A Basic Strategy for Biomineralization: Taking Advantage of Disorder

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Biologically formed minerals are renowned for their unique shapes, preferred orientations, organisation into ordered mineral-matrix composites, their remarkable mechanical properties and their beauty [1]. Organisms seem to have an ability to control mineral formation that far exceeds our own in vitro capabilities. Have organisms evolved a really novel underlying strategy for controlling mineral formation? Possibly, at least in the case of calcium carbonate deposition.

Three crystalline polymorphs of calcium carbonate exist (calcite, aragonite and vaterite), and 3 hydrated forms (monohydrocalcite, hexahydrate and amorphous calcium carbonate (ACC)) [2]. ACC is traditionally regarded as being amorphous because it is isotropic under crossed polarizers and it does not diffract X-rays. It does however have short range order that differs between ACC deposits formed by different organisms [3]. It is highly unstable and soluble in vitro. It is thus surprising that 16 species from 7 animal phyla are known to form ACC, in addition to quite a few plant taxa [4]. In most known cases, the ACC is stable at least during the lifetime of the organism. In 2 cases, the larval spicules of the sea urchin [5] and the larval shells of molluscan bivalves [6], the ACC is a precursor phase that transforms into one of the crystalline polymorphs. In this presentation I will focus on larval and adult mollusk shell formation processes, and in particular the possible role of ACC.

Since the beginning of the 20th Century it has been thought that all mollusk larval shells are composed of only aragonite crystals, with their c-axes perpendicular to the shell surface [7]. Using a high resolution ESEM we compared the ultrastructures of the larval shells of 2 bivalve species, *Mercenaria mercenaria* and *Crassostrea gigas*. We confirmed earlier observations that showed that they are both composed of an outer organic layer (periostracum), a relatively thick granular-homogeneous middle layer and an inner prismatic layer [8]. We noted that the larval shells are much more sensitive to radiation damage than the adult shells, and that the granular layer is composed of spherical sub-units; properties reminiscent of an amorphous phase. Using Raman and infrared spectroscopy, we demonstrated that the first-formed mineral phase is indeed ACC and that with time it converts into aragonite. This aragonite phase is much less crystalline than geological aragonite. Interestingly, there is no obvious correspondence between the observed changes in ultrastructure as the larval shell forms and the mineral transformation; apparently the amorphous mineral phase is initially moulded into specific shapes (for example prisms), and only subsequently does it transform into aragonite. We also noted that the aragonite in the adult *Mercenaria* shell is most surprisingly less crystalline than two different samples of inorganic aragonite we analysed. This may be a hint that the adult shell also contains some ACC and/or poorly crystalline aragonite, and if so, an indication that the adult shell also forms via an ACC precursor.

We used cryo-TEM to investigate the structure of the organic matrix of the nacreous layer of the adult bivalve *Atrina* [9]. We showed that the interlamellar sheets viewed in vitrified ice are composed mainly of highly ordered chitin that display lattice fringes. Aspartic acid-rich proteins are located on the surfaces of these sheets. The other major matrix component, silk fibroin, could not be imaged in the extracted organic matrices, or even when reconstituted on chitin in vitro. This suggests to us that it adopts a gel-like phase. Vitrified ice cross-sections of the interlamellar sheets showed that the spaces between sheets even after demineralization, contain organic material. We therefore proposed that this is the site of the silk fibroin. If so, this implies that the aragonite forms inside a hydrated silk fibroin gel. If the larval shell formation processes are any guide to the adult shell formation processes, it is conceivable that the silk is somehow involved in the formation and/or stabilization of a precursor ACC phase - an exciting prospect to be investigated.

Note that the two proven examples of ACC acting as a transient precursor phase, the larvae of the sea urchin and the larvae of bivalves, are from phyla that are on 2 quite different branches of the phylogenetic tree of the animal kingdom. If this strategy evolved divergently, this could imply that it is widespread in the animal kingdom. If so, we would need to understand the benefits of adopting this novel approach to crystallization, whereby the crystals nucleate and grow from a dense colloidal phase, possibly with its own tailor-made short range structure.

References

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