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Crystal lattice tilting in prismatic calcite

Ian C. Olson [a], Rebecca A. Metzler [b], Nobumichi Tamura [c], Martin Kunz [c], Christopher E. Killian [a], Pupa U.P.A. Gilbert [a,d,*]

[a] Department of Physics, University of Wisconsin–Madison, 1150 University Avenue, Madison, WI 53706, USA
[b] Department of Physics and Astronomy, Colgate University, Hamilton, NY 13346, USA
[c] Advanced Light Source, Lawrence Berkeley National Laboratory, 1 Cyclotron Road, Berkeley, CA 94720, USA
[d] Department of Chemistry, University of Wisconsin–Madison, 1101 University Avenue, Madison, WI 53706, USA

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ABSTRACT

We analyzed the calcitic prismatic layers in *Atrina rigida* (*Ar*), *Haliotis iris* (*Hi*), *Haliotis laevigata* (*HL*), *Haliotis rufescens* (*Hrf*), *Mytilus californianus* (*Mc*), *Pinctada fucata* (*Pf*), *Pinctada margaritifera* (*Pm*) shells, and the aragonitic prismatic layer in the *Nautilus pompilius* (*Np*) shell. Dramatic structural differences were observed across species, with 100-μm wide single-crystalline prisms in *Hi*, *HL* and *Hrf*, 1-μm wide needle-shaped calcite prisms in *Mc*, 1-μm wide spherulitic aragonite prisms in *Np*, 20-μm wide single-crystalline calcite prisms in *Ar*, and 20-μm wide polycrystalline calcite prisms in *Pf* and *Pm*. The calcite prisms in *Pf* and *Pm* are subdivided into sub-prismatic domains of orientations, and within each of these domains the calcite crystal lattice tilts gradually over long distances, on the order of 100 μm, with an angle spread of crystal orientation of 10–20°. Furthermore, prisms in *Pf* and *Pm* are harder than in any other calcite prisms analyzed, their nanoparticles are smaller, and the angle spread is strongly correlated with hardness in all shells that form calcitic prismatic layers. One can hypothesize a causal relationship of these correlated parameters: greater angle spread may confer greater hardness and resistance to wear, thus providing *Pf* and *Pm* with a structural advantage in their environment. This is the first structure–property relationship thus far hypothesized in mollusk shell prisms.

1. Introduction

Mollusks are prolific, diverse, and sophisticated mineralizing organisms. They are widely distributed, and inhabit very different environments, thus they offer the possibility of correlating the shell structure with local environmental conditions (Lowenstam, 1954a,b; Olson and Gilbert, 2012; Olson et al., 2012) with the mechanical properties of the shells (Bruet et al., 2005; Kearney et al., 2006; Launey and Ritchie, 2009; Munch et al., 2008; Ritchie, 2011). The mollusks produce a huge variety of mineralized tissues that are presumably adapted to specific functions (Lowenstam and Weiner, 1989). Bøggild (1930) identified seven major types of shell structures, and these have been further sub-divided into 50 or so variants (Carter, 1980, 1990). The main structures are simple prismatic, composite prismatic, sheet nacre, columnar nacre, foliated, crossed-lamellar, and homogeneous structure (Taylor and Layman, 1972).

Both aragonite and calcite are found in mollusk shell structures, and, in different species, different structures are composed of one or both of these polymorphs (Lowenstam and Weiner, 1989; Mann, 2001). Here we study the prismatic layer of 8 nacre-forming mollusk shell species, all forming simple prismatic structures, made of calcite, except for *Nautilus*, in which the prismatic layer is made of aragonite spherulites.

The first discussion of mollusk prismatic layers appeared in 1844 (Carpenter, 1844). In all species, each prism is enveloped in an organic peri-prismatic sheath. Intra-prismatic proteins are also present (Aizenberg et al., 1994; Gotliv et al., 2005; Ndao et al., 2010; Politi et al., 2007). These organic peri- and intra-prismatic organic matrix molecules are formed first, and the minerals are assembled between or around the organic matrix. This matrix, therefore, must fulfill both a chemical and structural role, and is believed to mediate the mineral formation process. The precise mechanism of how organic molecules enact and control crystal nucleation, mineral polymorph selection, and crystallization kinetics is not known. However, recently the first complete mollusk genome was published (Zhang et al., 2012), and complete proteomes of different mollusk shell layers have been assembled (Marie

Abbreviations: Ar, *Atrina rigida*; *Hi*, *Haliotis iris*; *HL*, *Haliotis laevigata*; *Hrf*, *Haliotis rufescens*; *Mc*, *Mytilus californianus*; *Np*, *Nautilus pompilius*; *Pf*, *Pinctada fucata*; *Pm*, *Pinctada margaritifera*.

* Corresponding author at: Department of Chemistry, University of Wisconsin–Madison, 1101 University Avenue, Madison, WI 53706, USA. Mobile: +1 (608) 358 0164; fax: +1 (608) 265 2334.
E-mail address: pupa@physics.wisc.edu (P.U.P.A. Gilbert).

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et al., 2012). With the identification of the proteins associated with different structures of a mollusk shell, experimental determination of functions of specific proteins or protein complexes during shell formation will be feasible in the near future.

Furthermore, newly developed high-resolution methods have assisted the analyses of shell mineral structures and their orientations. These methods include Raman microscopy (Nehrke and Nouet, 2011), electron back scattered diffraction (Checa et al., 2009; MacDonald et al., 2010; Perez-Huerta et al., 2011), and polarization-dependent imaging contrast mapping (Gilbert, 2012; Gilbert et al., 2008, 2011; Killian et al., 2009, 2011; Ma et al., 2009; Metzler et al., 2007, 2008a). Some of these methods can also map minerals and organic components simultaneously (Gilbert et al., 2005; Metzler et al., 2010, 2008b; Nehrke and Nouet, 2011).

Suzuki and Uozumi (1981) observed optically that the surfaces of prisms, whether calcitic or aragonitic, are rather smooth and structureless. However, observation with an electron microscope reveals that the prisms are built up of very small calcium carbonate crystals (Suzuki and Uozumi, 1981), as was previously observed by Watabe and Wada (1956), Tsujii et al. (1958), Taylor and Layman (1972), Nakahara and Bevelander (1971).

The subdivision of molluscan calcite prisms into 50–100 nm nanoparticles is a well-established observation (Bruet et al., 2005; Dauphin, 2001, 2008; Li et al., 2004; Wolf et al., 2012). Many authors had assumed that organics separate these nanoparticles (Rousseau et al., 2005; Wolf et al., 2012), however, the Estroff group showed with electron tomography that organic molecules within the calcite prisms of *Atrina rigida* are instead concentrated in sparse, disk-like nanopatches that are not connected (Li et al., 2011).

For this work we selected 8 mollusk shell species to observe structural differences for the different prismatic layers and attempt to correlate the observations with mechanical properties. All of the selected shells are also nacre-forming, but this is not directly relevant to this work. Seven of them have calcite prisms (*Ar*, *Hi*, *HL*, *Hrf*, *Mc*, *Pf*, *Pm*) and one has aragonite spherulites (*Np*) in their prismatic layers. These shells are representative of 3 classes of shell-forming mollusks: 4 are bivalves (*Ar*, *Mc*, *Pf*, *Pm*), 3 are gastropods (*Hi*, *HL*, *Hrf*), and 1 is a cephalopod (*Np*).

The structure, microstructure and crystallography of calcite and aragonite prisms here were analyzed with various high-resolution methods, in order to compare quantitative results with hardness values. These experiments are designed to reveal structure–property relationships, which, thus far, have been the subject of few studies in mollusk prisms.

2. Materials and methods

2.1. Samples

We analyzed a total of 13 shells from 8 different molluscan species, described here. *Haliotis laevigata* (*HL*): The *HL* 1 specimen, 156 mm length, was collected in Western Australia and purchased from Australian Seashells PTY Ltd (Kingsley, Australia); the *HL* 2 specimen, 148 mm length, was provided by Prof. Monika Fritz and originally purchased from Australian Abalone Exports PTY Ltd (Victoria, Australia). *Haliotis iris* (*Hi*): The *Hi* specimen, 107 mm length, was collected in New Zealand and purchased from Australian Seashells PTY Ltd (Kingsley, Australia). *Haliotis rufescens* (*Hrf*): The *Hrf* specimen, 78 mm length, was farm-raised in Santa Cruz, CA and purchased from the Tokyo Fish Market in Berkeley, CA. *Mytilus californianus* (*Mc*): The *Mc* specimen, 148 mm length, was collected from the wild in Bolinas, CA. *Nautilus pompilius* (*Np*): The *Np* 1 specimen, 183 mm length, was collected offshore Siquijor Island, Philippines and purchased from Conchology, Inc., Philippines; the *Np* 2 specimen, 142 mm length, was collected offshore Jolo Island, Philippines and purchased from Conchology, Inc., Philippines. *Atrina rigida* (*Ar*): The *Ar* 1 specimen, 161 mm length, was collected from Belleair Beach, Florida and purchased from the collection of Robert Marchiselli; the *Ar* 2 specimen, 165 mm length, was collected at low tide on Sanibel Island, Florida. *Pinctada fucata* (*Pf*): The *Pf* 1 specimen, 58 mm length, was purchased from Hai de Ming Pearl Co. Ltd. Liusha Town, Zhanjang, China; the *Pf* 2 specimen, 58 mm length, was purchased from Hai de Ming Pearl Co. Ltd. Liusha Town, Zhanjang, China. *Pinctada margaritifera* (*Pm*): The *Pm* 1 specimen, 99 mm length, was farm-raised in the inner lagoon of the Rangiroa atoll, French Polynesia and purchased from the Gauguin Pearl Farm; the *Pm* 2 specimen, 90 mm length, was farm-raised in the inner lagoon of the Rangiroa atoll, French Polynesia and purchased from the Gauguin Pearl Farm.

With one exception, all samples were cut with a jeweler's saw, embedded in epoxy (EpoFix, Electron Microscopy Sciences, PA), and polished with decreasing size alumina grit down to 50 nm (MasterPrep, Buehler, IL). This exposed the shell cross-sections as imaged in the visible light micrographs.

The *Np* 2 sample was cut with a jeweler's saw, and tripod-polished at an angle of 2° using diamond grinding discs, so the sample was wedge-shaped with a final maximum thickness <100 μm. After tripod-polishing the thin wedge sample was mounted on a washer for analysis.

2.2. VLM with crossed polarizers

Visible light microscopy (VLM) images were obtained using a Zeiss Axio Imager.A1m microscope that works in reflected light, with a mounted Jenoptik ProgRes C12plus camera. The illumination channel is equipped with a fixed linear polarizer, whereas the analysis channel has a rotating linear polarizer, with quantitative and accurate angle positioning and measurement. Birefringent samples, such as calcite and aragonite in the mollusk shells imaged here, generate crystal-orientation-dependent contrast when illuminated with polarized light. The angle of the analysis polarizer was selected to maximize contrast in the VLM images and was always around 90°, hence all images are acquired with crossed-polarizers. Partly overlapping VLM images were stitched and blended in Adobe Photoshop using the Auto-Blend Layers tool, and the color levels were also enhanced for display.

2.3. Microdiffraction

Synchrotron Laue micro-X-ray diffraction experiments were performed on beamline 12.3.2, at the Advanced Light Source at Lawrence Berkeley National Laboratory in Berkeley, CA. The instrument uses Kirkpatrick-Baez mirror optics to focus the X-ray beam down to a size of about $1 \times 1\ \mu m^2$ in cross-section at the sample position. The samples were mounted on a precision XY stage and illuminated with white beam X-ray radiation ($5\ keV < E < 22\ keV$, pink beam). Various sample geometries were used, as described below. In all cases, X-ray microdiffraction patterns were obtained using a Pilatus 1 M X-ray detector. The area detector was at a distance of ~140 mm from the sample. The exact detector position and sample orientations were calibrated using the Laue diffraction pattern of a silicon crystal. With the following exceptions, diffraction maps were 500 μm in horizontal, along the nacre-prismatic-boundary, and 200 μm in vertical, with a 5 μm step size. The *Np* 2 map was 100×100 μm with a 1 μm step size, the *Hrf* map was 300×300 μm with a 5 μm step size, and the *Pm* 2 map was 200×200 μm with a 5 μm step size. The *Np* 2 map was taken in transmission geometry with a 90° incident angle, and detector angle of $2\theta = 60°$, the *Hrf* and *Pm* 2 maps were taken in reflection geometry with a 25° glancing incidence angle and detector angle