

A HIERARCHICALLY STRUCTURED MODEL COMPOSITE: A TEM STUDY OF THE HARD TISSUE OF RED ABALONE

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ABSTRACT

The structure and crystallography of the nacre of red abalone, Haliotis rufescens, was studied by transmission electron microscopy imaging and diffraction. We found that the nacre structure is based upon hierarchical {110} twinning in aragonite with the following organization: (i) first generation twins between platelets having incoherent boundaries, (ii) second generation twins between domains having coherent boundaries within a given platelet, and (iii) nanometer-scale third generation twins within domains. Since the aragonite platelets nucleate and grow as separate crystals, this long-range crystallographic relationship between the inorganic units of a biological hard tissue indicates that the nucleation and growth process of crystals may be mediated by the organic matrix and that the organic template structure may also be long-range ordered. We propose a superlattice structure based on the possible twin variants and suggest that the organic matrix structure, or the arrangement of nucleation sites, is compatible with the superlattice. Multiple tiling based upon this superlattice allows all of the crystallographic and morphological platelet configurations observed in nacre.

INTRODUCTION

Nacre is a laminated ceramic-polymer composite material found in mollusk shells.¹⁻⁶ It has a highly ordered structure on a continuous scale¹ from the nanometer to the millimeter and has unique mechanical properties, such as high fracture toughness and strength.³⁻⁴ Understanding its structure, in particular the growth process and the interrelationships between the microstructure and properties, is valuable to biological sciences, materials science and engineering, and the electronic industry. Previous studies have suggested that the formation of the inorganic crystals was regulated by the organic matrix through epitaxial growth.⁶⁻⁹ But to date neither the structure of the inorganic phase with its detailed architecture nor the organic phase have been fully understood. In nacre, the inorganic phase, CaCO_3 in the aragonite form (Pmcn, No. 62), and the organic phase, a mixture of proteins and polysaccharides,⁸⁻⁹ are arranged in a "brick and mortar" microarchitecture (Figure 1).¹⁻⁵ The aragonite platelets, with hexagonal or square faces, are about 5 μm in edge length and 0.25 to 0.5 μm in thickness, and the organic phase is about 100-200 $\text{\AA}$ thick.¹⁰ Previous diffraction studies¹¹ illustrated that the platelets on a given layer are aligned in the c direction of the orthorhombic unit cell of the aragonite lattice. It was further suggested that the platelets are arranged in a "mosaic pattern" without definite crystallographic relationships between them in the a - b plane.¹² Similarly, it was suggested that only local ordering existed in the organic matrix, which was thought to be responsible for the mosaic polycrystalline pattern in the aragonite crystals.¹²

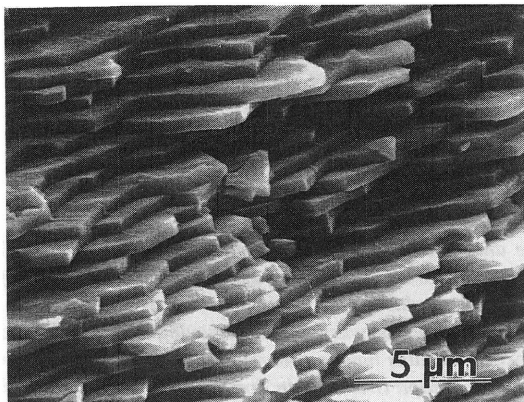


Figure 1. Secondary electron image of fractured surface of nacre reveals the brick and mortar microarchitecture. Nacre is from a pearl oyster sample (*Pinctada margaritifera*). Courtesy of Katie E. Gunnison.

Contrary to these earlier studies, in the following sections, we summarize our recent electron microscopic studies to show that platelets have definite crystallographic relationships to each other and are arranged with levels of hierarchical twins. Our findings also suggest the existence of a long-range order in the organic matrix.

CRYSTALLOGRAPHY OF ARAGONITE PLATELETS: HIERARCHICAL TILING IN THE NACRE STRUCTURE

We first studied both the geometrical arrangement of and the crystallographic relationship among the aragonite platelets.^{1,13} In the face-on view, each layer of the nacre is composed of closely packed platelets (Figures 2(a) and 2(b)). The platelets have either three, four, five, or six edges. The geometrical organization of the platelets often exhibits sixfold symmetry as shown in Figures 2(a) and 2(b) where six platelets are arranged with an approximate 60° angle between each pair (60° twin boundaries). The crystallographic orientations between the platelets on the a - b plane are not random, contrary to previous assumptions,¹¹⁻¹² but generally relate to one another by twinning, as shown by the diffraction patterns in Figures 2(c) and 2(d). The [001] single crystalline pattern in Figure 2(c) is from the interior of a platelet; the pattern in Figure 2(d), which was recorded from the boundary between two platelets, incorporates two superimposed patterns. Analysis of the two patterns reveals that they are correlated to each other by a twin relationship, with the twin plane $\{110\}$ parallel to the [001] direction of the crystal, i.e., either (110) or ($1\bar{1}0$). The images in Figures 2(a) and 2(b) were recorded by tilting the

sample a few degrees to bring each of the three alternate platelets into a strongly diffracting condition so that they exhibit a dark diffraction contrast. The platelets A, C, and E, therefore, are all in the same orientation and the remaining three, B, D, and F, are in a twin orientation with the first set. Although the diffraction patterns are slightly misaligned about the c direction, the twin relationships between them are preserved. In fact, the selected area diffraction (SAD) pattern recorded from all platelet boundaries shown in Figure 2 reveals the same twin reflections, indicating that each platelet is related to the one next to it by a $\{110\}$ twin relation. In this paper, we refer to the twinning between aragonite platelets as *incoherent first generation twinning*.

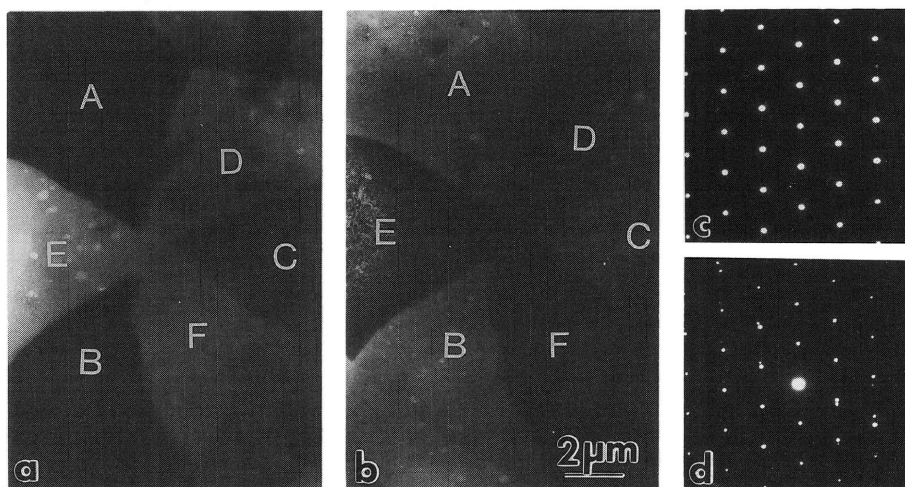


Figure 2. (a) and (b) show the face on view of nacre and were recorded by slightly tilting the specimen along the c direction. The nacreous layer consists of closely packed 3-, 4-, 5-, and 6-edged platelets, which often exhibit sixfold symmetry. In (c) and (d), diffraction revealed that the platelets are related to one another on $\{110\}$ planes. (c) is a single crystalline diffraction pattern from one platelet. (d) is the diffraction pattern from boundaries between platelets, showing twin relationships.

Further studies revealed that many of the the platelets are not single crystals but are comprised of several domains, and that the domains are related to each other by twinning as well. Figure 3(a) shows a four-domained platelet with 90° domains. The diffraction pattern in Figure 3(b), recorded from the interior of one of the domains, indicates that the platelet is again perpendicular to the $[001]$ electron beam direction. The diffraction pattern in Figure 3(c) recorded from a domain boundary exhibits twin splitting of (110) reflections. A close examination of the crystal structure of aragonite shows that the 90° twin boundaries can be accommodated by including two atomically flat (110) twin planes (reflection twins) and two zig-zag boundaries

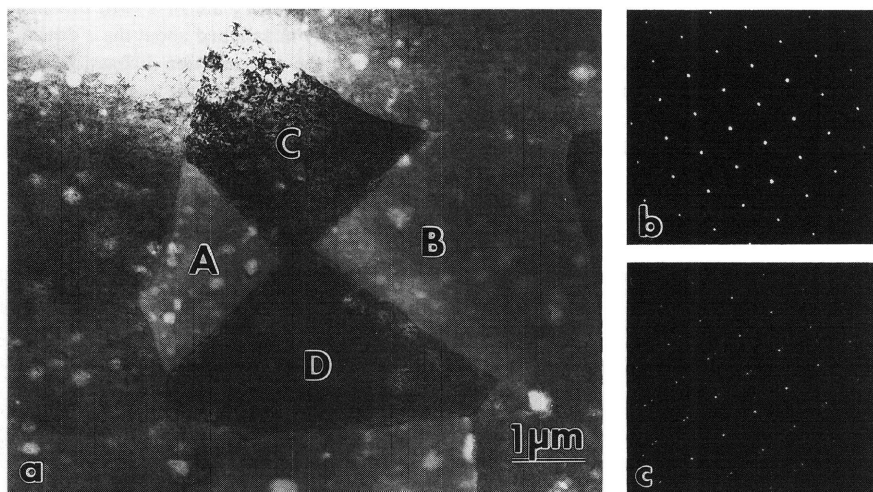


Figure 3. (a) shows the domain structures within platelets; (b) single crystalline diffraction pattern from one domain; (c) twin diffraction pattern from domain boundaries.

(180° -rotation twins). This kind of twinning is called *second generation coherent twins*, and there is no misalignment in the c direction among the domains within a platelet.

In an ideal hexagonally shaped platelet having six twin-related domains, the angle between each pair of domains must be 60° , with the six domains completing 360° for the whole platelet. This is not possible in aragonite since the outer edges of the platelets are parallel to $\{110\}$ planes and the angle between each pair of $\{110\}$ planes is 63.5° . This discrepancy, therefore, must be accommodated by lattice deformation during the formation of twin-related domains in an aragonite platelet and can be accomplished by either slipping (dislocation formation) or by twinning,¹ with the latter preferred in ionic crystals.¹ Imaging of the microstructure at higher magnifications shows that each domain in a given platelet actually contains two sets of nanometer-scale twins, each forming $\{110\}$ planes. Figure 4 shows two variants of ultrafine twins forming angles of about 63.5° or 127° , which are similar to growth twins in geological minerals.¹⁴ The accommodation of the 3.5° strain is possible by the formation of these nanometer scale defects on the $\{110\}$ planes, which allows the lattice to be deformed towards the periphery of the platelet. In fact, in most cases, the outer periphery of the platelets has a convex shape, the apex being in the middle of the edge, and the region of the edge where the two domains meet inwardly curved. We call these ultrafine twins *third generation nanometer-scale twins* as they take place at the smallest dimensional scale.

In summary, we found that there are three levels of twinning in the face-on configuration of the nacre section of red abalone shell: (i) first generation twins between platelets that have incoherent boundaries, (ii) second generation twins between domains with coherent boundaries within a given platelet, and (iii) nanometer-scale third generation twins within a domain. It should be noted that both twin and domain boundaries can be either 60° or 90° , although we only show examples of 60° twin boundaries and 90° domain boundaries. Therefore, these twin structures encompass a six-order magnitude size scale covering a range from the nanometer to

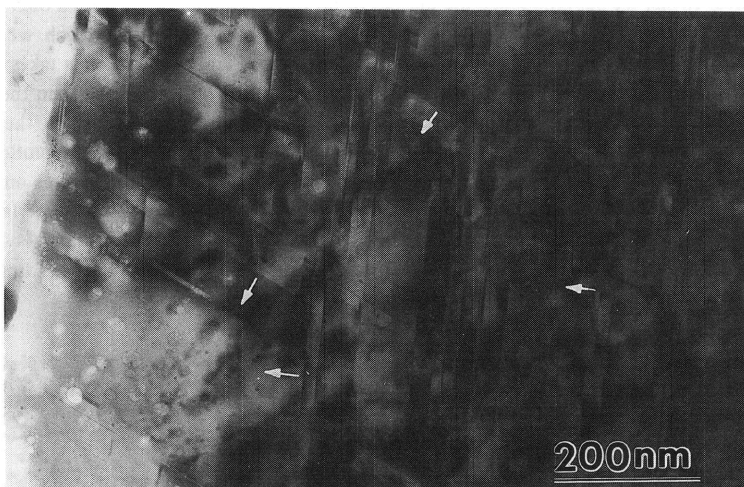


Figure 4. Nanotwins within domains.

the submillimeter and reveal a hierarchical structure for hard tissue in a biological material. Although sixfold twin structures also occur in geological aragonite, the hierarchical arrangement of twins in nacre is unique in the sense that each platelet is completely separate from the others during the early stage of growth. Even after crystallization is complete, the platelets are still separated from one another by an organic membrane. On the other hand, in geological aragonite¹⁹ the mimetic twin domains always grow one after another. The fact that the separate platelets grow simultaneously and yet retain a certain crystallographic relationship suggests that the growth process is mediated by the organic template beneath the crystals, and that the organic matrix also has long-range ordering.¹ In the following section we discuss how these hierarchical twins originate and the implications for the structure of the organic template on which the aragonite crystals are grown.

CONFORMATION OF MATRIX MACROMOLECULES

The interaction between the organic template and the crystals may include both electrostatic and stereochemical forces.^{8,15-17} If that is the case, then the nucleation and growth of the crystals will be influenced by both the nearest and higher order interactions. In aragonite, calcium ions give the crystal a pseudohexagonal symmetry in the [001] projection, but the CO_3^{2-} ions reduce the symmetry to an orthorhombic form.¹⁴ Although nucleation and growth may involve both the calcium⁸ and the CO_3^{2-} ions,¹² for simplicity, in this study only the arrangement of the calcium ions is examined in the [001] projection. To understand the origin of the hierarchical twins, we superimposed the lattices of all three possible twins with a 63.5° rotation with respect to each other and generated a new, higher-order structure, which we call the *superstructure* (Figure 5(a)). If the nucleation and growth of aragonite platelets takes place on this spatial configuration of the active sites for the binding of the Ca^{2+} ions, then the organic matrix must accommodate this superlattice and, thus, all twins in nacre since the organic template structure should also be aligned over all length scales. The most likely solution to this problem is that the organic matrix, or the arrangement of the active nucleation sites on it, has a single crystalline lattice structure over a wide area that is compatible with the superlattice. A local crystalline organization of the organic matrix, with no relationship between the neighboring areas, and, hence, without long-range order, would result in the formation of

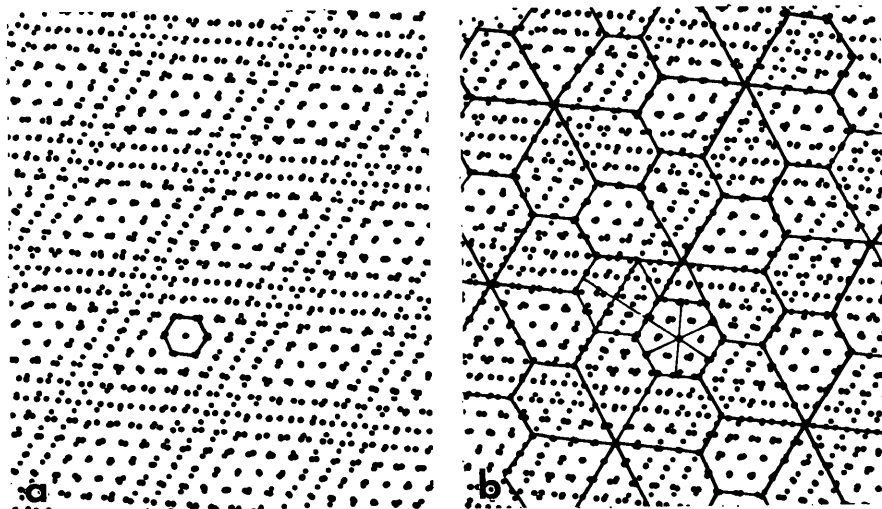


Figure 5. (a) Superlattice generated by superimposing possible twin crystal lattice structures. The circles are an imaginary lattice structure of the organic matrix; (b) All tiling patterns can be constructed on the superlattice by tracing along possible twin boundaries.

aragonite crystals without any definite crystallographic relationship among the platelets. For example, a pseudo-hexagonal arrangement, which is indicated by the circles in Figure 5(a), would satisfy the requirement for the organic matrix. The crystalline pseudo-hexagonal lattice is a possible solution since many two-dimensional protein lattices assume this organization during self-assembly.¹⁸

The superlattice shown in Figure 5(a) allows the generation of the overall hierarchical twin structures by tracing along the possible twin boundaries. One construction is illustrated in Figure 5(b) which contains all the shapes, geometry, and crystallography-related features discussed in this paper, such as five-edged platelets with 90° domains, sixfold symmetry of platelets, and six- and three-edged domains. The fact that all the possible configurations can be generated this way again illustrates the need for a compatible organic matrix with a wide range of length scales to accommodate all the twin relationships, rather than the lattice of a single domain or a single platelet.

The construction of the aragonite platelets discussed in this paper is the subject of multiple tiling in mathematics.¹⁹ Nature uses this technique to form a highly ordered structure compatible with both the soft tissue and the crystalline structural constraints of the hard tissue. This unique ordering, originating from the atomic or molecular structures of both the organic and inorganic tissues, extends from the molecular to the millimeter scales to form different shapes of shells.^{1,13}

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