Biological control of crystallographic architecture: Hierarchy and co-alignment parameters ☆

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ABSTRACT

Mytilus edulis prismatic calcite and nacre layers exhibit a crystallographic structural hierarchy which differs substantially from the morphological hierarchy. This makes these biomaterials fundamentally different from classical crystalline materials. Morphological building units are defined by their surrounding organic matrix membranes, e.g. calcite fibers or nacre tablets. The crystallographic building units are defined by crystallographic co-orientation. Electron backscatter diffraction quantitatively shows how crystallographic co-orientation propagates across matrix membranes to form highly co-oriented low-mosaic composite-crystal grains, i.e. calcite fiber bundles with an internal mosaic spread of $0.5°$ full width at half maximum (FWHM) or nacre towergrains with an internal mosaic spread of $2°$ FWHM. These low-mosaic composite crystals form much larger composite-crystal supergrains, which exhibit a high mosaicity due to misorientations of their constituting calcite fiber bundles or nacre towergrains. For the aragonite layer these supergrains nucleate in one of three aragonite $\{1\,1\,0\}$ twin orientations; as a consequence the nacre layer exhibits a twin-domain structure, i.e. the boundaries of adjacent supergrains exhibit a high probability for misorientations around the aragonite *c*-axis with an angle near $63.8°$. Within the supergrains, the constituting towergrains exhibit a high probability for misorientations around the aragonite *a*-axis with a geometric mean misorientation angle of $10.6°$. The calcite layer is composed of a single composite-crystal supergrain on at least the submillimeter length scale. Mutual misorientations of adjacent fiber bundles within the calcite supergrain are mainly around the calcite *c*-axis with a geometric mean misorientation angle of $9.4°$. The *c*-axis is not parallel to the long axis of the fibers but rather to the $(1\,0\,7)$ plane normal. The frequency distribution for the occurrence of misorientation angles within supergrains reflects the ability of the organism to maintain homoepitaxial crystallization over a certain length scale. This probability density is distributed log-normally which can be described by a geometric mean and a multiplicative standard deviation. Hence, those parameters are suggested to be a numerical measure for the biological control over crystallographic texture.

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1. Introduction

Mollusc shells are composite biomaterials which consist of biominerals embedded in an organic matrix. These hybrid composite materials exhibit extraordinary toughness as compared to the pure inorganic materials incorporated in the shells [1–4]. The mineral part usually exhibits a crystallographic texture [5] which is the key to understanding the interplay between mineral and organic matrix and therefore the materials design properties.

Many bivalve shells consist of an outer calcite and an inner aragonite layer. The calcite layer is composed of calcite fibers $\sim$1–2 μm in diameter, reaching lengths of more than 100 μm [6], which are embedded in an organic matrix. They are single-crystal-like aligned and the whole layer is considered as a composite crystal [5] in the sense of strongly co-aligned crystallites forming an ordered architecture of components which in turn forms the composite crystal. Here, each component, i.e. a single calcite fiber, is a mesocrystal [5,7] as it is composed of strongly co-aligned nanocrystals commonly found in prismatic calcite (see Ref. [8] and references therein).

The aragonite layer (nacre) is composed of aragonite tablets separated by organic membranes. It has been shown that nacre rapidly grows in towers and later expands sideways [9], and that the crystallographic lattice of adjacent tablets in such towers is co-oriented with [001] perpendicular to the platelet plane [10–14]. These findings strongly support the existence of mineral bridges across the interlamellar membranes which are responsible for the continuation of crystal orientation [15,16]. Recently we showed that nacre tablets in *Mytilus edulis* consist of spherical aragonite nanocrystals ~50 nm in size, which are embedded in a 3-D organic framework [17]. This is analogous to the membrane-coated granules found in pearls [18]. These granules, initially composed of amorphous calcium carbonate [18,19], were observed to crystallize rapidly [18,20], i.e. prior to platelet formation, in contrast to other observations which demonstrate compartment formation by membranes prior to crystallization [21–23]. The aragonite nanocrystals exhibit a misorientation spread of 2° full width at half maximum (FWHM) which spans over several (up to 20) mesocrystalline [7] tablets to form composite crystals [17]. These data support the hypothesis that aragonite nacre grows by nanoparticle attachment followed by semicoherent homoepitaxial crystallization.

The aim of this paper is to present a detailed orientation/misorientation analysis of the hierarchical crystalline building units present in the calcite and aragonite layers of the bivalve shell in order to establish qualitative numerical parameters for the degree of biological control over the crystallographic alignment of the mineral part, i.e. the crystallographic texture. For this purpose we have chosen to study the shell of *M. edulis*, which exhibits both calcite and nacre layers.

2. Materials and methods

Specimens of *M. edulis* from Naples harbor (Italy, Mediterranean Sea) were cut into 300 μm thick wafers. For the nacre measurement the cut was applied along the longest axis of the shell from the hinge to the commissure. Thus, the measurement plane is perpendicular to the shell surface, which results in a side view of the nacre tablets. For the calcite measurement, the cut was applied perpendicular to the longest axis of the shell, which results in a cross-sectional view of the calcite fibers. The wafer surfaces were treated with several mechanical polishing steps and finished using etch-polishing with colloidal silica in a vibratory polisher. The samples were coated with 4 nm of carbon. Electron backscatter diffraction (EBSD) maps were obtained using a field emission gun scanning electron microscope equipped with an Oxford Instruments NordlysNano EBSD detector and AZtecHKL software. EBSD patterns were collected at about one-third of the longest axis from the hinge towards the commissure. An acceleration voltage of 8 kV was applied with 125 and 200 nm step resolution for nacre and calcite, respectively. The patterns were indexed using aragonite lattice parameters of $a = 4.97$ Å, $b = 7.97$ Å and $c = 5.75$ Å for nacre and calcite lattice parameters of $a = 4.99$ Å and $c = 17.06$ Å for the calcite fibers. The EBSD maps were evaluated using the MTEX package [24].

In order to visualize the organic matrix in the nacre layer, samples were polished using an Ultracut ultramicrotome (Leica). Glass and diamond knives (Diatome, Switzerland) were used to polish planes across the shell, similar to the method described previously in Ref. [25]. The polished planes were subsequently etched for 30, 60 and 90 s with an aqueous solution composed of 2.5% glutaraldehyde (in order to stabilize the organic components) and $0.01 \text{ mol } l^{-1}$ MOPS buffer adjusted to a pH of 6.5. Etching was stopped by washing the samples three times with 100% isopropanol. After critical point drying (Bal-Tec CPD 030), the samples were rotary shadowed with 3–4 nm platinum at an angle of 45° using a BAF 300 (Balzers). The exposed organic matrix was analysed by field-emission scanning electron microscopy (FE-SEM) using a Hitachi S-5200 microscope.

3. Results

3.1. M. edulis nacre layer

From an EBSD map the constituting crystal grains can be identified as sets of adjacent measurement pixels which give similar lattice orientation, i.e. which are misoriented by less than a certain threshold angle. Fig. 1b shows that grains determined with a threshold misorientation angle of 3.5° reproduce the nacre tablets quite well. Here grains with less than three measurement pixels were removed from the initially determined grain map and the grains were subsequently recalculated. This procedure removes, for example, grains which have been falsely determined due to misindexed measurement pixels. The EBSD band contrast, i.e. the experimental signal strength of each individual EBSD pattern, is superimposed as an additional gray value on the grain map in Fig. 1b. This reveals the organic membranes between the tablets as white stripes. The distribution of the aragonite *c*-axis (see the {001} pole figure in Fig. 1a) shows a single density maximum roughly perpendicular to the surface of the shell. The *a*-axis distribution shows a band of distinct density maxima perpendicular to the mean *c*-axis direction. The grains in Fig. 1b are color-coded according to the θ angle of the {100} pole figure in Fig. 1a and therefore different color indicates different orientations rotated around the mean *c*-axis direction. Most of these grains are composed of stacks of several aragonite tablets, and these so-called towergrains can therefore be considered as composite crystals [5,17]. However, one should keep in mind that the threshold angle is arbitrary. A threshold angle smaller than 3.5° divides single aragonite tablets, while increasing the threshold angle increases the size of the towergrains until the whole nacre layer is a single grain at threshold angles above 60°. As a consequence, the following analysis carefully determines the properties of mutual misorientations for measurement pixels at grain boundaries of adjacent grains in order to establish meaningful threshold angles.

Fig. 2a shows the distribution of misorientation angles between neighboring pixels which are misoriented by >0.3°, the instrumental resolution for the orientation measurement. This distribution shows a dominant maximum for very small-angle misorientations. This maximum has a FWHM of 2°, and it tails out up to 30° [17]. In the high-angle regime there is a doublet peak near 60° which indicates cyclic aragonite {110} triplet twinning for which the theoretical misorientation angle is 63.8° around [001] for the first and 52.4° around [001] for the second twin. For the grain boundary misorientations between tablet grains defined by a 3.5° misorientation threshold only a single maximum near 64° remains (see Fig. 2b). Hence, there is no cyclic {110} triplet twinning between tablet grains but possibly within them.

To analyze the microstructure further, we also determine the rotation axes corresponding to the misorientations. Fig. 2c,d plot the rotation axis distribution, i.e. the frequency (probability) by which a certain crystallographic direction acts as a rotation axis at the grain boundary in the statistical ensemble provided by our map. The rotation axes are displayed in a symmetrical unique sector of the stereographic projection for the orthorhombic symmetry of the aragonite lattice. Fig. 2c shows results for boundaries with misorientation angles <30°, and Fig. 2d applies to boundaries with misorientation angles >0°. For the small-angle misorientations a high frequency of rotation axes close to the aragonite *a*-axis is revealed, with the *a*-axis itself showing the maximum probability. For large-angle misorientations a very high probability density is found for rotation axes close to the aragonite *c*-axis, which itself