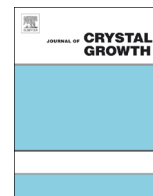




ELSEVIER

Contents lists available at ScienceDirect

Journal of Crystal Growth

journal homepage: www.elsevier.com/locate/jcrysgr

Microstructural control of calcite via incorporation of intracrystalline organic molecules in shells



Taiga Okumura^{a,*}, Michio Suzuki^b, Hiromichi Nagasawa^b, Toshihiro Kogure^a

^a Department of Earth and Planetary Science, Graduate School of Science, The University of Tokyo, 7-3-1 Hongo, Bunkyo-ku, Tokyo 113-0033, Japan

^b Department of Applied Biological Chemistry, Graduate School of Agricultural and Life Sciences, The University of Tokyo, 1-1-1 Yayoi, Bunkyo-ku, Tokyo 113-8657, Japan

ARTICLE INFO

Article history:

Received 12 April 2013

Received in revised form

25 June 2013

Accepted 17 July 2013

Communicated by: Dr. S. Veessler

Available online 25 July 2013

Keywords:

A1. Crystal structure

A1. Defects

A2. Growth from solutions

B1. Biological macromolecules

B1. Calcium compounds

B1. Minerals

ABSTRACT

It is widely accepted that organic substances regulate or influence the structure of biominerals, but its direct evidences are not plenty. Here we show that the crystallographic microstructures in biotic calcites arise from incorporated intracrystalline organic molecules (IOMs), through a comparison between biotic calcites in shells and synthetic ones with the IOMs extracted from the shells. Although the prismatic layers of a pearl oyster (*Pinctada fucata*) and a pen shell (*Atrina pectinata*) morphologically resemble each other, the crystallographic features of constituent calcites are considerably different; in *Pinctada*, the IOMs are distributed inhomogeneously to form small-angle grain boundaries and associated crystal defects, whereas in *Atrina*, the IOMs are distributed almost homogeneously and defects are rare in the calcite crystals. We conducted in vitro calcite syntheses in the presence of the IOMs in EDTA-soluble extracts from the prisms. The IOMs in the extracts from *Pinctada* and *Atrina* were incorporated into synthetic calcites in a different manner, exhibiting defect-rich/free features as observed in the natural shells. With regard to amino acid compositions of the IOMs, the extract from *Atrina* has a higher proportion of acidic amino acids than that from *Pinctada*, implying that acidic proteins do not correlate directly to their affinity for calcium carbonate crystals.

© 2013 Elsevier B.V. All rights reserved.

1. Introduction

Biominerals often possess elaborate and highly-regulated structure, and exhibit superior properties to abiotic counterparts [1,2]. For instance, single-crystalline geological calcites break easily along their perfect {104} cleavage planes. On the contrary, biotic calcites are much more resistant to fracturing because the {104} cleavages generally do not develop vastly [3–6]. Such superiorities are ascribed to the existence of organic components between and/or inside the crystals. Therefore biominerals as organic–inorganic composites are attracting a lot of attention in various research fields including recent nano-science and technologies [7–10].

Organic components in biominerals often exist as *intercrystalline* organic matrices, which partition and provide the environment for crystal growth, serve the nucleation sites of the crystals and constrain the crystal shapes [1]. On the other hand, some researchers are paying attention to *intracrystalline* organic molecules (abbreviated as IOMs in this study) which are incorporated *inside* inorganic crystals [11], because they are also considered to influence the structure and properties of the host crystals [2,12–15]. Although the amount of

IOMs is only a few percents of entire weight of biominerals at most [16], they can be visualized by using recent electron microscopy [6,17–21] or pulsed-laser atom-probe tomography [22]. These techniques enable us to determine the locations of IOMs in the host crystals, and to discuss their relationships with the microstructures of the crystals at nanoscale. For example, high-resolution tomography using scanning transmission electron microscopy (STEM) images gave the three-dimensional distribution and shapes of IOMs inside the calcite prisms of a mollusk shell (*Atrina rigida*), and revealed that the IOMs are aligned roughly perpendicular to the *c*-axis of calcites [23].

Meanwhile, the syntheses of calcium carbonate crystals in the presence of various organic molecules have been conducted to investigate the influence of the molecules on crystal growth [24–30]. These experiments reported that specific organic molecules can modify crystal morphologies probably owing to selective adsorption of the molecules onto specific crystallographic planes or sites.

In this article, we examined the microstructure of biotic calcites and synthetic ones with IOMs extracted from the biotic calcites, in order to clarify the influence of IOMs on the crystal structure. As biotic calcites, we adopted those constituting the prismatic layers of two bivalve shells, *P. fucata* and *A. pectinata*, because their crystallographic features are considerably different in spite of their morphological similarity. Each prism of *Pinctada* is composed of several smaller domains of a few micrometers, and shows a

* Corresponding author. Tel./fax: +81 3 5841 4019.

E-mail address: okumura@eps.s.u-tokyo.ac.jp (T. Okumura).

polycrystalline feature [18,31,32], whereas the prisms of *Atrina* have a monocrystalline feature [31]. At a smaller scale, the prisms of *Pinctada* have sub-grains of several hundred nanometers which are separated by small-angle grain boundaries caused by IOMs, whereas those of *Atrina* are monolithic single crystals without crystal defects and lattice strain [19]. Such structural differences have been reproduced to some extent in our synthetic experiments, demonstrating that certain IOMs themselves can induce specific crystallographic microstructure in biotic calcites.

2. Materials and methods

2.1. Materials

The fresh shells of *Pinctada* were supplied by the Fisheries Research Institute, Mie Prefecture, Japan, and those of *Atrina* were obtained at the Tsukiji Market, Tokyo, Japan. After their soft inner parts were removed, the shells were dried; then the prismatic layers were mechanically separated from the nacreous layers.

2.2. Synthetic experiments

2.2.1. Preparation of SAMs

For mimicking the processes of biomineralization, template-mediated crystallization is frequently utilized because many biominerals are formed on the templates of insoluble organic matrices via inhomogeneous nucleation. Among various artificial templates, self-assembled monolayers (SAMs) were often used recently [33–37]. The templates such as SAMs offer more-regulated crystal growth and serve to understand the influence of soluble organic molecules on crystal growth. In the present study, the SAMs of a thiol with a carboxyl group (11-mercaptoundecanoic acid, HS(CH₂)₁₀CO₂H, Aldrich) were used as template layers. Gold-coated coverslips (Phasis), a {111}-oriented Au thin film of 50 nm thick/a Ti thin film of 5 nm thick to promote adhesion/borosilicate glass, were used as substrates for the SAMs. Just before immersion in a solution of the thiol to form the SAMs, the substrates were cleaned with a strongly oxidizing chemical, a piranha solution (H₂SO₄:30% H₂O₂=7:3), for 15 min. The SAMs were formed on the substrates by immersing them in a 5 mM solution of the thiol in ethanol for 1 week. The atmosphere over the solution was replaced with inert argon gas during the immersion. The resulting surfaces were rinsed with ethanol and dried under an argon gas flow.

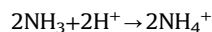
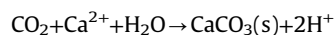
2.2.2. Extraction of IOMs

The IOMs, which were used as organic additives in the synthetic experiments, were extracted from the prisms of the shells. The prismatic layers were preliminarily bleached by a sodium hypochlorite (NaClO) solution, until their inter-prismatic organic walls were completely dissolved and individual prisms separated from one another. The separated prisms (0.5 g) were decalcified and dissolved by a 0.5 M ethylenediaminetetraacetic acid (EDTA) and a 0.01 wt% sodium azide solution (70 mL) at 4 °C for 1 week. Then the EDTA-soluble fractions were concentrated by ultrafiltration (Amicon Ultra centrifugal filter unit with a molecular weight cutoff 30,000 Da, Millipore), and desalted by a 10 mM Tris–HCl (pH 8.0) buffer. Finally, remained insoluble materials were filtered out using a 0.45 μm cutoff filter. The concentration of the IOMs extracts was measured using a NanoDrop 1000 spectrophotometer (Thermo Fisher Scientific).

2.2.3. Synthetic procedures

The substrates with the SAMs were placed upside-down in 2 mL of a 10 mM calcium chloride (CaCl₂) solution with 10 μg/mL

IOMs extracts. The initial pH of the CaCl₂ solution was adjusted to approximately 8.7 with a 10 mM sodium hydroxide (NaOH) before mixing. Instead of the IOMs extracts, a 10 μg/mL sodium polyacrylate (PANa, Mw~2100, Aldrich) solution whose pH was also adjusted to 8.7 with the NaOH was added for control experiments. The vials containing the solutions were placed in a closed desiccator with another vial containing 1 g of crushed ammonium carbonate [(NH₄)₂CO₃]. The precipitation of calcium carbonate crystals was achieved by the diffusion of carbon dioxide (CO₂) vapor into the solution, with the following reactions:



Thus (NH₄)₂CO₃ not only provides CO₂ to the solution, but also prevents pH change of the solution by the formation of ammonium ions. The crystal growth was carried out at room temperature for 1 day. After the reaction, the substrates were recovered from the solution and gently rinsed with ultrapure water, and dried under an argon gas flow. Additionally, the calcium carbonate crystals precipitated in the solution were also collected, washed three times with ultrapure water and twice with ethanol, air-dried, and analyzed using X-ray diffraction (XRD).

2.3. Analytical methods

2.3.1. SEM observation

Scanning electron microscopy (SEM) observations were carried out using an S-4500 SEM (Hitachi) with a cold field-emission gun at an acceleration voltage of 5 kV. SEM specimens were coated with Pt–Pd before observations.

2.3.2. TEM observation

The specimens for transmission electron microscopy (TEM) examination were prepared using a FB-2100 focused ion beam (FIB) system with a micro-sampling system (Hitachi). The specimens were locally coated by the deposition of tungsten to prevent beam damage during fabrication, and trimmed using a gallium ion beam of 30 kV, and then thinned down to be electron-transparent with a low energy beam of 10 kV as a final process. TEM was conducted using a JEM-2010UHR TEM (JEOL) operated at 200 kV, to observe the crystallographic features of calcite and the distribution of IOMs inside the crystals, which were imaged by the Fresnel contrasts.

2.3.3. STEM–EELS analysis

Electron energy-loss spectroscopy (EELS) analysis was conducted using an Enfina spectrometer (Gatan) equipped to a JEOL JEM-2010F TEM (JEOL) with a field-emission gun operated at 200 kV, in order to verify that Fresnel contrasts inside the crystals in under-focused TEM images correspond to IOMs. Specimens were prepared using FIB in the same manner as those for TEM observations, and cleaned using a JIC-410 ion cleaner (JEOL) just before the analyses to avoid contaminations. Since each Fresnel contrast was very small, EELS spectra were obtained from nanoscopic areas (< 10 nm) by converging an electron beam in a STEM mode. Each spectrum was collected within 5 s to avoid radiation damage, and approximately 30 spectra were collected and integrated with attention to an energy shift.

2.3.4. XRD analysis

As complementary and macroscopic characterization of the crystallographic aspect, XRD analysis was also conducted. Powder XRD patterns were obtained using a RINT-Ultima⁺ diffractometer

Download English Version:

<https://daneshyari.com/en/article/8152190>

Download Persian Version:

<https://daneshyari.com/article/8152190>

[Daneshyari.com](https://daneshyari.com)