Twinned aragonite crystals found in the bivalvian crossed lamellar shell structure

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Introduction

More than ten morphological types of internal microstructure are known to be present in mollusc shells. One of them, crossed lamellar shell structure is widely distributed among bivalves and gastropods. The shell structure has been examined by light and scanning electron microscopes (Bøggild, 1930; Kobayashi, 1964, 1971, 1988; Taylor et al., 1969; Uozumi et al., 1972; Carter, 1980; Uozumi and Suzuki, 1981; Togo and Suzuki, 1988).

This structure (Figs. 1-A and B) is composed of first-order lamellae which assemble themselves normally to inner shell surface. The regularly arranged first-order lamellae contain second-order lamellae described by Bøggild (1930) at first, which consist of third-order lamellae (Kobayashi, 1964). The third-order lamellae have a shape of rectangular rod and are oriented parallel with each first-order lamella. In addition, the third-order lamellae of two adjoining first-order lamellae incline in opposite directions. The longest axis of third-order lamellae lies obliquely to the inner shell surface. Third-order lamellae are composed of aragonite crystals (Kobayashi, 1971). However, it is not known whether each third-order lamella consists of a single crystal or polycrystalline aggregate. Moreover, Uozumi et al. (1972) made an important electron microscopic observation of the inner texture of the third-order lamellae. They found minute banded structure in the third-order lamellae and pointed out that determining the nature of this structure is important. However, the banded structure has not been properly interpreted.

The purpose of this study is to restudy the fine structures of crystals of the crossed lamellar structure

by transmission electron microscopy and to find their crystallographic characteristics of the banded structure in the third order lamellae by electron diffraction.

Specimens

Bivalves with crossed lamellar structure selected for this study included *Barbatia virescens* (Reeve) (Recent specimen; Loc. Kashikojima Island, Mie Prefecture) and *Glycymeris yessoensis* (Sowerby) (Recent specimen; Loc. Rebunto Island, Hokkaido; Late Pleistocene specimen; the Narita Formation, Loc. Shisui, Chiba Prefecture; Early Pleistocene specimen; the Sawane Formation, Loc. Sawane, Sado Island, Niigata Prefecture).

Methods

Above-mentioned materials of the crossed lamellar layer were prepared for transmission electron microscopy with following two methods.

1) Ion-etching method: Radial thin sections of a shell were made by a diamond saw. Parts of the sections were cut off and then thinned by ion-etching method. 2) Powder method: Specimens were crushed into small fragments and subjected directly to the electron microscopic observation.

For transmission electron microscopy, a JEM 200 CX (JEOL) microscope was operated at 200 kV. Selected area electron diffraction analyses were also carried out.

Besides transmission electron microscopy, light and scanning electron microscopes were used for low magnification observations. The specimens for the microscopy were prepared by conventional methods.

Results and discussions

A light micrograph of the crossed lamellar structure of *Glycymeris* is shown in Fig. 2. A scanning electron micrograph of the same structure is shown in Fig. 3. The third-order lamella is about 0.2-0.5 μm in width.

The banded structure is seen in the ion-etched rectangular fragments of third-order lamellae of recent *Glycymeris yessoensis* and *Barbatia virescens* under a transmission electron microscope (Figs. 4 and 5). The banded structure consists of alternative arrangements of electron translucent and opaque bands standing side by side. Each band is 100-500 Å in width and nearly normal to the oneside of a rectangular fragment. The width of one band is mostly constant.

Fig. 1. Schematic diagram of crossed lamellar structure.

A : Arrangement of lamellae, VD : Direction of ventral shell margin, IS : Section nearly parallel to inner shell surface, B-a : First-order lamella, B-b : Second-order lamella, B-c : Third-order lamella (rectangular rod), C : Banded structure of third-order lamella, twinning plane and crystallographic orientations are indicated.

Fig. 2. Crossed lamellar structure of *Glycymeris yessoensis* (Sowerby) from the Narita Formation. Light micrograph of vertical thin section, showing a number of light and dark bands which run parallel with each other. Each band corresponds to a first-order lamella. IS : Direction of inner shell surface.

Fig. 3. Crossed lamellar structure of *Glycymeris yessoensis* (Sowerby) from the Sawane Formation. Scanning electron micrograph of fractional surface, showing three first-order lamellae (FL). Each lamella consists of aggregates of many third-order lamellae (TL) consisting of rectangular rods.

Fig. 4. Dark field transmission electron micrograph of ion-etched crossed lamellar layer of *Glycymeris yessoensis* (Sowerby) from Rebunto Island. Banded structure is very clear. Spotted images may be due to the existence of organic matters.

Fig. 5. Transmission electron micrograph of ion-etched crossed lamellar layer of *Barbatia virescens* (Reeve) from Kashikojima Island. Banded structure is found in the fragment of one third-order lamella. The fragment nearly corresponds to a transverse section of the third-order lamella.

Fig. 6. Transmission electron micrograph of ion-etched crossed lamellar layer of *Glycymeris yessoensis* (Sowerby) from the Sawane Formation. Banded structure is also observed in the fragment of a third-order lamella, however, the structure is a little obscure.

This banded structure was found not only in recent specimens of *Glycymeris yessoensis*, but also in the ion-etched crossed lamellar layer of fossil specimens of the same species (Fig. 6). The similar banded structure was also observed in the crossed lamellar structure of Pliocene and Holocene bivalved fossils by Uozumi et al. (1972). These indicate that the finest original shell structure has still remained in the fossil specimen. The banded structure of fossil specimen observed in Fig. 6 is a little obscured when compared with that of recent specimens. It is difficult to explain the cause.

Selected area electron diffraction patterns obtained from fragments of the third-order lamellae of *Barbatia virescens* by the powder method (Fig. 7) as well as electron microscopic images of the banded structure clearly revealed that this structure is of polysynthetic twins of aragonite with twinning planes of (110) (Fig. 1-C). The electron diffraction patterns consist of two sets of diffraction spots. One set of them is due to a single crystal and is correlated with the other set by twin operation. The twin operation is the same as that shown by Bragg (1924). Based on the determination of crystal orientation from the electron diffraction patterns, the twinning plane was confirmed to be (110).

We came to the conclusion that the banded structure of third-order lamellae reported by Uozumi et al. (1972) is composed of polysynthetic twins of aragonite. Each third-order lamella of crossed lamellar structure does not consist of a single crystal but a twinned crystal.

Aragonite of inorganic origin often has twinned structure (e. g., Dana, 1914). Watabe (1981) reviewed the crystal growth of calcium carbonate in the invertebrates and noted the existence of twinned crystals. It is known that aragonite crystals in the hinge ligament of *Mya arenaria* and *Spisula solidissima*

Fig. 7. Transmission electron micrograph and electron diffraction pattern of the powdered crossed lamellar layer of *Barbatia virescens* (Reeve) from Kashikojima Island. The fragment with banded structure corresponds to a longitudinal section of the third-order lamella. Electron diffraction pattern clearly indicates that the third-order lamella has thin polysynthetic twinned structure.

(Bivalvia) are twinned (Marsh and Sass, 1980). Moreover, twinning of biogenic aragonite was reported in the nacreous layer of cultured pearls from *Pinctada martensii* (Watabe, 1954) and in the nacreous shell layer of monoplacophora *Neopilina* (Meenakshi et al., 1970). Although Mutvei (1977, 1978, 1980) reported that aragonite tablets of various mollusc nacreous layers indicated twinned structure when etched with a glutaraldehyde-acetic acid solution, Watabe (1984) had a doubt about Mutvei's interpretation and said that etch figures shown by Mutvei do not necessarily reflect the twinning nature of crystals.

Thus, twinned crystals of aragonite have been reported previously in the ligament and nacreous structure of mollusc shells, but they were newly found in the crossed lamellar structure of bivalves by this study.

Our present result may be important to reveal the mechanism of biomineralization in a mollusc shell. In general, twinned crystals may be formed in the process of growth, transformation or gliding (Buerger, 1945). The twinned structure found in this study may be due to growth twinning, because transformation twinning is impossible in aragonite crystal and there is no evidence of stress or mechanical process for the formation of gliding twinning. Polysynthetic twinning pattern in growth twinning of inorganic aragonite (Dana, 1914) resembles the twinning pattern observed in this study. However, the latter twinning pattern is much smaller than that in inorganic aragonite.

In the case of bivalvian biomineralization, the crystal growth of a shell occurs in the narrow extrapallial space between the mantle and shell. The extrapallial fluid in the space contains many organic matters. The existence of these organic matters may be important for the formation of twinned aragonite crystals.

The twinning pattern newly found by this study in

the third-order lamellae is very characteristic in biogenetic aragonite crystals, but we could not fully explain the mechanism of the twinned crystal formation.

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