Calcite to Aragonite Phase Switching Mediated by Soluble Mollusc-Shell Proteins

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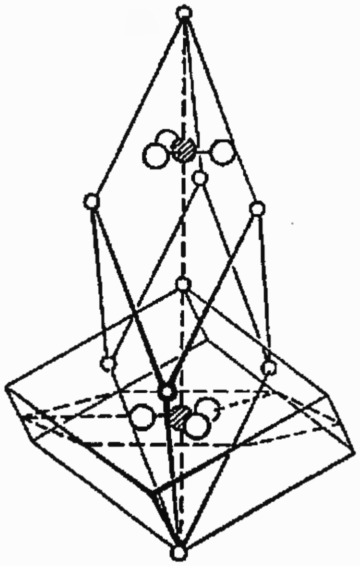
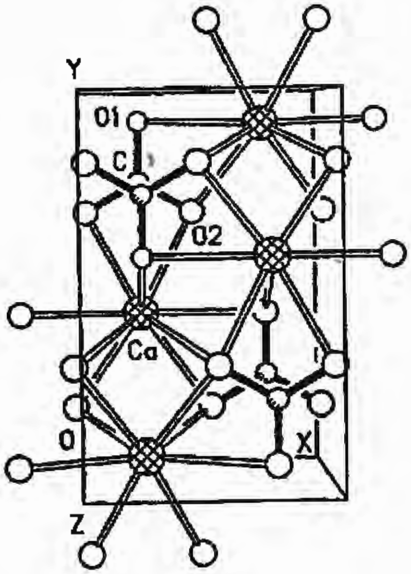
Biomineralization, simply stated, is the process by which organisms convert ions in solutions into solid minerals.[1] For decades, we have been aware of the diversity of biomineralization products as well as the organisms which form them.[2] This controlled formation of inorganic materials has inspired new approaches to the controlled fabrication of synthetic materials such as nanoparticles, polymer-mineral composites and templated crystals.[3-9] Recently, this biomimetic approach has gained momentum,[3,10] although the biological mechanisms involved in the biomineralization process remain relatively unexplored.[11,12]

The marine gastropod *Haliotis rufescens* [13] has been chosen to be the subject of recent investigations into the mechanisms involved in the biofabrication of aragonitic shell nacre.[11,14,15] The growth front of the shell is governed by epithelial cells which secrete organic macromolecules as well as inorganic ions into the extrapallial space between the mantle and the shell.[1,16] Insertion of an abiotic substrate, (glass, mica or MoS_2), between the mantle and the shell results in the formation of a highly organized composite material termed the 'flat pearl'.[11,14,15] It has been shown that the fabrication of this substance closely mimics that of the natural shell both temporally as well as spatially.[11,14,15]

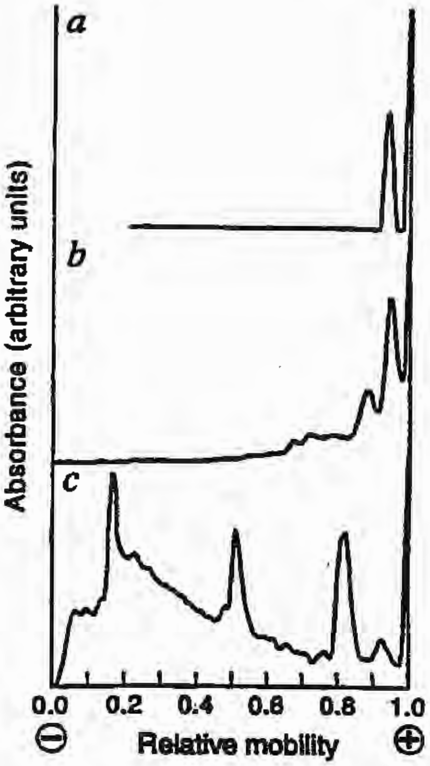




Upon analysis of the flat pearls, three distinct regions were found: a red outer band, an iridescent central region and a green inner zone. The size of these regions varied with the exact placement of the abiotic substrate, but typically had widths of 1-3 mm for the distal red zone, 6-10 mm for the central region and 8-10 mm for the green proximal area. X-ray diffraction studies characterized the composition of the three zones.[11] The two outer regions were found to be mineralized primarily with calcite, while the central zone was heavily covered in aragonite. The aragonite was composed of pseudo-hexagonal tablets which were surprisingly oriented along the (001) crystallographic plane. All tablets were found to be coated with a polymeric organic sheet.[11]

Subsequent analysis of this polymeric material revealed the contents to consist of a polyanionic protein with an approximate molecular weight (M_r) of 16K. Demineralization of the aragonite tablets allowed for subsequent extraction of two more polyanionc species with relative molecular weights of 20K and 31K. Similar analysis of the calcitic layer revealed a complex mixture of six similar proteins of molecular weights of 14K, 28K, 35K, 48K and 55K (data not available).[14]

In vitro mixing of the initiating protein sheet, (found intercalated among the aragonite tablets), and the soluble mineral-phase-specific polyanionic proteins allowed for growth of the calcium carbonate phase corresponding to the region from which the proteins were isolated.[11,17] In the case of calcite, the crystals grown from a solution containing the calcitic proteins revealed the characteristic spherulitic morphology of calcite found in the native shell. However, in the case of the crystals grown from a solution of the polyanionic proteins solubilized from the aragonitic composite, a very different morphology was grown than that found in the native shell. It should be noted however, that crystals grown from a mixture of proteins from the aragonitic and calcitic composites proved to be aragonite. The morphology of these crystals was identical to that found in the native shell.[11,14,15]



The abrupt transition from calcite to aragonite seen in biogenic nacre as well as the flat pearl can be induced by the addition of mineral-specific proteins. Calcite crystals grown in the presence of the calcitic polyanionic proteins can be converted to the aragonite morphology upon the addition of the soluble protein mixture isolated from the aragonitic composite. Depletion of these proteins results in the reformation of the initial calcite morphology. It should also be noted that this proteins-directed mineralization is stereospecific in the case of extensive crystal overgrowth on the calcite rhombohedral faces. The structures generated maintain six-fold symmetry about the [001] (c-axis) direction.[14]

The presence or absence of the aragonite-specific polyanionic proteins is thus sufficient to switch the polymorph of growing crystals at the micrometer scale, without the addition of magnesium or a change in temperature or pressure.[7,14]

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