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Hierarchically structured scleractinian coral biocrystals

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Abstract

Microscopic (AFM and FESEM) observations show that scleractinian coral biomineral fibers in extant *Desmophyllum* and *Favia*, and fossil Jurassic *Isastrea* are composed of nanocrystalline grains of about 30–100 nm in size. In contrast to these findings, SR diffraction data on the same coral materials exhibit narrow Bragg peaks suggesting much larger crystallite size. These seemingly contradicting results of microscopic and diffraction studies are reconciled within a new, minute-scale model of scleractinian biomineral fibers. In this model, nanocrystalline aragonite units are interconnected by mineral bridges and form aggregates usually larger than 200 nm. Most likely, the size of the aggregates is resulting from physiological biomineralization cycles that control cellular secretion of ions and biopolymeric species. Intercalation of biopolymers into crystal lattice may influence consistently several structural parameters of the scleractinian coral bio-aragonite in all studied samples: (i) the lattice parameters and internal strains of the bio-aragonite are larger than in mineral aragonite, (ii) lattice parameter elongations and internal strains reveal directional anisotropy with respect to crystallographic axes.

Keywords: Biominerals; Scleractinian corals; *Desmophyllum*; *Favia*; *Isastrea*; $CaCO_3$; Aragonite; Synchrotron radiation diffraction; X-ray diffraction; SEM; AFM

1. Introduction

Reef-building scleractinian corals belong to the most extensive natural producers of $CaCO_3$ (Milliman, 1993). Modern scleractinian corals produce carbonate skeleton whose principal polymorph is aragonite (see also Stolarski et al., 2007a). Until recently it has been commonly assumed that biological control of skeleton formation is confined to the initiation of its growth (in the regions called calcification centers) whereas the successive growth of biomineral fibers follows strictly physico-chemical rules of abiotic crystal growth resembling the formation of $CaCO_3$ marine cements (Bryan and Hill, 1942; Constantz, 1986). It was a "single orthorhombic crystal of aragonite" that was considered fundamental unit of coral skeleton (Bryan and Hill, 1942: 84). This purely inorganic model of fibers growth is particularly persuasive when the optical behavior (extinction every 90° as the specimen is rotated) of fibers in the polarized light is observed (Wainwright, 1964).

However, the early claims as to the monocrystalline aragonite ($CaCO_3$) structure of fibers have been challenged by the results of X-ray micro-diffraction that documented their polycrystalline nature. Wainwright (1964) observed the orientation of the aragonite crystallites along the crystallographic *c* axis, the *a* and *b* directions being randomly distributed. The interpretation of fibers as purely inorganic polycrystals has been also challenged by nanostructural atomic force microscopy (AFM) observations which point to their nanocomposite structure. Mineral nanograins, ca. 30–100 nm in diameter (Stolarski, 2003; Cuif et al., 2004; Cuif and Dauphin, 2005a,b; Stolarski and Mazur, 2005;

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Dauphin et al., 2006), are embedded in a "thin layer of a highly interactive (i.e., organic) material" (Cuif and Dauphin, 2005b). Even deeper penetration of the mineral structure by the organic components is suggested by high-resolution synchrotron radiation (SR) diffraction studies. Bio-aragonite extracted from corals (Stolarski et al., 2007b) reveal anisotropic elongation of the lattice parameters *a*/*b*/*c* similar to that previously reported for biogenic aragonite extracted from bivalve, gastropod and cephalopod shells (Pokroy et al., 2004, 2006). Also chemical and isotopic composition of scleractinian skeletons is under strong biological control (Rollion-Bard et al., 2003; Allison et al., 2005; Meibom et al., 2006, 2007). These new evidences support the view that in contrast to synthetic, abiotically precipitated $CaCO_3$, structure of scleractinian coral bio-aragonite is, from the macroscopic down to the atomic length scales, influenced by organic macromolecules present in biomineralization region. Similar biological control on hierarchical structure of biominerals has been described in variety of organisms (Aizenberg et al., 2005; Fantner et al., 2005; Tai et al., 2006; Oaki et al., 2006).

One of the basic questions raised by nanostructural observations concerns the mechanisms that arrange nanocrystals into crystallographically continuous units ("skeletal fibers"). Up to now, the nanoscale morphology of scleractinian coral biocrystals has been investigated only over relatively small areas (ca. 2 μm^2), and the larger scale, hierarchical arrangement of the nanograins (i.e., their organization in higher level structural units) has not been accurately described and/or still needs to be verified by preparation-free methods (Dauphin et al., 2006). In this paper, we present for the first time the quantitative analysis of the mineral nanocomponents based on high-resolution synchrotron radiation diffraction studies. We also propose a model of the minute-scale hierarchical structure of the scleractinian coral skeleton that is based on combined SR diffraction data and nanotopographic field emission scanning electron microscopy (FESEM) and AFM observations performed over micrometer range scales.

2. Material and methods

2.1. Samples

Biomineral samples were extracted from the skeletons of three scleractinian coral species: (1) *Favia stelligera* (Dana, 1846), extant colonial, shallow-water, and zooxanthellate coral (collection site: Lizard Island, Great Barrier Reef, Pacific Ocean, 5–10 m; ZPAL V.31/1), (2) *Desmophyllum dianthus* (Esper, 1794), extant solitary, deep-water, azooxanthellate coral (collection site: off Chile, station: 51052, Pacific Ocean, 51°52,0′S/73°41,0′W; 636 m; ZPAL H.25/5-Car), and unusually well preserved (3) *Isastrea* cf. *bernensis* Etallon, 1864, fossil, colonial, presumably shallow-water coral from the Oxfordian (Upper Jurassic) deposits of western Pomerania that still preserves aragonite skeleton mineralogy (collection site: Ostromice, Poland, see Roniewicz, 1984; ZPAL H.IV/303). In the text, for brevity, only generic names of species are mentioned (i.e., *Favia*, *Desmophyllum* and *Isastrea*). The reference Geological Aragonite (ZPAL V.31/10/VMIN08) came from Tazouta Mine in the Atlas Mountians, Morocco (Sefrou, Sefrou Prefecture, Fès-Boulemane Region). All samples are housed in the Institute of Paleobiology, Polish Academy of Sciences, Warsaw (abbreviation ZPAL).

2.2. Microscopic techniques

Atomic Force Microscopy was performed on MultiMode Nanoscope IIIa (Digital Instruments, Veeco). Standard silicone nitride cantilevers were used for measurements in contact mode. The coral samples extracted *en bloc* from the skeleton were polished with diamond suspension of grain sizes 5 and 1 μm, and then with aluminium oxide (Buehler Topol 3 final polishing suspension with particle size 0.25 μm). After polishing, the sections were rinsed in Milli-Q water and washed in an ultrasonic cleaner for 10 s. The polished samples were then etched in 1% ammonium persulfate in McIlvain buffer (pH 8) for 10 min. Next, they were rinsed with deionized water and dried.

Scanning Electron Microscopy was performed on Philips XL 20 SEM or Field Emission SEM LEO1530. For standard SEM measurements polished and etched blocks of corals skeleton were used. The preparation procedure involved polishing the samples with diamond powder 1200 Grit and aluminium oxide (Buehler Topol) followed by etching for 10 s in 0.1% formic acid. High resolution FESEM imaging was performed on fractured native septa extracted from coral skeletons.

Laser Confocal Scanning Fluorescence Microscope observations of *Desmophyllum* section (Fig. 6) were acquired on a Leica TCS SP1 Confocal Microscope at the Natural History Museum, London. Polished section was stained in a 0.45 μm filtered 1% acridine orange aqueous solution for 5 min, then briefly rinsed in distilled water and air dried. Argon laser operating at 488 nm was used to excite fluorescence which was detected at 500–600 nm.

2.3. Synchrotron radiation high resolution powder diffraction

Measurements were performed at the beamline ID31 at (Fitch, 2004) ESRF Grenoble. Samples of the extant *Desmophyllum* and *Favia*, were extracted *en bloc* from the skeleton as approximately parallelepiped pieces ($4 \times 4 \times 1$ mm) and mounted directly in transmission mode in the SR beam. The samples consisted of septa/wall parts whose microstructural units (fibers) have different orientation. The coral sample was immobile during the measurements. SR measurements were also performed for pulverized biogenic fossil *Isastrea* and pulverized reference Geological Aragonite. Both pulverized samples were sealed in borosilicate capillaries 0.7 mm and 1.0 mm in diameters, respectively. The capillaries were rotated during the