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Cidaroids spines facing ocean acidification

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ABSTRACT

When facing seawater undersaturated towards calcium carbonates, spines of classical sea urchins (euechinoids) show traces of corrosion although they are covered by an epidermis. Cidaroids (a sister clade of euechinoids) are provided with mature spines devoid of epidermis, which makes them, at first sight, more sensitive to dissolution when facing undersaturated seawater. A recent study showed that spines of a tropical cidaroid are resistant to dissolution due to the high density and the low magnesium concentration of the peculiar external spine layer, the cortex. The biofilm and epibionts covering the spines was also suggested to take part in the spine protection. Here, we investigate the protective role of these factors in different cidaroid species from a broad range of latitude, temperature and depth. The high density of the cortical layer and the cover of biofilm and epibionts were confirmed as key protection against dissolution. The low magnesium concentration of cidaroid spines compared to that of euechinoid ones makes them less soluble in general.

1. Introduction

Anthropogenic atmospheric CO_2 dissolves in the ocean and modifies the chemistry of seawater. The main effects are a decrease of the pH and calcium carbonate saturation state, the whole phenomenon being called ocean acidification (OA). These changes might jeopardize organisms building a calcium carbonate skeleton in two ways: (1) by increasing the energetic cost of eliminating protons from the calcification site, thereby reducing growth rate and (2) by inducing the dissolution of the skeletal structures in contact with seawater undersaturated with respect to calcium carbonate. Dissolution depends on the presence of protecting organic layers and on the solubility of the considered calcium carbonate polymorph, aragonite and high-magnesium calcites being the most soluble among crystalline forms. The solubility of magnesium calcite varies according to the amount of magnesium ions substituted to calcium in the calcite lattice. The relationship between solubility of magnesium calcites and magnesium concentrations is debated (Morse and Mackenzie, 1990; Morse et al., 2006; Andersson et al., 2008). Recent developments favored a view that low-magnesium calcites (up to 4 mole % $MgCO_3$) have a solubility equal or close to that of pure calcite while high-magnesium calcites (from 6 mole % $MgCO_3$) have a solubility equal or higher to that of aragonite (Fig. S1 in Lebrato et al., 2016). Furthermore, other factors, as crystal size or other structural characteristics, also influence the solubility of calcite resulting sometimes in large effects in solubility between abiotic and biogenic magnesium calcites (Morse et al., 2006).

Sea urchins build an extensive high-magnesium calcite skeleton and have been considered at risk due to OA. However, recent results question this paradigm, at least in adults (Calosi et al., 2013; Dubois, 2014; Hazan et al., 2014; Uthicke et al., 2014;Collard et al. 2015, 2016; Moulin et al., 2015; Dery et al., 2017). Indeed, the main component of the skeleton (the test encasing the urchin body) appeared as protected from both undersaturated seawater and extracellular fluids (see Dery et al., 2017 and references therein). On the contrary, spines showed evidence of dissolution and reduced fracture force in the same condition, despite the fact that they are separated from seawater by an epidermis (Dery et al., 2017 and references therein). Considering these results, one would expect that spines of cidaroids (the sister clade to all other echinoids, the latter being called euechinoids) which have mature primary spines devoid of epidermis and whose skeleton is in direct contact with seawater (Märkel and Röser, 1983) would even more suffer from undersaturation. These primary spines are composed of three concentric layers: a central zone, the medulla, a median layer and the peripheral cortex. The two central layers are composed of monocrystalline stereom, like in all sea urchins, while the cortex is polycrystalline. Their initial development takes place under an epidermis but the latter disappears after the formation of the cortex. This cortex may be then colonized by a biofilm and epibionts (Märkel and Röser, 1983; Dery et al., 2014) among them many are calcified. When submitted to undersaturated seawater for 3 weeks, the cortex of the cidaroid *Prionocidaris baculosa* spines showed only few traces of corrosion while the central and median stereom layers were completely corroded

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by the same treatment (Dery et al., 2014, *P. baculosa* wrongly determined as *Phyllacanthus imperialis*). This resistance was attributed to the significantly lower porosity and magnesium concentration of the cortex compared to the central and median layer and possibly to the coverage of biofilm and epibionts avoiding a direct contact of the cortex with sea water (Dery et al., 2014). However, the latter study was conducted on a single shallow tropical species while cidaroids are present in all oceans and depths with some populations or species living below the saturation horizon of their skeletal mineral (Sewell and Hofmann, 2010; Lebrato et al., 2016). Much too few other data is available to determine if these properties are general adaptative or preadaptive features of this clade or particular to *P. baculosa*. Slightly lower magnesium concentration in the cortex was reported for the Antarctic species *Ctenocidaris speciosa* and the temperate *Stylocidaris affinis* but those differences were not statistically tested (Märkel et al., 1971; Catarino et al., 2013). Interestingly, Catarino et al. (2013) reported significantly lower magnesium concentration in the cortex of spines from *C. speciosa* collected below the saturation horizon for aragonite (used as a proxy of the saturation horizon for high-magnesium calcite). All other studies reporting Mg concentration in cidaroid spines did not differentiate between cortex and central and median layers (reviewed in Smith et al., 2016 and Lebrato et al., 2016). Epibionts growing on cidaroid spines are generally viewed as parasitic, at least when considered collectively (David et al., 2009) but their real impact has never been addressed beyond bioerosion. Therefore, the aim of the present study was to carry out a comparative study of the cortex magnesium concentration and porosity of a large number of cidaroids species from a broad range of latitudes, temperatures and environments to assess if these characteristics are general adaptive features of the clade. Specimens from different depths of two species were also analyzed. Finally we assessed experimentally the possible protection offered by epibionts and biofilm when the spines face corrosive water.

2. Material and methods

2.1. $MgCO_3$ concentration and density of the different layers of mature primary spines of cidaroids

Three mature primary spines were collected from each of three individuals per species. We selected 11 species from different locations covering a temperature range from 0.8 °C to 29.1 °C (Table 1). Two species were collected at different depth (*Cidaris cidaris* and *Stylocidaris affinis*). Two species were collected at the same location but at different depths (*Cidaris cidaris* and *Stylocidaris affinis*). Saturation state of calcite was calculated using data from the databases GLODAP (Global Ocean Data Analysis Project) and NOAA (National Centre for environmental information).

Spines were cleaned in 3% sodium hypochlorite for 60–90 min and rinsed in 3 successive baths of Ultrapur water (Arium® Pro, Sartorius) and finally air-dried. Each spine was cut transversally at the middle of the shaft in two segments of similar length. The apical segment of the shaft was used for the measurements. The apical extremity of the segment was removed in order to obtain segments of identical length. The segments were placed on Multiclip (Struers) in screw capsules (Rotilabo®) and immerged in 2 successive ethanol100% solutions for 30 min followed by a solution of 1,2-epoxypropane 99% for 30 min and a solution of acetone 99.8% for 60 min. The segments were then embedded in the hard mixture of Agar Low Viscosity Resin (R1078 kit, Agar Scientific). Samples were placed under vacuum for 2 sessions of 60 min and the polymerisation was completed in an oven at 60 °C for 48 h. The embedded segments of spines were cut transversally into 5 mm slices with a diamond saw. The resulting sections were polished with sandpapers of decreasing grit size (P80, P200, P400, P800, P1200, P2400 and P4000) using an automatic polisher Rotopol-2 (Struers, Germany). The final polishing was performed using 1 µm diamond suspension (non-aqueous, 1PS-1MIC, ESCIL, France). The sections were laid on a stub and bridged to the latter by aluminium sticker and quick drying silver paint (AGAR, G302). Elemental energy dispersive X-ray (EDX) analysis was performed in an environmental SEM (FEI ESEM-FEG XL30) operating at 20 kV under low vacuum condition (0.3–0.4 Torr). Spectra were acquired on uncoated polished surfaces by a Bruker 129 eV silicon drift detector of 10 mm^2 with an S-UTW window for the detection of light elements. They were treated by the QUANTAX Esprit 2.1 (Bruker, USA). One spectrum was acquired in each of the three concentric zones or layers on the spines cross sections (cortex, median layer and medulla) of each of the three sampled spines (Fig. 1).

Spectra showed the presence of carbon, oxygen, sodium, magnesium, silicon, sulphur, chlorine and calcium. The silicon atoms derived

Table 1

Taxonomy, origin and seawater conditions in collecting sites of used specimens. (*) Collection from the "Université Libre de Bruxelles" (**) collection of the "Muséum national d'Histoire naturelle de Paris" (***) collection of the "Université de Bourgogne – Franche-Comté" at Dijon and (****) individuals kindly provided by Maria Byrne.

collection number	species	region	Location	Latitude	Longitude	Temperature	Ωcal	depth	Preservation	sampling
(***)	*Ctenocidaris spinosa*	Antarctic	Weddell sea	−71.1	−11.46	0.8	1.99	277	alcohol	2003
(***)	*Homalocidaris gigantea*	Antarctic	Weddell sea	−71.1	−11.46	0.8	1.99	277	alcohol	2003
2013022737 (*)	*Notocidaris mortenseni*	Antarctic	Bransfield strait	−62.95	−58.24	1.7	1.88	325	alcohol	2013
190810-26; CP2773-01(02) (***)	*Stereocidaris granularis*	Tropical	Papoua-New Guinea	−9.41	160.53	6.3	1.55	537–619	alcohol	2007
MNHN-IE-2013-16014(15,16) (**)	*Cidaris cidaris*	Temperate	Gran Canaria, Spain	31.87	−28.06	12.1	3.39	480	dry	1993
(**)	*Cidaris cidaris*	Temperate	Gran Canaria, Spain	31.87	−28.06	10	2.71	750	dry	1993
(**)	*Cidaris cidaris*	Temperate	Gran Canaria, Spain	31.87	−28.06	8	2.25	1060–1380	dry	1993 (2) 1987 (1)
(*)	*Stylocidaris affinis*	Temperate	Antiparos, Greace	37.01	25.04	16.5	6.44	60	dry	2007
MNHN-IE-2013-16017(-18,-19) (**)	*Stylocidaris affinis*	Temperate	Gran Canaria, Spain	28.01	−15.9	15.3	3.87	215	dry	1993
MNHN-IE-2013-16007(-8,-9) (**)	*Stylocidaris affinis*	Temperate	Gran Canaria, Spain	33.98	−30.19	9.9	2.66	665	dry	1993
100193-01(02,03) (*)	*Stylocidaris lineata*	Temperate	Gran Canaria, Spain	30.11	−28.27	15	3.98	340	alcohol	1993
(****)	*Phyllacanthus parvispinus*	Temperate	Sydney, Australia	−33.97	151.25	17	5.22	1.7	dry	2013
190810-29; CP2804-01 (***)	*Stylocidaris bracteata*	Tropical	Malaita, Solomon Islands	−9.15	161.21	23	4.27	150–175	alcohol	2007
(*)	*Eucidaris tribuloides*	Tropical	Montego Bay, Jamaica	18.47	−77.89	29.1	6.47	10	dry	2012
(*)	*Prionocidaris baculosa*	Tropical	Philippines	10.21	123.85	28.7	6.16	10	dry	2008