nitrate were subjected to pH increases until the intravesicular concentration of freely diffusing OH\(^{-}\) was high enough to initiate precipitation of Ag\(_2\)O. The crystals that resulted, which only formed inside the liposomes, were essentially of homogeneous size, unlike precipitates formed directly from solution. Since these early papers, this approach has been used to generate iron oxide microparticles (Mann and Hannington 1987), aluminum oxide particles (Bhandarkar and Bose 1989, Yaacob \textit{et al.} 1992), and nanocomposite particles of aluminum, magnesium, and calcium hydroxides (Bhandarkar and Bose 1990). Also, the generation of nanoparticles is of particular interest in semiconductor engineering, as Fendler and coworkers have used compartmental synthesis techniques in creating semiconductor particles with unique properties (Tricot and Fendler 1986, Chang \textit{et al.} 1990). Eanes and coworkers used liposomes as tools to model the mineral precipitation sequence believed to occur during matrix vesicle calcification (Eanes and Heywood 1989). Phosphate-loaded liposomes of size similar to matrix vesicles were prepared by encapsulating aqueous inorganic phosphate solutions (up to 50 mM) within the aqueous cores of liposomes constructed from phosphatidylcholine, dicetyl phosphate, and cholesterol. The lipid bilayer, which serves as an ion conductor, is essential for the formation of mesoporous structures. An early synthetic approach to mimic compartmental biomineralization and take advantage of calcium- and phosphate-loaded liposomes constructed of phospholipids that exhibit a phase transition at a specific, tailorable temperature; significant increases in bilayer permeability at the phase transition temperature permit the release of liposome-entrapped ions into the aqueous medium, resulting in supersaturation and rapid mineral formation. In the future it may be possible to use such a mineral-forming system for the repair or remineralization of skeletal tissue.

2.2 Biomimetic Mineralization in Cubic, Hexagonal, and Bicontinuous Mesophases

Considerable attention has been given to the use of organized assemblies of surfactant molecules to direct the formation of mesostructured inorganic solids (Firouzi \textit{et al.} 1995, Stupp and Braun 1997). Surfactants are amphiphilic organic molecules consisting of one or more hydrocarbon chains (hydrophobic tails) attached to a polar or ionic group (hydrophilic head). These molecules have the characteristic property of adsorbing strongly at interfaces, such as air/water, oil/water, and water/solid, and are capable of spontaneously assembling into a variety of lyotropic phases in solution. Micelles, reverse micelles, rod-like aggregates, and lamellar phases are examples of common surfactant assemblies. Generally speaking, the type of structure formed varies with temperature, the molecular structure, chemical composition, and concentration of the surfactant, and the composition (ionic strength, pH, etc.) of the aqueous phase. In laboratory efforts involving surfactant assemblies, the general goal is to use the complex structure of the assembly of organic molecules to direct the formation of inorganic phases of controlled mesostructure.

A number of workers have taken advantage of liquid crystalline order in aqueous surfactant systems to synthesize mesostructured silicates with lamellar, cubic, and hexagonal topologies. Typically, monomeric species present in the aqueous phase polymerize to form an inorganic mesostructure with shape primarily determined by the organization of the surfactant/water array. In some systems, however, it appears that chemical interactions between surfactant molecules and solvated inorganic species play an important role in determining the topology of the surfactant/water system (Firouzi \textit{et al.} 1995). A prototypical example of this type of approach is the use of cationic surfactant assemblies to template the formation of mesoporous silicates for use as high surface area catalysts (Kresge \textit{et al.} 1992). These materials contain uniform channels varying from 15 Å to roughly 100 Å in size, which are remnants of the hexagonal or bicontinuous cubic phases formed by
the aqueous surfactant system. Recent extensions of this strategy to include block copolymers as structure-directing phases have greatly increased the diversity of structures attainable (Bagshaw et al. 1995, Zhao et al. 1998), and the range of inorganic materials accessible via these approaches continues to increase. For example, three-dimensional networks of calcium phosphate and calcium carbonate minerals have been created with the use of a bicontinuous surfactant/oil/water reverse microemulsion (Walsh et al. 1994, Walsh and Mann 1995, Mann and Ozin 1996). This approach makes use of the interconnecting aqueous channels of the bicontinuous network to define, through nanoscale architecture of the microemulsion, a framework within which formation of mineral phase may occur. In these systems, reactions between mineralizing species are confined to the interconnecting water channels of the bicontinuous network, leading to highly complex, interconnected mineral frameworks. Such complex inorganic scaffolds could be used for repair of bone defects or as scaffolds for tissue engineering applications. Finally, synthesis of mesostructured inorganic semiconductors, such as CdS, CdSe, and ZnS have been accomplished through the use of lyotropic organic liquid crystalline phases (Braun et al. 1996).

See also: Bone Mineralization; Shell: Properties; Ivories

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