Molecular recognition in biomineralization

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The deposition of precise arrays of inorganic crystals in many organisms involves controlled nucleation at interfaces between the crystals and substrate macromolecules. These inorganic-organic molecular recognition processes have potential technological application.

SURPRISING though it may seem inorganic solid state chemistry is as much an integral aspect of biology as the aqueous phase organic and inorganic reactions of conventional biochemistry. More than 40 different minerals have been identified in organisms (Table 1), the most common being the phosphate and carbonate salts of calcium that are used in conjunction with organic polymers such as collagen and chitin to give structural support to bones and shells. Salts of barium, strontium, silicon and iron are also common. The minerals are formed by normal cellular processes and replicate to produce precisely organized structures in cells ranging from bacteria, algae and protozoa to the osteoblasts of bone. The minerals may be in membrane-bound vesicles within cells, in the mucilaginous layers of cell walls in bacteria, or impregnated in biopolymers in extracellular space. In all these structures, the inorganic crystals are laid down in orderly arrays in association with a matrix of organic macromolecules. There is a growing body of information on the structure, composition and synthesis of the macromolecules, but the key and exciting question is to identify the molecular processes that produce minerals of precise form with uniform particle size, novel crystal morphology and specific crystallographic orientation. How do the proteins and polysaccharides of the substrate interact to regulate solid-state chemical reactions?

Distribution

Calcium salts are not the only minerals found in biological systems, and minerals serve several functions other than providing support. Many unicellular organisms build cytoskeletons by depositing hydrated silica, a form of amorphous glass; in protozoa and algae this inorganic polymer is often sculpted into exquisite rods, perforated disks and reticular frameworks that are species-specific and have long been used as diagnostic characters for classification. Minerals are also put to special uses, for example, as magnetic sensors in magnetotactic bacteria (Fe_3O_4), gravity balance devices ($CaCO_3$, $CaSO_4$, $BaSO_4$), deterrence against predators (SiO_2, $CaCO_3$), iron storage and mobilization ($Fe_2O_3 \cdot nH_2O$ in the protein ferritin), love darts in snails ($CaCO_3$) and eye lenses ($CaCO_3$ in fossilized trilobites). The hard parts formed by these minerals are living structures that may undergo active demineralization and remodelling in response to environmental and biological stress. Bone, for example, provides an essential store of calcium in vertebrates and calcium oxalate is a calcium source in plants.

Controlled crystallization

Because the interactions resulting in controlled crystallization are tailored to biological function, the underlying molecular processes must have a specificity analogous to those involved in the reactions of aqueous-phase biological molecules. An understanding of biological solid-state interactions would therefore be of immense value in structural biology and medicine (for example, in the pathological mineralization of bones and teeth and formation of kidney stones), in crystal growth, colloidal and solid-state science (as in the prevention of industrial scaling and the controlled synthesis of electronic, magnetic and catalytic devices) and in materials and engineering technology (organized composites and ceramic precursors, and the interrelationships between microstructure and mechanical properties, for example).

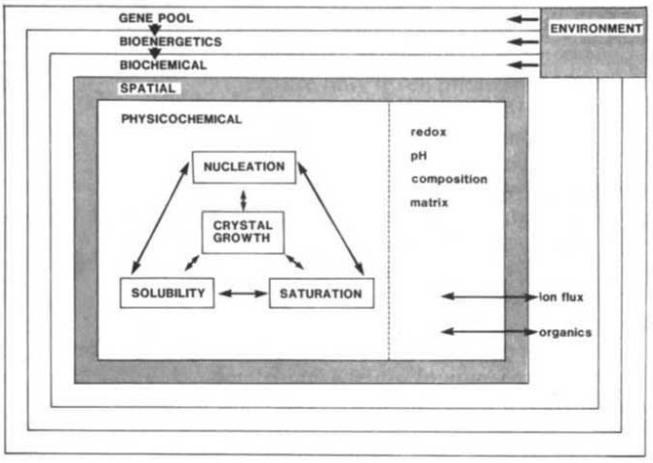

Fig. 1 The control processes in biomineralization[3]. In organisms, there are several different interconnecting levels that regulate the physical chemical properties of mineralization (solubility, supersaturation, nucleation and crystal growth). An essential condition for controlled mineralization is spatial localization arising from the compartmentalization of biological space. This permits direct regulation of physicochemical and biochemical properties in the mineralization zone. Nucleation, in particular, can be mediated by organic polymeric substrates in or on the spatial boundary. At a higher level of organization, mineralization is under biochemical and bioenegertic constraints and, ultimately, under control at the gene level. The interplay of these control processes with the external environment is also of fundamental importance.

The influence of organic macromolecules is important in the regulation of growth of the mineral[1-4,5,6] and in the resulting specificity in crystal morphology[7] and particle aggregation[8], but the molecular interactions inherent in these processes are not well understood. Here we will focus primarily on current ideas about the role of organic macromolecules in initiating the formation (nucleation) of inorganic solids in biology. Because these ideas are potentially important in industrial processes where nucleation control is a critical factor, for instance in the reproducible synthesis of materials, emphasis will be placed on the molecular interactions at the interface between the mineral and organic matrix. It is in the area of biological mechanisms of nucleation control that significant novel concepts have arisen, and the investigation of these ideas in simplified physical chemical systems should provide information about the regulation of nucleation in biomineralization.

Nucleation control

The processes controlling biomineralization are summarized in Fig. 1. Organized biopolymers at the sites of mineralization are

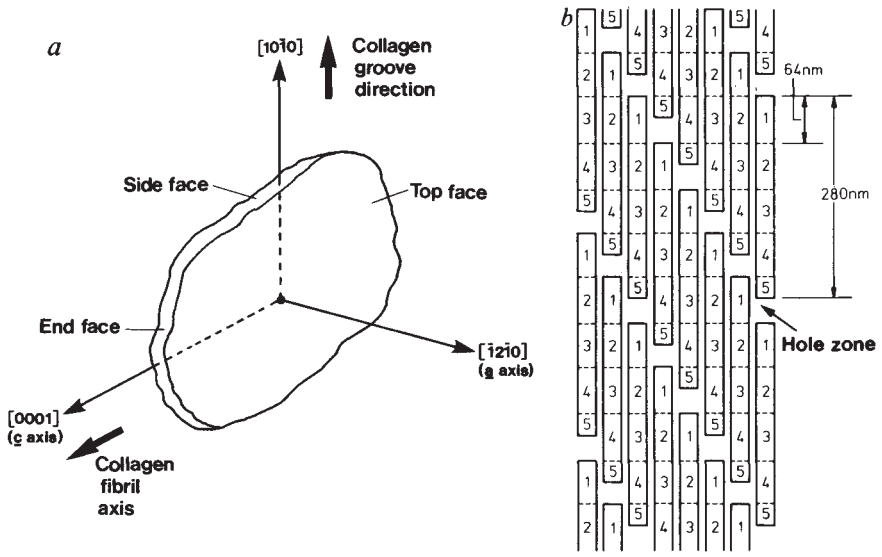

Fig. 2 The relationship between the crystallographic properties of calcium phosphate crystals of bone and the preorganization of collagen fibrils, as determined by high resolution transmission electron microscopy[9]. Numbers in square brackets refer to the Miller index notation for describing crystallographic directions. *a*, A single plate-like crystal from calcified turkey tendon showing its orientation with respect to the collagen fibrils. The crystallographic *a* ([$\bar{1}$2$\bar{1}$0]) and *c* ([0001]) axes are aligned perpendicular and parallel to the collagen fibril axis respectively. The [10$\bar{1}$0] crystallographic direction runs parallel to the collagen groove direction. *b*, The revised quarter-stagger model for collagen (in planar form), showing the intrafibrillar regiospecific hole zones which are the sites of hydroxyapatite nucleation in mature bone. In this arrangement each molecule is divided into five zones, the first four of equal length (64 nm) and the fifth only 25 nm. Adjacent molecules are transposed by 64 nm along their length by strong intermolecular cross linking to produce a 30–40 nm × 3–5 nm space (the hole zone) between the ends of each molecule. Several models of the collagen fibril arrangement predict that grooves are formed by the overlap of adjacent hole zones and that the grooves are organised in parallel rows at the same height in the fibrils[14,15]. (Reproduced with permission from ref. 40).

essential to these processes. In unicellular organisms these macromolecules act primarily as spatial boundaries through which ions are selectively transported to produce localized supersaturation within discrete cellular compartments. In many instances, particularly in organisms such as the diatoms that deposit shells of amorphous silica, the final shape of the mineral appears to be dictated by the ultrastructure of the membrane-bound compartment. Thus a diversity of mineral shapes can be biologically moulded by constraining the space available to the growing mineral and there is no immediate reason to infer that specialized interactions between the mineral and organic macromolecules exist.

There is clear evidence in many multicellular organisms for a much closer relationship between macromolecules and crystallographic properties. Two well-studied examples are bone and mollusc shells. Bone is made up of microscopic plate-like calcium phosphate crystals (~450 Å × 200 Å × 30 Å) formed within and between fibrils of collagen; in calcified turkey tendon, each crystal is deposited with the *a* axis of the crystal perpendicular to the plane of the plate, the length of each plate corresponding to the *c* axis of the crystal, which is also oriented parallel to the long fibre axis of the protein[9] (Fig. 2*a*). There is similar relationship in the nacreous inner layers of some mollusc shells, where alternating layers of polygonal blocks of aragonite are sandwiched between thin (30–300 nm) sheets of a protein-polysaccharide organic matrix; each crystal is aligned with the *c* axis perpendicular to the plane of the organic sheets (Fig. 3*a* and *b*). Although the biopolymers in bone and shells certainly have important functional roles in the mechanical strength and toughness of the mineralized composite, the well-defined crystallographic relationships imply there is an underlying molecular function for the macromolecules. In particular, the formation of crystals with preferred orientations at specific sites on the organic matrix indicates that interactions at the mineral-matrix interface are the key to the regulation of nucleation.

Nucleation, in general, represents an activation energy barrier to the spontaneous formation of a solid phase from a supersaturated solution. This kinetic constraint may be sufficient to offset the thermodynamic driving force for precipitation, resulting in metastable solutions which do not undergo phase transformations over a long period of time. Clusters of the solid phase will continue to grow only if the energy required to form the new interface, ΔG_I, is overcome by the energy released in the formation of bonds in the bulk of the aggregate, ΔG_B. Nucleation is favoured only at a critical cluster size, which is directly proportional to the ratio $\Delta G_I/\Delta G_B$. The activation energy for nucleation (ΔG_N) is related to ΔG_I and ΔG_B by the equation

$$\Delta G_N = \frac{16\pi(\Delta G_I)^3}{3(\Delta G_B)^2}$$

and

$$\Delta G_B = kT \log_e S$$

where k is the Boltzmann constant, T the temperature and S the relative supersaturation of the medium.

In biological environments, the activation energy for nucleation can be reduced by lowering the interfacial energy (ΔG_I) and/or increasing the supersaturation[1–3]. Supersaturation levels are regulated by confining the mineralization reactions to diffusion-limited sites (Fig. 1), such as intracellular vesicles and membrane-bound extracellular compartments, where selective ion-transport to and from the mineralization zone can effectively be controlled[4,10]. Interfacial energies, on the other hand, can be lowered by the presence of organic surfaces at the nucleation site. A factor of fundamental importance is the existence of a discrete energy minimum for nucleation on the organic subtrate, corresponding to a discrete nucleus structure and orientation. If there were a range of minima corresponding to the formation of nuclei of varying structure and morphology on the organic substrate, then crystallochemical specificity in biomineralization simply would not arise.

Because the chemical bonding at the surface of mineral nuclei is primarily ionic, the organic interface must contain areas of high local charge where electrostatic, dipolar and hydrogen bonding interactions can take place during nucleation. If these forces provide molecular recognition between the stereochemical requirements of ions in the nucleus surface and charged groups at the macromolecular interface, then both the structure and orientation of the mineral deposit can be precisely determined. Two features of the organic matrix are essential to generate this crystallochemical specificity: an existing organization of the organic matrix (molecular preorganization) and molecular complementarity between the inorganic ions and the local binding sites on the matrix. To limit the number of discrete nucleation sites at the matrix surface, the matrix must be organized at both the microscopic and molecular level before mineral

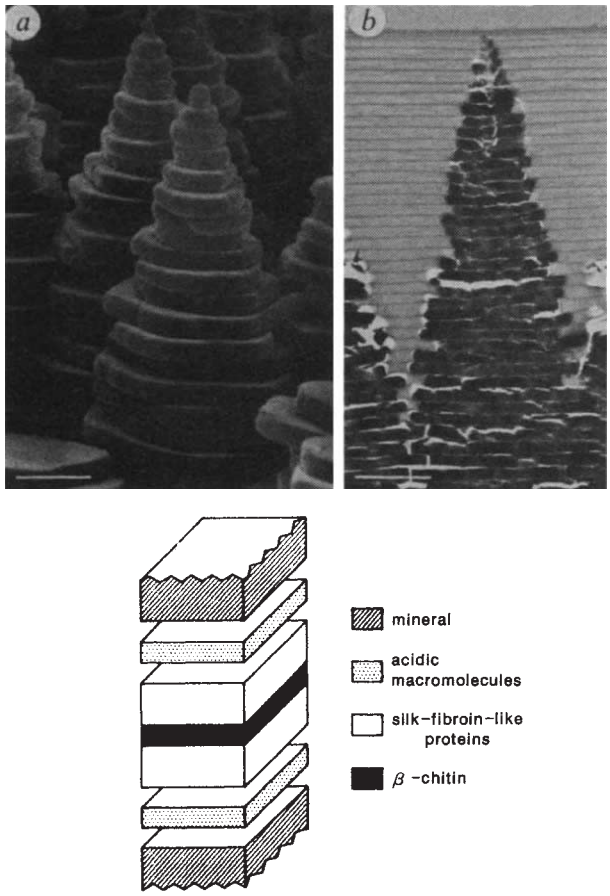

Fig. 3 The relationship between aragonite crystals and planar sheets of organic matrix in the mollusc shell. *a*, Scanning electron micrograph of the growth surface of the nacreous layer from the mollusc *Monodonta labio* showing the stacked arrangement of aragonite crystals. Scale bar, 2.5 μm. *b*, Transmission electron micrograph of an unstained section showing growing stack (*T*) with sheets of organic material (*S*) periodically interspaced between the aragonite crystals. Scale bar, 4 μm. (Reproduced with permission from ref. 41). *c*, Schematic representation of a composite section of a mollusc shell showing one sheet of organic matrix bounded by the mineral aragonite. The diagram illustrates the simplified two-component mode for the organization of the matrix, with hydrophobic biopolymers (chitin and silk-fibroin proteins) forming a framework on which the hydrophilic nucleator acidic macromolecules are arranged. (Reproduced with permission from ref. 23).

deposition starts. The disposition of the nucleation centres is regiospecific in that they are spatially confined to localized domains in the polymeric structure of the organic framework; the distribution of the sites may be random or periodic (Fig. 4). The specificity of the sites for nucleation arises from the organization of the matrix to produce distinct structural, topographical and chemical domains across its surface. The interaction between ions in the crystal nuclei and the localized surface binding sites on the matrix is cooperative and results in crystallochemical specificity in the mineral deposit. Complementarity at the inorganic-organic interface is both electronic and steric; thus the matrix nucleation centres can be considered analogous to receptor sites in enzymes, genes and antibodies, and the nucleation ion-clusters analogous to enzyme substrates, cofactors and antigens.

Matrix preorganization

The preorganization of extracellular macromolecular substrates for regiospecific nucleation and the subsequent development of

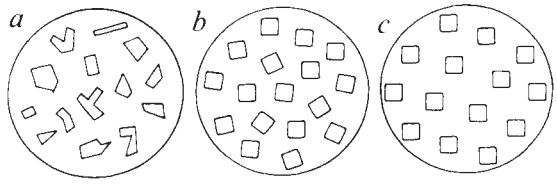

Fig. 4 Nucleation of biominerals on organic polymeric substrates. The organic surface within the area delinated by the circles consists of a limited number of discrete sites at which crystal nucleation takes place. *a*, Non-specific nucleation in which crystal nuclei of varying orientation are randomly formed across the organic surface. *b*, *c*, Site-directed nucleation involving molecular specificity at the organic/crystal interface. The crystals have a preferred orientation and morphology and may be organized randomly as shown in *b* or periodically as in *c*.

biominerals with controlled micro-architecture can be clearly demonstrated in many systems. In avian eggshells, the nucleation sites are spherulitic protein masses organized on the fibrous disulphide-linked proteins of the inner shell membranes. Oriented calcite nucleation occurs at these loci with the crystals developing preferentially along the crystallographic *c* axis. This process occurs only once in the development of the avian eggshell and is therefore confined to the two-dimensional surface of the outer membrane; in contrast, nucleation sites in mollusc shells form repeatedly throughout the lifetime of the animal through the episodic deposition of the matrix surface, and so result in three-dimensional stack-of-coins (Fig. 3*a* and *b*) or brick-wall arrangements of aragonite crystals, each with the crystal *c* axis oriented perpendicular to the matrix sheets.

The organization of nucleation at discrete sites along the organic matrix is determined by the surface and bulk structure of the substrate. These properties are dictated by the underlying molecular processes involved in the polymerization and self-assembly of the organic macromolecules. The best-studied example is collagen in bone, which comprises a rope-like arrangement of three, non-coaxial, helical polypeptides rich in glycine, proline, hydroxyproline and alanine; the small glycine side-chains at every third amino acid allow close-packing of the polypeptides. This configuration is the basic subunit for the assembly of the collagen fibre, which can be generated from a variety of packing permutations, depending on the chemical composition of the polymerization medium[11]. This gives the collagens involved in biomineralization the specific revised quarter-stagger configuration[12,13], in which holes and grooves[14,15] are organized in parallel rows across the fibrils (Fig. 2*b*). These spaces are the sites for calcium phosphate mineralization in mature bone and the localized structure, chemistry and topography of these regions must be important factors in controlling nucleation; for example, native-type reconstituted collagen fibres and collagen fibres obtained after decalcification of bone tissue can be mineralized *in vitro* with crystal formation specifically in the hole zones, whereas reconstituted collagen fibres with different (non-native) packing arrangements fail to nucleate calcium phosphate crystals under identical physical chemical conditions[16].

There is, however, much discussion about the precise function of structural proteins such as collagen in biomineralization. In particular, old ideas that the molecular nature of the hole zone directly promoted calcium phosphate nucleation have to some extent been replaced by schemes that consider collagen as providing the necessary architecture (in the form of holes and grooves) for the location of non-collagenous nucleator molecules, such as glyco- and phosphoproteins. This two-component model of the organic matrix, with an essentially hydrophobic constituent that acts as an architectural framework on to which hydrophilic constituents directly involved in the control of mineral nucleation are attached (Fig. 3*c*), has been

Table 1 The types and functions of the main inorganic solids found in biological systems

Mineral	Formula	Organism/function
Calcium carbonate:		
Calcite	$CaCO_3$*	Algae/exoskeletons
		Trilobites/eye lens
Aragonite	$CaCO_3$	Fish/gravity device
		Molluscs/exoskeleton
Vaterite	$CaCO_3$	Ascidians/spicules
Amorphous	$CaCO_3 \cdot nH_2O$	Plants/Ca store
Ca phosphate:		
Hydroxyapatite	$Ca_{10}(PO_4)_6(OH)_2$	Vertebrates/endoskeletons teeth, Ca store
Octa-calcium phosphate	$Ca_8H_2(PO_4)_6$	Vertebrates/precursor phase in bone?
Amorphous	?	Mussels/Ca store
		Vertebrates/precursor phases in bone?
Calcium oxalate:		
Whewellite	$CaC_2O_4 \cdot H_2O$	Plants/Ca store
Weddellite	$CaC_2O_4 \cdot 2H_2O$	Plants/Ca store
Group IIA metal sulphates:		
Gypsum	$CaSO_4$	Jellyfish larvae/gravity device
Barite	$BaSO_4$	Algae/gravity device
Celestite	$SrSO_4$	Acantharia/cellular support
Silicon dioxide:		
Silica	$SiO_2 \cdot nH_2O$	Algae/exoskeletons
Iron oxides:		
Magnetite	Fe_3O_4	Bacteria/magnetotaxis
		Chitons/teeth
Goethite	α-FeOOH	Limpets/teeth
Lepidocrocite	γ-FeOOH	Chitons (Mollusca) teeth
Ferrihydrite	$5Fe_2O_3 \cdot 9H_2O$	Animals and plants/ Fe storage proteins

* A range of magnesium-substituted calcites are also formed.

applied to a range of biomineralized structures in both vertebrates and invertebrates. Much of the present information comes from investigations on mollusc shells. Biochemical studies of the matrix composition show that there is a complex assemblage of macromolecular constituents at the mineralization site and that these constituents may differ between different species[17] and between different layers in the same shell[18]. But comparative studies of shells decalcified at neutral *p*H in the presence of EDTA indicate that the EDTA-soluble extract is remarkably similar in all species studied. It is a heterogeneous mixture of two classes of proteins[19]: one rich in aspartic acid and, to a lesser extent, glutamic acid (representative amino acid composition Asp 30%, Glu 17%, Ser 10%, Gly 7%, Thr 7%), possibly with some covalently bound polysaccharides, and the other rich in serine and associated with relatively large amounts of polysaccharide, possibly proteoglycans. In contrast, the EDTA-insoluble fraction from mollusc shells varies considerably in composition between species. The major components are proteins rich in glycine, alanine, phenylalanine and tyrosine[20].

On the basis of these results, combined with ultrastructural data, the mollusc-shell matrix is now considered to be highly organized before the nucleation of aragonite crystals (Fig. 3*c*). The EDTA-insoluble constituents provide an architectural framework based on a core of β-chitin sandwiched between two layers of silk-fibroin-like proteins; these are covered on both sides by layers of EDTA-soluble matrix constituents which are aligned with the underlying anti-parallel β-pleated sheet polypeptide chains[21,22] to provide a structurally well-defined charged interface for nucleation[23]. This organization of acidic and hydrophobic macromolecules explains both the mechanical and crystallochemical properties observed in shells.

Molecular complementarity

Having established that organic biopolymers are organized so that their surfaces consist of localized sites tailored to the control of mineral nucleation, what type of interactions are possible at the inorganic-organic interfaces?

The key to specificity in nucleation is the presence of some form of molecular complementarity at an interface between functional groups on the organic macromolecules and ions in the surface of a crystal nucleus. It is postulated that polarity, charge and stereochemical relationships at sites that are restricted in size and topology can induce matching between the potential fields surrounding the inorganic and organic surfaces. This would account for the specific lowering of the activation energy for nucleation that is observed as a function of the structure and orientation of the nuclei. In this perspective, the molecular specificity of organic surfaces in nucleation resembles that inherent in protein-protein or protein-membrane associations. Precise chemical, configurational and topographical organization of hydrophilic and hydrophobic domains at the surface of the macromolecules in each system generates similar specific changes in the conformation of multi-component complexes.

Three aspects of recognition at inorganic–organic interfaces leading to specificity in the nucleation of biominerals are of particular interest here: electrostatic accumulation, structural correspondence and stereochemical requirements. Although these factors probably act cooperatively, we will discuss them separately to highlight the essential properties of the matrix surface.

Electrostatic accumulation. Organic macromolecules extracted from shell and dentine bind Ca^{2+} *in vitro* with stoichiometries often exceeding the number of potential anionic binding sites on the matrix[24–26]. This is taken to indicate that the electrostatic accumulation of ionic charge at specific sites on an organic substrate—the so-called ionotropic effect[27]—induces crystal nucleation. It is unlikely that this supra-stoichiometric binding involves multiple coordination complexes between organic ligands and Ca^{2+} because the stereochemical requirements of multiple ligands associated with distinct amino-acid sequences and molecular folding would suggest higher affinities and lower binding capacities than those reported. A more feasible interpretation is that the accumulation of charge at surface (anionic) sites on the matrix occurs by primary cation-binding. This would generate a loosely associated anion-coordination sphere and in turn attract a secondary cation-coordination sphere. This effect has been demonstrated by ^{31}P NMR spectroscopy of the binding of Ca^{2+} to phosphorylated serine residues in the presence of inorganic phosphate in a phosphoprotein isolated from fetal dentine[28]. (Phosphoproteins have also been isolated from chicken and bovine bone[29,30].)

Both high capacity and low binding affinity are critical for controlling nucleation. A matrix with high affinity for Ca^{2+} would probably inhibit nucleation because strong polymer binding would effectively remove aqueous ions from solution and render them unreactive. On the other hand, high capacity is required to localize a sufficient number of ions at the matrix surface to exceed the critical nucleus size. Crystallochemical selectivity will arise at the interface if the polarity and charge density at the matrix nucleation sites complement the electrostatic field generated around a particular crystal face of the forming nucleus. Important factors are the size of the nucleation site, the number of active functional groups on the matrix and their disposition, determined by the macromolecular structure and topography of the sites. For example, one model for hydroxyapatite nucleation in bone proposes that phosphoprotein molecules present in the regiospecific hole zones of collagen electrostatically accumulate large amounts of Ca^{2+} and

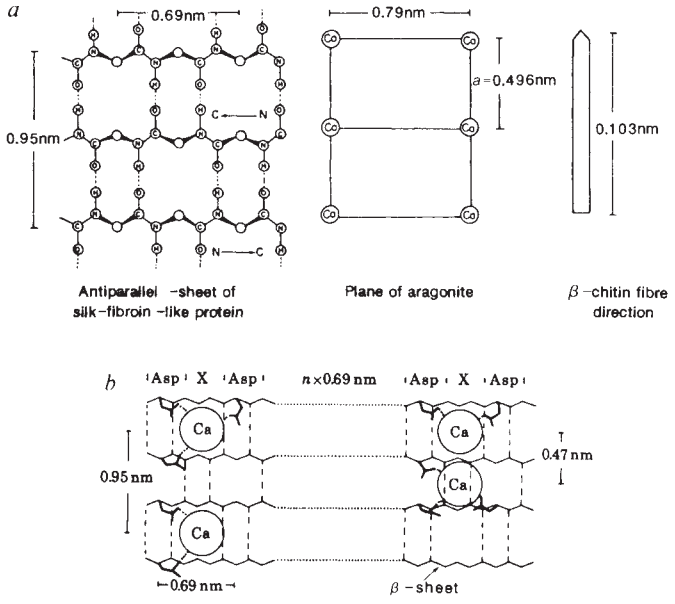

Fig. 5 Molecular correspondence at the inorganic-organic interface in the nacreous shell layer of *Nautilus repertus*. *a*, Schematic representation of the structual relationships between protein sheets, aragonite crystals and chitin fibres. There is a close geometric match between the periodicity of the β-sheet and the lattice spacings in the *ab* face of aragonite. *b*, Possible modes of molecular complementarity between Ca atoms in the aragonite *ab* face and aspartic acid residues organized in the sequence Asp-X-Asp (X, neutral residue) along the β-sheet matrix interface. Ca binding involves two or three ligands and is regulated along the interface to produce oriented crystal nuclei. (Reproduced with permission from refs 21 and 23 respectively).

phosphate ions[31]. This effect could be enhanced by the distinct size and topology of the hole zones to increase the structural order of the transient nucleus in its second and higher coordination spheres. This would facilitate deposition of hydroxyapatite rather than of a less-ordered material, such as amorphous calcium phosphate or microcrystalline brushite. Furthermore, it is feasible that the topographical organization of ion charge in the hole zone favours orientational anisotropy in the development of the mineral nuclei, resulting in the hydroxyapatite crystals being preferentially aligned, with the crystallographic *c* axis running parallel to the length of the hole zone, namely the collagen fibre axis (Fig. 2*a*).

Structural correspondence. Structural correspondence, or epitaxy, between the macromolecular surface and crystal nuclei implies a one-to-one geometrical matching between the functional groups of the matrix and the crystallographic lattice dimensions of a specific crystal face in the overlying mineral phase. Although structural relationships at the mineral-matrix interface have been implicated in a wide range of biomineralization processes, they have received only limited support from experimental studies. Some of the most elegant work has involved electron diffraction by small (6 μm^2) regions of partially demineralized mollusc shell fragments which in many species show that both the *a* and *b* axes of the antiparallel β-pleated sheet of the matrix and the overlying aragonite crystal lattice are matched in orientation at the interface[22] (Fig. 5*a*). Although this crystallographic correspondence is present throughout each matrix sheet the alignment of the *ab* plane of the β-pleated sheets can change, with the result that the mineral layer consists of a mosaic of aragonite crystals all with their *c* axis perpendicular to the matrix surface, but with different crystallographical alignment of the *a* and *b* axes parallel to the matrix interface[23].

A plausible mechanism has been suggested for the crystallochemical correspondence observed in mollusc shells[21-23]: the presence of a local sequence of negatively charged residues such as aspartic acid, linked to the β-sheet conformation, could act as a structurally organized nucleation site by binding Ca^{2+} in a configuration corresponding to the *ab* plane of aragonite. Comparison of the Ca-Ca distance in the *ab* plane of aragonite with the matrix periodicity indicates that a close matching occurs along the *a* axes (4.96 Å and 4.7 Å respectively), whereas a greater mismatch is observed along the *b* axes (7.97 Å and 6.9 Å respectively). In contrast, the periodicities along the *b* axes are essentially commensurate over a distance of seven calcium atoms (48 Å). As Ca^{2+} binding to carboxylate groups is generally cooperative, involving at least two or three ligands[32], an amino-acid sequence of Asp-X-Asp (where X is a neutral residue) along the β-sheet framework could present optimum binding (and hence nucleation) configuration for Ca^{2+} at the matrix interface[23] (Fig. 5*b*). Sequences of this type are common in EDTA-soluble proteins of both aragonite and calcite layers of mollusc shells[33,34]. Domains along the β-sheet that do not contain this sequence would be inactive in nucleation. Thus the coding of such sequences at intervals along the β-sheet framework would provide regiospecific sites for mineralization over considerable distances in extracellular space.

Stereochemical requirements. Structural correlations (as described above) depend essentially on numerological relationships in one or two dimensions at the mineral-matrix interface. Although some consideration of cation coordination geometry is implied by specific amino-acid sequences, these factors are secondary because of the flexible stereochemistry of Ca-ligand interactions. No emphasis is placed, however, on the stereochemical requirement for optimal completion of the anionic coordination geometry in the developing crystal face. This is an important aspect of recognition at inorganic-organic interfaces leading to specificity in nucleation.

Many biologically deposited calcite and aragonite crystals have their crystallographic *c* axes oriented along preferred directions. The disposition of Ca atoms in lattice sites running perpendicular to the *c* axis is almost identical in both structures and an argument based on structural correspondence can be invoked for the preferential adoption of the *c* axis orientation in these biominerals. This mechanism fails to explain, however, why selectivity between these two crystal structures is such a common feature of biomineralized systems; for example, many shells have inner pearly layers of aragonite and outer prismatic layers of calcite. It turns out that the difference between the calcite and aragonite structures along the *c* axis lies in the stereochemistry of the CO_3^{2-} anions sited between adjacent Ca layers running perpendicular to the *c* axis. In calcite, the most favoured stereochemical approach for the three binding oxygens of carbonate is parallel to the calcium plane and the optimum face for this presentation is the *ab* or (001) face. It follows that a matrix surface with binding sites in a configuration that is stereochemically equivalent to the anion motif of the crystal structure provides the optimum local coordination geometry to induce oriented nucleation for a specific crystal face in the nucleus. This stereochemical requirement explains the selectivity of interfacial association between crystal faces with similar Ca-Ca distance (structurally equivalent) but with different anion stereochemistry (for example, calcite and aragonite). It also explains why faces of maximum charge density do not always interact with the matrix surface, as might be predicted for a purely electrostatic recognition at the interface.

The importance of stereochemical complementarity has been demonstrated experimentally *in vitro*[7]. Acidic protein macromolecules in the β-sheet conformation extracted from mollusc shells and adsorbed at low concentration (<0.5 $\mu g\ ml^{-1}$) on to rigid glass or plastic substrates developed rhombohedral calcite crystals, which were often nucleated on a small triangular (001) face at the rigid substrate surface. Indirect immunofluorescence

staining of the crystals clearly showed the presence of aspartic acid-rich protein on the (001) face only, indicating that the organization of carboxylate residues attached to the β-pleated sheet surface satisfies the important stereochemical requirements of this face.

Cooperativity is also important in the regulation of crystal nucleation on ordered organic substrates[35]. In outline, the mechanism proposed for oriented (001) calcite nucleation involves a cooperative effect arising from enhanced electrostatic interaction between sulphonated residues of the substrate and the structural and stereochemical requirements of polyaspartate macromolecules. Evidence for this mechanism comes from recent *in vitro* experiments using polyaspartate in the β-sheet conformation adsorbed onto polystyrene films. It did not induce nucleation of oriented calcite crystals unless the substrate had previously been sulphonated. The effect is clearly cooperative because only a few oriented crystals form on sulphonated films without polyaspartate, whereas many form on films with both components, and the affinity of Ca for sulphonated films with adsorbed polyaspartate is more than twice that for sulphonated films without the polypeptide. These results are consistent with observations on acidic macromolecules isolated from mollusc shells, which contain covalently bound sulphate residues. When adsorbed on to solid substrates the macromolecules induce oriented calcite nucleation. As many mineralized tissues contain macromolecules with sulphate and carboxylate ligands, the demonstration of cooperativity in these model systems has wide-ranging implications in the understanding of nucleation in living systems.

Potential applications

The study of biomineralization is relevant to a range of industrial processes, including the direct biotechnological exploitation of biogenic materials[36], the design of novel composite materials by *in situ* precipitation[37], and the mimicking of biological processes in an inorganic context. The underlying mechanisms of controlled nucleation in biological systems has important implications for the third of these approaches, for fabricating inorganic materials with technological applications. In principle, the aim is to use stereospecific polymer surfaces, prepared either synthetically or, in the longer term, by molecular biology techniques, on which crystals of defined shape, size, structure and orientation could develop. Crystal properties would be regulated by the molecular interactions at the interface, with systematic changes in the substrate surface reflected in changes in crystal chemistry. If this could be achieved, a range of useful materials could be fabricated, including magnetic (Fe_3O_4, γ-Fe_2O_3), catalytic (doped metal oxides), optical (SiO_2, $LiNbO_3$), and piezoelectric ($BaTiO_3$ analogues) devices. Furthermore, the growth of materials as oriented thin sheets on polymeric substrates has potential value for making piezoelectric and electroactive sensors, as well as in the production of organized precursors for chemical and ceramic support beds.

Apart from the *in vitro* work described above on the nucleation properties of β-sheet proteins attached to sulphonated polymer films[35], so far only a few studies have investigated the nucleation potential of organic substrates. Perhaps the most promising is the system based on compressed surfactant or phospholipid monolayers where control of both the polarity and the head-group spacing of these molecules could provide a way to engineer the interactions involved in nucleation at an organic-inorganic interface. Landau *et al.*[38,39] have shown that oriented growth of glycine and sodium chloride takes place at the air/water interface of structured monolayers. The controlled formation of chiral crystals such as glycine is valuable because homochiral monolayers will induce the nucleation only of crystal faces with corresponding symmetry. Although the nucleation of NaCl on compressed monolayers is less specific, probably because the surfactant molecules cannot simulate the stereochemistry of the NaCl surfaces, particular faces are stabilized in the presence of charged monolayers, presumably by cooperative electrostatic and structural correspondence[39]. For example, close-packed films of octadecylamine favour nucleation of NaCl on the cubic (100) face, whereas stearic acid monolayers induce nucleation mainly on the uni-charged (111) faces. Using stearic acid, complete inhibition of crystal nucleation under the monolayer results from increasing the distance between the carboxylate groups of the monolayer, which can be achieve by using molecules with bulky hydrophobic chains, such as cholesteryl succinate. Although only a limited number of crystal types have been studied, this is a novel approach in engineering crystal nucleation that can be extended into fields such as crystallization science and materials technology.

The significance of studying inorganic solid-state chemistry in biology is highlighted by the importance of molecular recognition at interfaces comprising organic polymeric substrates and inorganic crystal surfaces, particularly for controlling the crystallochemical properties of mineral nuclei forming at the matrix surface. The key features of the interface are that it is chemically, structurally and topographically organized for site-directed nucleation, and that the molecular interactions at each nucleation site involve a high degree of complementarity between the electrostatic, structural and stereochemical requirements of functional groups at the matrix surface and ions in the crystal faces of the developing nuclei. The *in vitro* investigation of these factors and their theoretical simulation by molecular modelling together with the investigation of possible industrial and biotechnological applications, are important directions for future work.

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