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Biomineralization on the wavy substrate: Shape transition of nacreous tablets from pyramids of amorphous nanoparticles to dome-capped prisms of single crystals

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ABSTRACT

Nacre has long served as a model for understanding the biomineralization process and designing bio-inspired materials. However, our current knowledge about nacre is essentially based on the investigation of the flat nacre, where its building blocks, the aragonite tablets, grow on the flat substrate. Here, using field-emission scanning (SEM) and transmission electron microscopy (TEM), we investigate a new type of nacre, where the tablets grow on the wavy substrate. We first show that: (1) with growth, the tablet undergoes a shape transition from a pyramid to a frustum and finally to a dome-capped prism; (2) the shape transition occurs earlier at the downslope side of the tablet than at the upslope due to the slope effect; and (3) the shape of the top and base facet of the mature tablet depends on that of the substrate surface. In addition, we report that the tablet initially consists of amorphous calcium carbonate (ACC) nanoparticles, which gradually transforms into a single crystal of aragonite with time. Finally, we propose that the shape transition is induced by the crystal lattice mismatch between the tablet and substrate. We conclude that the topography and strain of the substrate play key roles in the biomineralization process of nacre.

Statement of Significance

Nacre is the iridescent inner lining of many mollusk shells, consisting of more than 95 wt% aragonite tablets and minor biopolymers. Owing to its superior mechanical properties, nacre has been extensively studied. However, nearly all previous works focused on the flat tablets. Here, we focus on the curved tablets grown on the wavy substrate. The main finding is that the topography and strain of the substrate play key roles in the growth process of the tablets. They not only induce the shape transition of the tablets from pyramids to dome-capped prisms, but also control the final shape of the tablets. The finding advances our understanding of the biomineralization process of nacre.

1. Introduction

Biominerals are a special class of minerals produced by living organisms for a variety of biological functions, such as protection and mechanical support [1–2]. They have been in existence since the Cambrian period (about 540 million years ago). As a result, they have evolved many unique features that distinguish them from their abiotic counterparts, such as non-crystallographic shapes [3], hierarchical structures [4], superior material properties [5], and unusual crystallization pathway [6]. These features have attracted widespread interest in the field of chemistry, biology, and materials science.

Among various biominerals, nacre serves as a model for understanding the biomineralization process [7] and designing bio-inspired materials [8]. Nacre is an iridescent structure present in the inner part of most mollusk shells [9]. Its basic building blocks are named aragonite tablets (2–10 μm wide and 0.4–2 μm thick), which are organized into horizontal mineral laminae interleaved by organic interlamellar membranes (abbreviated as IMs hereafter) (~20–30 nm thick) [7,10–13]. In general, the individual free-standing tablet looks like a flat hexagonal prism that behaves as a single crystal when probed by electron diffraction [14–16]. In other words, it exhibits a regular crystallographic shape bounded

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by flat facets. This flat tablet, as well as corresponding flat nacre, has been extensively investigated since the beginning of last century [17]. However, the curved tablet has been reported only by several authors.

To our knowledge, the curved tablets were first reported by Taylor et al. in 1969 [10], which show "irregular variation in the thickness" and are "distorted with wavy upper and lower surfaces". Later in 1977, Mutvei [18] found that the tablets from blue mussel have a clearly concave top facet. In 2007, Barthelat et al. [5] observed that part tablets from red abalone have microscale wavy facets, which are different from those with nanoscale wavy facets [19] and thus named "wavy tablets". This microscale waviness may contribute to the superior mechanical properties of nacre. However, the detailed shape and nanostructure of the curved platelets were not investigated until very recently. In a communication, Zhang and Li [20] showed that the mature curved tablets from green mussel have a convex top and concave bottom facet, indicative of non-crystallographic shape. Surprisingly, they have six flat lateral facets nearly identical to those of the flat tablets, indicative of crystallographic shape. Therefore, the curved tablets exhibit both the crystallographic and non-crystallographic shape, which is unique among all known biogenic crystals. Subsequently, Xu and Zhang [21] reported that the curved tablets vary in shape from the shell margin to interior. Despite these studies, the detailed growth process of the curved tablets is still unknown. Moreover, these tablets always grow on the wavy substrate. However, whether their growth is affected by the latter has not been studied before.

Here, we investigate the detailed growth process of the curved tablets at the posterior margin of green mussel shells, where the tablets grow faster than those at the shell interior and so display the richest transitional shapes [21]. We particularly focus on those tablets nucleated on the slopes of the wavy substrate, which show an unusual shape transition from pyramids to dome-capped prisms. We show this transition is analogous to that of the epitaxial semiconductor nanocrystals grown at high temperature. Therefore, we predict that the strain (or stress) and substrate topography play important roles in the biomineralization of nacre.

2. Materials and methods

The green mussel, *Perna viridis*, is a bivalve mollusk native to the Indo-Pacific region. Its shell consists of three layers: an inner nacreous layer (nacre), a middle prismatic layer, and an outer organic layer called the periostracum. In this work, the mussel samples were collected alive on the coast of Beibu Gulf in Guangxi, southern China. They were processed as follows: (1) the soft tissues and periostraca were removed from the shells with a scalpel; (2) the shells were cleaned with tap water followed by distilled water and air-dried at room temperature for 2 h; (3) shell fragments, about 5 mm × 5 mm in size, were mechanically broken away from the posterior margin of the shells; and (4) some fragments were treated by either immersion in 6 vol.% NaClO solution for 10 min or sonication in distilled water for 2 min. Meanwhile, for some shell fragments, their prismatic layer was ground away with 400- to 1200-grid SiC paper, so that the nacreous outer surface (the boundary with the prismatic layer) was exposed for FIB (focused ion beam) sample preparation.

For SEM analysis, the formerly prepared shell fragments, including the pristine, NaClO-immersed, and sonicated, were sputtered with gold and then observed with a field emission SEM (Hitachi, SU8020) operated at 8–10 kV.

For TEM analysis, the samples were prepared by two methods. One is the scratch method as described elsewhere [22,23]. The untreated shell fragments were scratched along their inner surfaces with a scalpel to obtain powdered samples, which were then dispersed in distilled water by sonication and dropped on a carbon-coated copper grid. The second is the FIB method. A vertical cross-section slice was prepared from the nacreous outer surface, using a dual-beam FIB system (FEI Helios NanoLab 600i). After that, TEM images and selected area electron diffraction (SAED) patterns were obtained with a field emission TEM (FEI, Tecnai G2 F20 S-TWIN) operated at 200 kV. Meanwhile, TEM images were analyzed with the software Digital Micrograph (Gatan).

3. Results

3.1. General shape of the tablets and their growth substrate

On the nacreous inner surface, the aragonite tablets exhibit a variety of shapes which depend on their growth stages. From the left to right in Fig. 1a, we can see that the tablets initially look like an oblique pyramid, which, when reaching a critical height, become truncated with the shape of an oblique frustum. As growth progresses, the frustums transform into dome-capped prisms (DP in Fig. 1b), on which the new tablets are seen to grow. Finally the dome-capped prisms are embedded by the new tablets (Fig. 1c). In short, with growth, the tablets undergo a distinct shape transition which will be detailed later.

Interestingly, although the dome-capped prisms always display asymmetrical shapes as represented by their irregular base facets, their dome caps are surprisingly symmetrical and well oriented. Particularly, in the same mineral lamina, all caps approximately lie in the same plane (called the girdle plane) (GP in Fig. 1b), which therefore form a wavy substrate with a wave length λ of ~2 μm and height h of ~400 nm. Such wavy substrate can also be understood as the top surface of a preformed mineral lamina.

Finally, for future convenience, the height of a tablet is defined as the height along its vertical axis, the aspect ratio (AR) of a tablet as the ratio between its height and base width, and the base width as the width of the tablet's bottom surface projected on the horizontal plane. Particularly, the term "horizontal plane" refers to the plane passing through the apexes of the tablets in the same mineral lamina (HP in Fig. 1a and c), and the "tablet base" refers to the tablet's bottom surface situated on the top surface of the underlying IM.

3.2. Detailed shape of the growing tablets

At the shell margin, the aragonite tablets can nucleate either on the peaks (Fig. S1), slopes (Fig. 2a), or in the pits (front tablet in Fig. 2f) of the wavy substrate. In general, the nucleation sites depend on the distance between the tablet and shell edge. With the distance increasing, the tablets in turn tend to nucleate on the peaks, slopes, and in the pits of the wavy substrate; meanwhile, the nucleation rate of the tablets has been reported to decrease gradually [24]. Therefore, it is probable that the nucleation sites depend on the rate. The high rate favors the nucleation on the peaks, while the opposite favors the nucleation in the pits. A more detailed discussion is beyond the scope of this work.

Here, we focus on the tablets nucleated on the slopes since they can display the effect of the slope on the shape transition. In addition, please note that the pristine tablets are usually covered by the organic materials (black arrows in Fig. 1a) and so exhibit smooth facets, while the NaClO-immersed ones show granular facets made of nanoparticles of 15–40 nm in diameter (e.g. Fig. 2a).

At the early growth stage, the tablets appear as oblique pyramids (Fig. 2a–d) with a constant AR of 1.1–1.2. Their axes are almost vertical but with obviously inclined bases. Besides, their lateral facets are asymmetrical. In particular, their downslope and