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Amorphous calcium carbonate: A precursor phase for aragonite in shell disease of the pearl oyster

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ABSTRACT

Amorphous calcium carbonate (ACC) has long been shown to act as an important constituent or precursor phase for crystalline material in mollusks. However, the presence and the role of ACC in bivalve shell formation are not fully studied. In this study, we found that brown deposits containing heterogeneous calcium carbonates were precipitated when a shell disease occurred in the pearl oyster *Pinctada fucata*. Calcein-staining of the brown deposits indicated that numerous amorphous calcium deposits were present, which was further confirmed by Fourier-transform infrared spectroscopy (FTIR), Raman spectrum and X-ray difraction (XRD) analyses. So we speculate that ACC plays an important role in rapid calcium carbonate precipitation during shell repair process in diseased oysters.

1. Introduction

Calcium carbonate has six mineral forms, calcite, aragonite, vaterite, monohydrocalcite, calcium carbonate hexahydrate and amorphous calcium carbonate (ACC). Biominerals composed of calcium carbonate are mostly found in thermal stable forms of calcite and aragonite. Interestingly, biogenic ACC is widely spread in the crustaceans cuticle [1,2], coral skeleton [3], molluscan shells [4], sea urchin spines [5] and plants [6], despite the fact that ACC is a metastable phase and tends to transform into aragonite and calcite. The utilization of ACC may benefit from the isotropy (equal mechanical properties in all direction), water-solubility (cuticle molting and reabsorption) or particle attachment (fast-growing skeleton) [3,7].

In molluscan shell formation, ACC has long been suspected acting as a transient precursor phase for aragonite or calcite. A continuous layer of amorphous calcium carbonate was found to cover the aragonite platelets without the interaction of matrix proteins in *Haliotis laevigata* [8]. Consistently, indication of ACC surrounding each growing nacre tablet in *Pinctada margaritifera* and *Atrina rigida* was observed by environmental- and cryo-scanning electron microscopy [9]. In addition, the finding of ACC in larval shells of diverse molluscan species led to the hypothetic basic strategy of shell growth from a precursor of ACC [4,10]. Yet, more confirmative evidences are still needed to support this idea.

Acceleration of $CaCO_3$ precipitation has been found in emergency cases, such as shell damage [11] and shell disease [12]. We speculate that the ACC synthesis in diseased oyster will be accelerated to promote shell repair. In previous studies, we characterized a shell disease in the pearl oyster *Pinctada fucata*. Serious infection was found in the extrapallial space (EPS), leading to highly disordered deposition on the nacreous layer. In the present study, we will focus on addressing the potential role of ACC in shell repair.

2. Materials and methods

2.1. Shell samples collection

Live adult pearl oysters were sacrificed by cutting the adductor muscle, and diseased individuals with serious infection on the inner surface of the nacreous layer were picked out. The fresh shells were washed with tap water and prepared for Light microscopy. For other analysis, the brown deposits were dehydrated with alcohol gradients (30, 50, 70, 90 and absolute alcohol).

2.2. Light microscopy

Small pieces of the brown deposit at the infection sites were peeled off using a nipper and subjected to Calcein-staining (0.1 g/L)

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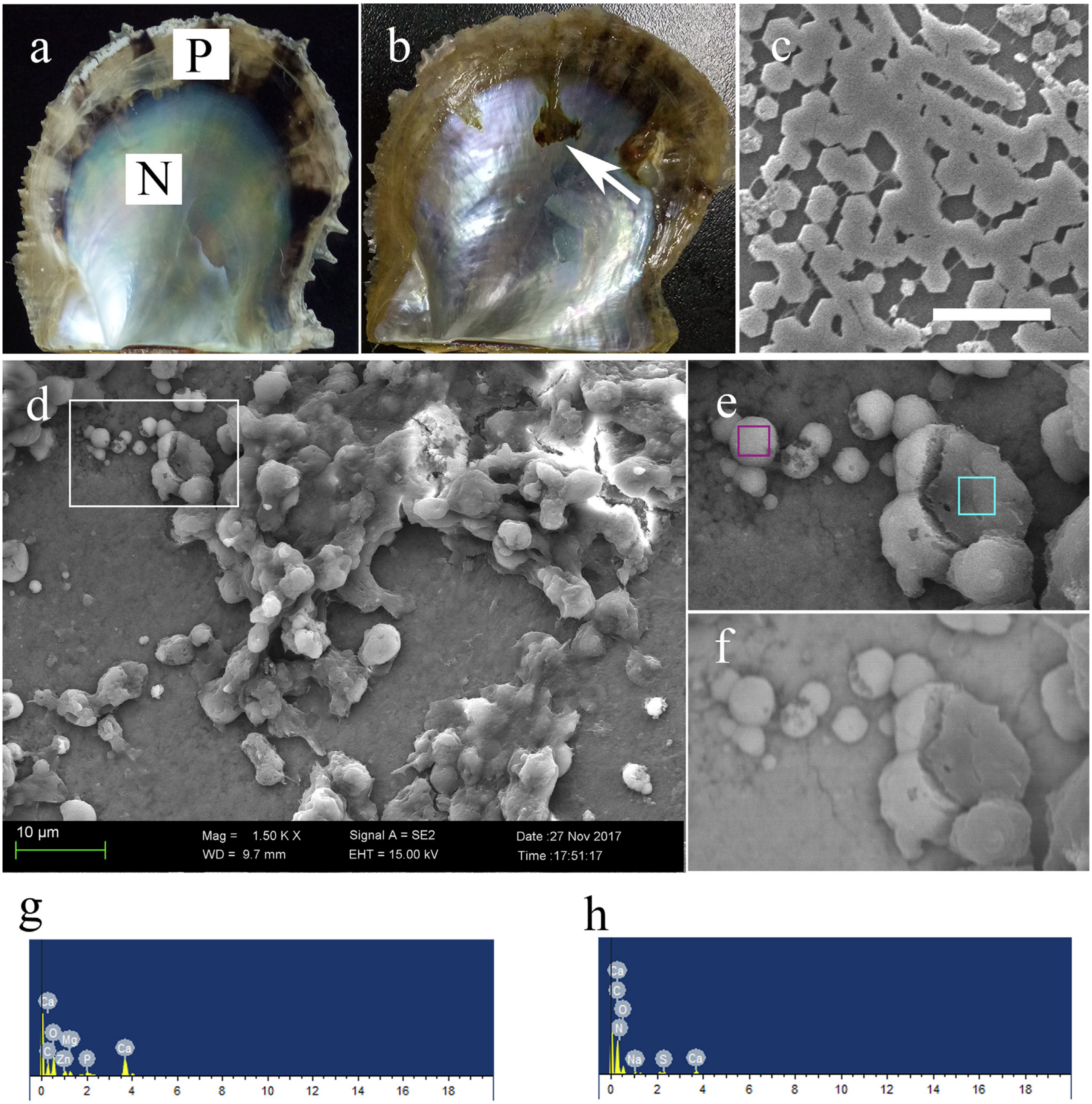

Element	Weight (%)	Atom (%)
C K	21.83	33.01
O K	46.45	52.71
Mg K	2.55	1.90
P K	1.97	1.16
Ca K	20.95	9.49
Zn L	6.25	1.74
Total	100.00	100.00

Element	Weight (%)	Atom (%)
C K	36.14	42.15
N K	35.81	35.81
O K	22.66	19.84
Na K	0.93	0.57
S K	0.75	0.33
Ca K	3.72	1.30
Total	100.00	100.00

Fig. 1. Scanning electron microscopy of the shell surface in *Pinctada fucata*. a, normal oyster shell. P, prismatic layer; N, nacreous layer. b, diseased oyster shell, note the brown material on the nacreous layer (white arrow). c, SEM of the normal nacreous layer, bar = 10 µm d, SEM of shell surface containing irregular particles in diseased oyster. e, magnification of white frame in d. f, back-scattered pattern of the same area in e. g and h, EDS analysis of the purple and light blue frame in e, respectively. Tables under the energy spectrums show the quantitative results of elemental composition. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)