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| Author(s) | Uozumi, Satoru; Togo, Yoshihiro |
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FORMATION OF THE NACREOUS AND THE INNERMOST PRISMATIC LAYER IN OMPHALIUS RUSTICUS (GMELIN) (GASTROPODA)

by

Satoru Uozumi and Yoshihiro Togo

(with 2 text-figures and 6 plates)

(Contribution from the Department of Geology and Mineralogy, Faculty of Science, Hokkaido University, No. 1397)

The initiation and the growth of aragonite crystals which make up the nacreous layer have been studied from morphological, histological and biochemical view points. Recent studies carried out with the aid of an electron microscope have shown details of the sequential processes which lead to the formation of aragonite crystals. Bevelander and Nakahara (1959) have reported that the initiation and the growth of aragonite occur within the domain of compartments composed of the inter-lamellar conchiolin membranes. This conclusion is well-known as the "compartment hypothesis". Mutvei (1972) confirmed this hypothesis from scanning electron microscopic observations on the developing surfaces of the aragonite nacreous and the calcite prismatic layers of *Mytilus edulis*.

Most of the works on the shell formation have been concentrated in the nacre (sheet nacre) of lamellibranchs, and only a few studies have been reported on that of marine gastropods (Erben *et al.*, 1972a, 1972b; Wise, 1970).

The purpose of the research presented in this paper was to examine with a scanning electron microscope the formation of the aragonite crystals on the developing nacre surface of *Omphalius rusticus* (Gastropoda), and also to describe the formation of aragonite crystals in the innermost prismatic layer, which develops directly on the inner surface of the nacre.

Materials and Methods

Specimens of *Omphalius rusticus* were obtained from the coast of Shukuzu, Otaru-City, Southwest Hokkaido, and kept in a glass aquaria, filled with artificial sea water (19–20°C, pH 8.5).

Pieses of shell approximately $1-2 \text{ cm}^2$ in size were removed from the body whorl of the living specimens, using an electric dental machine. These pieces

were thoroughly washed in sea water and repeatedly in distilled water to remove the extrapallial fluid and then divided into three groups. One of them was kept in the desiccator with no treatment, another was treated with a 5% sodium hypochlorite solution at several different time intervals to remove the proteinaceous materials in different degrees, and the last one was fixed in a 2.5% glutaraldehyde-sea water solution. Then, the pieces of the latter two groups were washed in distilled water and air dried. A few pieces of the former group were randomly irradiated by an electron beam to break the last-formed membrane after coating with carbon.

All of them were directly mounted on the specimen-holders using silver paint, followed by a double coating with carbon and gold, and studied with a scanning electron microscope JEM-U3 (Japan Electron Optics Lab. Ltd.) at the Department of Hokkaido University.

Observations and Results

Nacreous layer

The so-called nacreous layer of marine shells has roughly been divided into two types based on the difference in construction of the nacre tablets: one is



Text-Fig. 1. Scanning electron micrographs showing the nacreous layer in vertical polished section (left) and growing stacks of tablets (right). Note the tablets with parallel arrangement, and the inter-lamellar conchiolin membranes (arrows) lying between the tablets. X 2,000 (left); X 6,000 (insert); X 6,000 (right).



Text-Fig. 2. Electron micrograph showing inter-lamellar conchiolin membrane; demineralized in EDTA. X 15,000.

called the "sheet nacre" (Taylor, 1969) or "Backsteinbau" (Schmidt, 1923) of the lamellibranchs; the other is the "columnar nacre" (Taylor, 1969) or "Treppen" (Schmidt, 1923) of the gastropods.

The nacre of *Omphalius* in not an exception to these rules: it consists of aragonite tablets arranged into columns. The growing surface of the nacre was covered by tall conical stacks of aragonite tablets: the early crystals form at the tops of the stacks and then, as growing proceeds, they expand laterally as more crystals form on top. Their lateral growths generate flat tablets that ultimately get in contact with the crystals of the adjacent stacks. Then, they coalsce to form thin mineral lamellae characterizing the nacre of molluscs. In our preparations of surface of nacre also, conical stacks are covered by the last-formed inter-lamellar conchiolin membrane, lying parallel to the shell inner surface. This membrane is very thin and fragile, whereas the conical termination of stacks which contact with this membrane are very sharp. Accordingly, these terminal parts of the stacks are occasionally seen as if protruding on the inter-lamellar membrane. This aspect is caused by the artificial distortion or constriction of the membrane produced in the course of the drying of the preparations.

The preparations in which the last-formed membrane was broken by a random irradiation of an electron beam clearly show that this membrane covers the terminations of conical stacks (Pl. 2, Figs. 4, 6). In the preparations in which the membrane was constricted and remove from the foreground, the conical terminal parts were pulled down in the same direction (Pl. 1, Figs. 3-6).

Also, from the observations under the low accelerating voltage 10 kv, the conical terminal parts are photographed as the soft-focus picture, and the last-formed membrane is draped roughly on these terminations (Pl. 2, Figs. 1, 2).

From the foregoing observations, it is clear that all growing terminations of stacks are always covered by the last-formed membrane.

Incidentally, most of the membranes examined have an smooth surface but it is noticed that a few of them have a granular surface with many scattered "openings" (Pl. 2, Fig. 5). The granular ones appear to be more dense and thicker than the smooth ones, and seem to be continuous with them (Pl. 2, Fig. 2). It seems, if this observation is correct, that either of the two aspects of membrane shows an artificial pattern. The present writers will re-examine more precisely the natural appearance of the membrane by freeze-drying and critical drying methods recently developed for the preparation of these samples for the electron microscope.

Innermost Prismatic Layer

The junior author (1974) has described the way of growing and the shell structure of the innermost layer of gastropods based on the ontogenetic study of the shell structure.

This layer is composed of aragonite crystals and represents a rather disordered construction of crystals which is clearly distingushed from other structural layers, nacreous, crossed-lamellar and so on. Moreover, this layer decreases rather smoothly in thickness towards the aperture of the shell, although the inner and the outer layers increase inversely in thickness.

The innermost layer of *Omphalius rusticus* consists of aragonite prisms: in the vertical section of this layer various sized prisms, 3 to 20μ in width, are standing close together and inclining with certain angles to the mineral lamellae of nacre underlying this layer. When the developing surface of this layer is observed, the fan-shaped aragonite tablets appear arranged in roughly parallel overlapping rows (Pl. 3, Figs. 1, 3). In some places, their marginal portions increase in thickness and rise as a ridge, 4μ to 5μ in width and 10μ to 20μ in length (Pl. 4, Fig. 5). In the areas where the innermost layer is just being formed many spherical crystallites, $0.1-0.4\mu$ in diameter, can be observed on the nacre crystals or on the immature fan-shaped tablets (Pl. 3, Figs. 2, 5). In some parts, many acicular crystals, approximately 5μ in length and 0.3μ in diameter, are visible on or around the fan-shaped tablets (Pl. 3, Figs. 3-5, Pl. 5, Fig. 3). Three shapes of aragonite crystals, spherical, acicular and tabular, are observable in one portion of the preparation, although the proportion of these crystals is variable in places. When spherical crystallites are predominant, the other types have an ill-development. Same relationship is valid between acicular and tabular crystals respectively.

More detailed observations show that the formation of new tabular crystals is initiated from spherical crystallites: these experiment a dendritic growth produced by aggregation and ultimately generate the acicular crystals (Pl. 3, Figs. 5, 6; Pl. 5, Figs. 2,3). With the increase in size and the number of the acicular crystals they make contact with the neighbouring ones, and form a fan-shaped tablet (Pl. 3, Fig. 6; Pl. 4, Figs. 2, 3). Sequentially, the fan-shaped tablets increase their length and width by further accumulation of acicular crystals. Moreover, they generate larger crystals through pseudohexagonal intergrowth and so on (Pl. 4, Fig. 1; Pl. 5, Fig. 5). When the fan-shaped tablets are being almost completed, new minute crystallites occur at the rims or on the central portions of their surfaces. These new crystallites are rounded, measuring 0.2μ to 0.7μ in diameter at an early stage (Pl. 4, Fig. 1; Pl. 5, Fig. 6) and grow rapidly in a vertical direction from the surface of the fan-shaped tablets. Through growth these crystallites become fine prisms (Pl. 6, Figs. 1-3). Some of them grow independently and some coalesce with adjacent ones to form a larger prism. They are approximately 0.2μ to 0.7μ in width and arrange with their long axes nearly normal to the surface of the fan-shaped tablets. They finally overgrow all over the surface of the tablets (Pl. 6, Fig. 6). Vertical growth and coalescence of these forms give rise to an increase of the thickness of the fan-shaped tablets and to the generation of various sized prisms.

Summary

The present study has revealed that the initiation and the growth of nacre crystals in *Omphalius* take place within the interspaces between inter-lamellar conchiolin membranes, which have been already polymerized prior to crystal growth, just as in the case of the lamellibranch nacre. All, or a part, of the extrapallial fluid that passed through the several sheets of the inter-lamellar membranes is thought to influence the further increase in size of the tablets of stacks until the growing tablets make contact with tablets of adjacent stacks. In this case, it is still unknown exactly what site of the membrane influences the transportation of the extrapallial fluid. However, it may be possible to some extent that the passage of the fluid takes place through the minute "openings", scattered on the conchiolin membrane (Pl. 2, Fig. 5).

The study of the developing surface of the innermost layer has revealed the following two phases in the process by which this innermost layer is formed: 1) initiation of spherical crystallites, their growth to form acicular crystal, the accumulation and coalescence of the acicular crystals to form fan-shaped

tablets and 2) the vertical growth of tablets and the resultant formation of prisms.

The crystal growth in the latter phase is strikingly different from that in the former one, and rather similar to that in lamellibranch nacre which will be described in other paper in the near future.

Meanwhile, it is difficult to explain what is the significance suggested by the formation of the two different shell layers, nacreous and prismatic, built up from the same mantle tissue. It may be assumed that the difference between them is not due to basic but to slight differences in the secretory activity of the mantle tissue at the respective portion. Namely, the differences may be dependent on the amounts, the degree of supersaturation and the viscosity of the extrapallial fluid.

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Explanation of Plates

All figures are scanning electron micrographs of *Omphalius rusticus* (Gmelin). Bar scale in microns.

Plate 1

Outcrop pattern of the growing stack ends of tablets on the inner surface of the nacreous layer.

- Figs. 1, 2. The growing stacks are covered by the last-formed inter-lamellar conchiolin membrane which somewhat hangs down artificially along the surface of stacks. Fig. 1: X 3,000; Fig. 2: X 5,000.
- Figs. 3-6. The growing stacks are under the horizontal inter-lamellar membrane. The 3-5 tablets of the stack ends are pulled down artificially in the same direction by the constriction of the inter-lamellar membrane.

The outline of the stacks is obscurely photographed for the cover of this membrane. Fig. 3: X 2,000; Fig. 4: X 3,000; Fig. 5: X 2,000; Fig. 6: X 5,000.



- Fig. 1, 2. Outcrop pattern of the growing stack ends on the inner surface of the nacreous layer. The outline of the stacks is photographed as the soft-focus picture for the cover of inter-lamellar membrane under the low accelerating voltage 10 kv of the electron beam. The membrane is stretched like a thread between some stack ends. Fig. 1: X 3,000; Fig. 2: X 3,000.
- Fig. 3, 4. Oblique view of a right-angle fractured surface of the nacreous layer showing a two-dimensional aspect of the growing stacks. Fig. 3: X 1,000; Fig. 4: X 1,000.
- Fig. 5. Inner surface of the nacreous layer showing the inter-lamellar membrane which covers the stacks without being constricted. Note the granular surface and the numerous minute "openings" of the membrane. X 3,000.
- Fig. 6. Oblique view of the cracked inner surface with stacks intersecting conchiolin membranes at an angle. Note the last-formed inter-lamellar conchiolin membrane and next underlying one. X 2,000.



Fig. 1. Outcrop pattern of growing crystals on the inner surface of the innermost layer. Spherical crystallites develop on and among immature fan-shaped tablets. X 1,000.

Fig. 2. Same preparation as in Fig. 1 with a higher magnification. X 3,000.

Fig. 3. Outcrop pattern of the growing crystals on the inner surface of the innermost layer. Acicular crystals develop on and among immature fan-shaped tablets. X 1,000.

Fig. 4. Same preparation as in Fig. 3 with a higher magnification. X 3,000.

Fig. 5. Same preparation as in Fig. 2; a higher magnificated micrograph showing acicular crystals formed from spherical crystallites by dendritic intergrowth. X 3,000.

Fig. 6. Immature fan-shaped crystals growing from acicular crystals. X 3,000.



- Fig. 1. Outcrop pattern of growing crystals on the inner surface of the innermost layer. All growing acicular and fan-shaped crystals are developing in a dense organic matrix. Fan-shaped tablets represent the phenomenon of pseudohexagonal intergrowth which is observable in the natural growth of aragonite crystals. X 6,000.
- Figs. 2-5. Micrographs showing the aspect of fan-shaped tablets in various stages of their growth on the inner surface of the innermost layer. Note tips of acicular crystals protruding from the side face of the tablets. Tablets shown in Fig. 3 are in a more advanced stage than those in Fig. 2. Fan-shaped tablets in Fig. 4 correspond to an almost mature stage of their growth, although they remain as the tips of acicular crystals in their side face. Figs. 2, 3, 4: X 3,000; Fig. 5: X 1,000.
- Fig. 6. Full developed fan-shaped tablets of the inner surface of the innermost layer showing the smooth side face and an increase in thickness. X 1,000.



- Fig. 1. Oblique view of a right-angle fractured surface showing a two-dimensional aspect of the innermost layer. Various sized spherical crystallites are seen on the fan-shaped tablets which overlie the nacreous layer (lower right). X 6,000.
- Fig. 2, 3, 5. Outcrop pattern of immature fan-shaped tablets on the inner surface of the innermost layer. Note the acicular or rod-like crystals which are formed by aggregation of spherical crystallites. Figs. 2, 3, 5: X 3,000.
- **Fig. 4.** Outcrop pattern of the inner surface of the inner-most layer. Most of the organic materials have been removed with a 5% sodium hypochlorite solution. Note the coalescent acicular crystals directly lying on the mineral lamellae of nacre (lower left) and overlaid in turn by immature fan-shaped tablets. X 3,000.
- Fig. 6. A high magnificated micrograph showing spherical crystallites covered by organic materials located on fan-shaped tablets. X 10,000.



- Figs. 1-3. Outcrop pattern of minute prismatic crystals growing on the rims or central parts of the fan-shaped tablets. Note the pattern of growth and the linear arrangement of the minute prisms. Fig. 1-3: X 6,000.
- Fig. 4. Outcrop pattern of mature fan-shaped tablets. The pattern of the inner surface of the fan-shaped tablets is made up of many minute prismatic crystals. Organic material has been partially removed with sea water. X 3,000.
- Fig. 5. Outcrop pattern of mature fan-shaped tablets. Note the interior structure of the fan-shaped tablets. Organic material has been almost perfectly removed with a 5% sodium hypochlorite solution. X 3,000.
- Fig. 6. Outcrop pattern of irregular prisms at the final stage of the formation of the innermost layer. The surface of the fan-shaped tablets is almost completely occupied by prismatic crystals which have grown vertically from this surface. X 3,000

