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Journal of Structural Biology 156 (2006) 355–362
Journal of Structural Biology
www.elsevier.com/locate/yjsbi

Microstructure and crystallographic-texture of giant barnacle (*Austromegabalanus psittacus*) shell

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Received 3 February 2006; received in revised form 26 April 2006; accepted 28 April 2006
Available online 7 May 2006

Abstract

Barnacle shell is a very complex and strong composite bioceramic composed of different structural units which consist of calcite 15 microcrystals of very uniform size. In the study reported herein, the microstructural organization of these units has been examinated in detail with optical and scanning electron microscopy, and X-ray diffraction techniques. These analyses showed that the external part of the shell has a massive microstructure consisting of randomly oriented crystals. Toward the interior, the shell became organized in mineral layers separated by thin organic sheets. Each of these mineral layers has a massive microstructure constituted by highly oriented calcite microcrystals with their *c*-axes aligned [(001) fibre texture] perpendicular to the organic sheets and the shell surface. Interestingly, in another structural unit, the shell shield, the orientation of the *c*-axis calcite crystals shifts from being perpendicular to being parallel to the shell surface across its thickness. This study provides evidence that the organic matrix is responsible for the organization of the shell mineral and exterts strong a strict control on the polymorphic type, size and orientation of shell-forming crystals.

Keywords: Biomineralization; Calcite; Organic matrix; Pole figures; XRD; X-ray diffraction; Crystallite size

1. Introduction

Austromegabalanus psittacus is a large (normally up to 30 cm high) sessile balanomorph barnacle from the coast of Chile and South Perú. Its mineralized shell is composed of calcite ($CaCO_3$) (Fernandez et al., 2001a,b; Arias, 2002). The shells consists of several structural components including twelve side plates, síx parietes, and síx radii which are cemented forming a truncated cone opened at the top (Fig. 1). The cone-shaped shell stands on a basal disk firmly cemented to the substratum to which the barnacle is attached. The conical aperture is partially obstructed by six oblique mineralized structures, referred as to the shell shield. Each of the components constituting the shell has different microstructural and crystallographic characteristics which will be described in detail in this study.

Shell mineral is deposited by mantle epithelial cells of the crustracean body which are in contact with the inner shell surface. The epithelial cells supply Ca^{2+} and HCO_3^- ions, which are necessary for the precipitation of calcium carbonate, and also supply specific organic components which regulate crystal growth. This is similar to the way mollusks mineralize their shel, although mollusks deposit their shell continuously (Nousek, 1984; Lowenstam and Weiner, 1989). In contrast, crustraceans grow by shedding their exoskeleton every molting cycle and depositing a new exoskeleton, which later mineralizes. Although they are crustraceans, barnacles only deminerlize and shed part

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doi:10.1016/j.jsb.2006.04.009

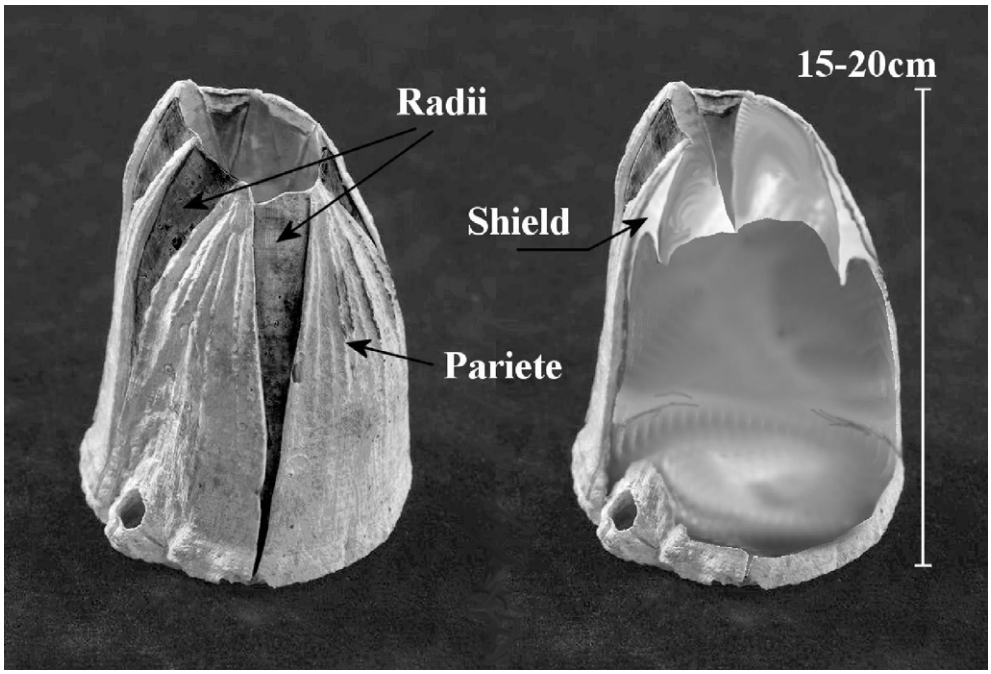

Fig. 1. Barnacle shell showing its different structural parts. (A) External shell (left), (B) Internal shell (right).

of the shell and build a quite stable and heavily mineralized wall made of a series of thick plates that completely surround the animal and are firmly cemented to the substratum. Thus, barnacle shell mineralization is quite different from other well studied systems (i.e., mollusk shell, eggshell). It is an interesting alternative model for studying biomineralization processes and deserves to be studied more intensively. Besides Darwin's pioneering studies (Darwin, 1854) and a few studies on barnacle's shell formation and structure (Bourget, 1977; Fernandez et al., 2001a,b), there is no detailed study of the organization of barnacle shell microstructure and the relationship between the organic matrix and the mineral. In this paper, we focus on the microstructure and crystallographic-texture of the barnacle shell to understand its formation and to determine which mechanisms control the development of its ordered microarchitectures. This study provides an insight into the influence of the organic matrix on shell mineral organization. The findings may also be applied to the fabrication of biomimetic materials, which are composed of highly oriented crystals of the same size and morphologies (Aksay et al., 1996). Also, this biomaterial could be of interest for biomedical applications as an alternative to nacre which has been successfully used in bone implants (Camprase et al., 1990; Atlan et al., 1997). In contrast to nacre, the barnacle shell contains calcite microcrystals (instead of aragonite microcrystals) and has considerable porosity (compared to nacre which is very dense material). The different composition and microstructural characteristics should affect dissolution behaviour in the body fluids (calcite being less soluble than aragonite) and porosity could favour bonding of this material with bone.

2. Materials and methods

2.1. Samples

Several shells from specimens of *Austromegabalanus psittacus* collected along in the coast of Chile were used for this study.

2.2. Optical and electron microscopy

The microstructure of different parts of barnacle shell was observed by optical (OM) and scanning electron microscopy (SEM). For transmitted-light microscopy, petrographic thin-sections (30 μm thick) of shell pieces cut along different orientations were prepared. SEM observation was carried out with both in fractured specimens and on the inner and outer of the different structural units of the shell. Samples were observed intact and after partially removing the organic material (with 5% sodium hypochlorite for 1–2 h at room temperature). Samples were coated with gold (Polaron E5000 sputtering) and observed using a variable-pressure SEM microscope (LEO 1430-VP, Germany).

2.3. Crystallographic-texture analysis

The three-dimensional orientation of the barnacle shells' crystals was determined using an X-ray single diffractometer equipped with an area detector (Bruker D8 SMART APEX, Germany). For diffraction experiments the working conditions were: Mo Kα, 50 KV and 30 mA, a pin-hole collimator of 0.5 mm in diameter and an exposure time of 20 s per frame. A set of frames (2-D diffraction patterns) was