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Control of aragonite deposition in colonial corals by intra-skeletal macromolecules

Giuseppe Falini [a,*], Michela Reggi [a], Simona Fermani [a], Francesca Sparla [b], Stefano Goffredo [c], Zvy Dubinsky [d], Oren Levi [d], Yannicke Dauphin [e,*], Jean-Pierre Cuif [e]

[a] Dipartimento di Chimica 'G. Ciamician', via Selmi 2, Alma Mater Studiorum, Università di Bologna, 340126 Bologna, Italy
[b] Dipartimento di Farmacia e Biotecnologie, via Irnerio 42, Alma Mater Studiorum, Università di Bologna, 340126 Bologna, Italy
[c] Dipartimento di Scienze Biologiche, Geologiche e Ambientali, via Selmi 3, Alma Mater Studiorum, Università di Bologna, 340126 Bologna, Italy
[d] The Mina and Everard Goodman Faculty of Life Sciences, Bar Ilan University, Ramat Gan, Israel
[e] Université Paris-Sud, Orsay, Bat. 504, UMR IDES, F-91405 Orsay, France

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ABSTRACT

Scleractinian coral skeletons are composed mainly of aragonite in which a small percentage of organic matrix (OM) molecules is entrapped. It is well known that in corals the mineral deposition occurs in a biological confined nucleation site, but it is still unclear to what extent the calcification is controlled by OM molecules. Hence, the shape, size and organization of skeletal crystals from the fiber level through the colony architecture, were also attributed to factors as diverse as nucleation site mineral supersaturation and environmental factors in the habitat. In this work the OMs were extracted from the skeleton of three colonial corals, *Acropora digitifera*, *Lophelia pertusa* and *Montipora caliculata*. *A. digitifera* has a higher calcification rate than the other two species. OM molecules were characterized and their $CaCO_3$ mineralization activity was evaluated by experiments of overgrowth on coral skeletons and of precipitation from solutions containing OM soluble and insoluble fractions and magnesium ions. The precipitates were characterized by spectroscopic and microscopic techniques. The results showed that the OM molecules of the three coral share similar features, but differ from those associated with mollusk shells. However, *A. digitifera* OM shows peculiarities from those from *L. pertusa* and *M. caliculata*. The $CaCO_3$ overgrowth and precipitation experiments confirm the singularity of *A. digitifera* OM molecules as mineralizers. Moreover, their comparison indicates that only specific molecules are involved in the polymorphism control and suggests that when the whole extracted materials are used the OM's main effect is on the control of particles' shape and morphology.

1. Introduction

Scleractinia corals possess several records in terms of their mineralization activity. They represent the biggest source of biogenic calcium carbonate (Spalding et al., 2001; Cohen and McConnaughey, 2003) and they are among the fastest marine mineralizing organisms (Marshall and Clode, 2004). Despite their great relevance in biomineralization, and the plethora of articles published in this field (Tambutté et al., 2011 and references therein), many aspects of their mechanism of mineralization are still a source of discussions and controversies. The main issue is the relative level of biological and environmental control over calcification.

It has long been recognized that coral skeletons comprise both inorganic (aragonite) and organic components (e.g. Wilfert and Peters, 1969; Young, 1971), but the main issue is the relative level of biological and environmental control over calcification. In geochemical research area, opinions inspired by the spherulitic crystallization model (Bryan and Hill, 1941) are still prevalent. If presence of an organic component has become accepted, its active role in the biomineralization process is still considered doubtful (Holcomb et al., 2009). Among biologists, organic components entrapped within coral skeletons are usually referred to as "organic matrices (OM)" and owing to an unusual amino-acid composition (high concentrations of acidic amino acids) have been suggested to play an important role in the coral mineralization process (Mitterer, 1978; Constantz and Weiner, 1988; Goffredo et al., 2011). Morphology of coral colonies are dramatically affected by habitat conditions (Jokiel, 1978), nevertheless the changes always remain within the species-specific "vocabulary" controlled by the taxon's DNA (Cuif et al., 2003a).

* Corresponding authors. Fax: +33 (0)1 69 15 61 23 (Y. Dauphin), +39 051 2099456 (G. Falini).
E-mail addresses: giuseppe.falini@unibo.it (G. Falini), yannicke.dauphin@u-psud.fr (Y. Dauphin).

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Regarding the fine scale features of coral skeletons, structural analysis has long been carried out by using optical microscope with polarized light. This led to valorization of the "sclerodermite" as the microstructural unit of the skeletons: a group of radiating fibers with crystal-like appearance (Ogilvie, 1896; Wells, 1956). However, investigations conducted by scanning electron microscopy, microprobe analysis and synchrotron-based mapping (Cuif and Dauphin, 1998) have clearly established that the actual building unit of the skeleton is a few micrometer thick, mineralizing growth layer, synchronically produced for a given septa. This repeatedly produced unit comprises two distinct areas with specific modes of mineralization (Fig. 1). At the growing edge of the septa the mineral phase is in the form of tiny randomly oriented crystals, deposited at the top of the septal spines or growing edge with species-specific geometry, chemical and isotopic signatures. These distal structures, producing the septal framework, are then covered by the successive mineral layers which progressively create the fibers (i.e. constituting the major part of the corallite structure).

The biochemical composition of the organic compounds associated with the mineral units are essentially known from numerous analyses carried out on the organic components extracted from coral skeletons after decalcification and purification processes. From Young (1971) to Constantz and Weiner (1988) proteins and glycosaminoglycans but also sulfated polysaccharides (Dauphin, 2001; Cuif et al., 2003b) have been consistently found in the skeletons of the numerous species covering the whole taxonomic diversity of the phylum. Lipids are also present in the OM (Farre et al., 2010).

However, the mode of action of these organic compounds is still debatable. Initial models favored the "template mechanism" in which organic layers were acting as a scaffold for Ca-carbonate oriented crystallization. In contrast to models involving a liquid layer with chemical properties close to sea-water (McConnaughey and Whelan, 1997; Adkins et al., 2003) as the place where crystallization occurs, Clode and Marshall (2002) have established the existence of direct contact between cells and skeleton. Calcification occurs within a gel secreted, or a high viscous sol, at the interface between the calicoblastic cells and the skeleton. In situ XANES mapping of organic components (proteins and sulfated polysaccharides) have shown that organic and mineral components were not distributed among alternating layers, but associated within each growth layer at a submicrometer scale (Cuif et al., 2003b).

Molecular models suggest formation of a glycoproteic architectural framework bearing sites for development of a crystalline structure (Addadi et al., 2006). More recently attention was drawn to the potential role of OMs as carriers of the mineral component, possibly in amorphous status, crystallization itself trapping the organic phases along the boundaries of the grains (Cuif et al., 2008; Weiner and Addadi, 2011; Motai et al., 2012). This model is supported by $CaCO_3$ crystallization experiments in gel and partial gels (Asenath-Smith et al., 2012).

The real role of the OM in the dynamics of skeleton formation of corals is enigmatic and is related to the information on its properties once extracted from the skeleton. However, bearing in mind these limits, but supported by many analogous studies on mollusk shells, it has been recently demonstrated, by *in vitro* studies, that the OM from a coral species (*Balanophyllia europaea*) influences the polymorphism and morphology of $CaCO_3$ (Goffredo et al., 2011).

Here, we extended this study to different species of colonial corals, *Montipora caliculata*, *Acropora digitifera* (French Polynesia) and *Lophelia pertusa* (Atlantic Ocean), living in different habitats and having diverse calcification rates. Respectively, first and second largest coral genus in the present-day seas with respect to taxonomical diversity, *Acropora* and *Montipora* belong to the most successful and most studied coral family *Acroporidae*. According to Veron (2000), *Acroporidae* and *Caryophyllidae* (the Family to which the deep-sea coral *Lophelia* belongs) are distributed into two major supra-ordinal lineages, possibly distinct since the Triassic times. The aim of the present study is to determine whether the OM extracted from different corals shows a different capability to interact with $CaCO_3$ and if this is related with the coral species.

2. Results

2.1. Skeletal structures in corals

Scanning electron microscope (SEM) images of the studied species showed the common structural pattern of the coral skeleton (Fig. 1): the early mineralization zone (EMZ) was composed of small rounded granules. EMZs were more sensitive to etching than the fibers (Cuif and Dauphin, 2005). Then, fibers were produced with a rhythmic growth. Growth layers are about 2–3 μm thick. However, the size and arrangement of EMZ and fibers depend upon the species (Fig. 1a, b, f, j). The inner structure of EMZ and fibers becomes visible using atomic force microscopy. Both EMZ and fibers are composed of rounded small granules (Fig. 1d, g–i, k, l). These granules are surrounded by a cortex, the exact nature of which remains still unknown. Nevertheless, from phase image contrast, it can be said that the cortex is probably a mixture of organic and amorphous components. The inner part of the granules is also heterogeneous, as shown by the changes in colors in phase images.

2.2. Studies on the intra-skeletal organic matrix

In *M. caliculata*, *A. digitifera* and *L. pertusa* the OM was embedded in a skeleton of pure aragonite, as shown by the X-ray powder diffraction patterns (Fig. 1SI). A first quantification of the OM content in the skeleton was carried out by thermogravimetric analysis (TGA). The skeletons' thermograms showed a first weight loss in a range around 150–220 °C followed by one between about 280 and 450 °C (Fig. 2SI). The total weight loss (water + OM; see Cuif et al., 2004) was of 3.4 ± 0.1 (0.9 ± 0.1 + 2.5 ± 0.1), 2.7 ± 0.1 (1.0 ± 0.1 + 1.7 ± 0.1) and 3.9 ± 0.1 (1.3 ± 0.1 + 2.6 ± 0.1) % (*w*/*w*) in *M. caliculata*, *A. digitifera* and *L. pertusa*, respectively. The OM concentration was also determined after acetic acid extraction from the skeleton. This material, formed by the soluble (SOM) and insoluble (IOM) fractions of OM, was about 0.3, 0.2 and 0.1% (*w*/*w*) in *M. caliculata*, *A. digitifera* and *L. pertusa*, respectively. The quantification of the relative amount of the OM fractions, SOM and IOM, was a difficult task, since a significant variability was observed from one extraction to another. However, as the result of six extractions the mass ratio between SOM and IOM was roughly estimated to be 1.4 in *M. caliculata*, 5.0 in *A. digitifera*, and 1.5 in *L. pertusa*.

The chemical–physical characterization of the OM fractions was performed by Fourier transform infrared spectroscopy (FTIR), polyacrylamide gel electrophoresis (SDS–PAGE) and amino-acidic analyses (AAA). Table 1 summarizes the observations from the FTIR spectra of SOM and IOM obtained from six extraction processes from different coral skeletons of each species. In Fig. 2 the most representative spectra are shown. In general SOM and IOM showed the same absorption bands, regardless of the coral species; however, differences were observed in their relative intensities, and to a minor extent in their maxima. Two marked bands at about 2920 cm^{-1} and 2851 cm^{-1} and a weak one at 1737/35 cm^{-1} were observed in all fractions. In the SOMs the amide I bands were centered around 1654 cm^{-1}, this band partially shifted towards lower wavenumbers, around 1636 cm^{-1}, in the IOMs. The amide II band, at around 1542 cm^{-1}, was weak with respect to the amide I band, in both SOM and IOM, particularly in *M. caliculata*. The band at

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