NOTICE

**CERTAIN DATA
CONTAINED IN THIS
DOCUMENT MAY BE
DIFFICULT TO READ
IN MICROFICHE
PRODUCTS.**

CONF-871119 172

PNL-SA--17900

DE91 004885

Received by OST
DEC 14 1990

AN ELECTRON MICROSCOPY STUDY OF THE
MICROSTRUCTURE AND MICROARCHITECTURE
OF THE STROMBUS GIGAS SHELL

P. C. Rieke A. H. Heuer(a)
V. J. Laraia M. Aindow(b)

November, December 1989

Presented at the
1989 Fall Meeting Symposium R of the
Materials Research Society
Boston, MA
November 27-December 2, 1989

Work Supported by
the U. S. Department Of Energy
under Contract DE-AC06-76RLO 1830

Pacific Northwest Laboratory
Richland, Washington 99352

- (a) Case Western Reserve University
Cleveland, Ohio 44106
- (b) Ohio State University
Columbus, Ohio 43210-1179

MASTER

DISTRIBUTION OF THIS DOCUMENT IS UNLIMITED

482

DISCLAIMER

This report was prepared as an account of work sponsored by an agency of the United States Government. Neither the United States Government nor any agency thereof, nor any of their employees, makes any warranty, express or implied, or assumes any legal liability or responsibility for the accuracy, completeness, or usefulness of any information, apparatus, product, or process disclosed, or represents that its use would not infringe privately owned rights. Reference herein to any specific commercial product, process, or service by trade name, trademark, manufacturer, or otherwise does not necessarily constitute or imply its endorsement, recommendation, or favoring by the United States Government or any agency thereof. The views and opinions of authors expressed herein do not necessarily state or reflect those of the United States Government or any agency thereof.

AN ELECTRON MICROSCOPY STUDY OF THE MICROSTRUCTURE AND MICROARCHITECTURE OF THE *STROMBUS GIGAS* SHELL

V.J. Laraia, M. Aindow* and A.H. Heuer

Department of Materials Science and Engineering, Case Western Reserve University, University Circle, Cleveland, OH 44106, USA.

* Present address: Department of Materials Science and Engineering, Ohio State University, 116 West 19th Avenue, Columbus, OH 43210-1179, USA.

ABSTRACT

A scanning and transmission electron microscopy study is presented of the microstructure of the *Strombus gigas* shell. The hierarchical nature of this crossed-lamellar structure and the defect content of the mineral component are described. This mineral component consists of small single crystal grains of aragonite, the metastable orthorhombic polymorph of CaCO_3 . The habit and morphology of the grains discussed here have not been determined previously. The observed habit and defect structure suggest that the organic matrix exerts a high degree of control over the crystal growth of the mineral phase and is responsible for the long range order in the microarchitecture. Electron beam heating of the mineral component leads to certain phase changes and these are discussed.

INTRODUCTION

Numerous organisms exhibit the ability to manufacture structural composites, such as bone or shell, that contain a high volume fraction of mineral. These biological hard tissues are of considerable materials science interest, as they are fabricated from abundant precursors at near ambient conditions. Such materials often possess room temperature mechanical properties which are comparable or superior to those of man-made structural ceramics [1,2,3]. It is therefore of interest to understand the mechanisms by which these tissues are formed, not only because of their considerable biological and medical significance, but also as model systems for the manufacture of structural composites utilizing analogous processing routes. Shell structures typically contain >95% of the mineral phase and can be divided into 9 distinct structural types (nacreous, crossed-lamellar, etc.) according to the way in which the mineral is organized [4]. Although the general morphological features of these microstructural types are known, the details of the formation mechanisms are not well understood. Many hard tissues form by precipitation of a mineral phase upon an organic matrix that is assembled in advance of the "mineralization front." [5]. However, the structure of the mineral-organic interface has yet to be determined for any biological hard tissue, and the morphology, structure and location of the mineral nuclei are unknown. A great deal of additional information can be obtained about these issues by the use of standard materials characterisation techniques to study the mineral component

In the present paper, we describe electron microscopy studies on the conch shell shown in Figure 1 (from an adult specimen of the saltwater mollusk *Strombus gigas*). This shell is of the crossed-lamellar type and contains less than 0.5% protein. The mineral component is aragonite, a metastable orthorhombic polymorph

Figure 1. The *Strombus gigas* shell.

of CaCO_3 , and the protein exists as a fine grain boundary phase. Scanning Electron Microscopy (SEM) and diffraction contrast Transmission Electron Microscopy (TEM) imaging is used to reveal the hierarchical nature of the microstructure. Particular emphasis is placed on the orientation relationships between the microstructural units. Diffraction contrast and High Resolution Electron Microscopy (HREM) imaging modes are used to characterise the defects within the mineral phase.

The defect structures in this biogenic mineral are compared to those in geological aragonite and the significance of these observations for growth mechanisms is discussed. Finally, diffraction and imaging experiments are described which illustrate the nature of beam-specimen interactions.

EXPERIMENTAL

For our studies, an air-dried specimen of the *Strombus gigas* shell was used. Specimens for scanning electron microscopy were prepared by fracturing portions of the shell and mounting on aluminum stubs with epoxy. As CaCO_3 is an insulator, the specimens were coated with C or Pd to reduce charging under the electron beam. Secondary electron images were obtained in a JEOL 35CF SEM at 25kV. TEM specimens were prepared from crushed portions from the region of interest, as argon ion milling produces many artifacts in electron transparent regions. Specimens were prepared by depositing a drop of a suspension of the powder in distilled water onto a holey carbon film. Upon drying, this procedure yielded specimens with very large amounts of thin area. As the particles were in direct contact with the C film no subsequent coating was required. Bright field imaging and selected area diffraction were performed in a Philips EM400T and a JEOL 200CX at accelerating voltages of 120kV and 200kV respectively. HREM images were obtained in a JEOL 4000EX at 400kV. Low temperature experiments were performed in the Phillips 400T using a Gatan cold stage with liquid nitrogen.

RESULTS AND DISCUSSION

Hierarchy of Microstructural Units

The size of the shell used for this study is typical of adult specimens of *Strombus*; about 18cm along the anterior-posterior axis. The shell is approximately 3mm thick over most of its area. Figure 2a is a secondary electron image of a fracture surface running perpendicular to the original surfaces of the shell. Three distinct layers are revealed each of which exhibit the crossed-lamellar microstructure described below. The inner layer (i.e. that which was closest to the animal) is $\approx 0.5\text{mm}$ thick and the central and outer layers are ≈ 1.75 and $\approx 0.75\text{mm}$ thick respectively. The central layer is rotated by $\approx 90^\circ$ with respect to the inner and outer layers. The rotation axis is approximately normal to the original shell surface. It should be emphasized that the thickness and orientation of these coarse-scaled microstructural features may vary locally in the shell. Each of the macroscopic layers is comprised of large sheets $25\text{--}50\mu\text{m}$ thick and many hundreds of microns in extent. These microstructural units are known as first-order lamellae [6] and are oriented parallel to the shell surfaces as shown in Figure 2b. Two distinct orientations (labelled A and B in Fig. 2b) exist and are related by a rotation of $\approx 85^\circ$ about the normal to the plate.

First-order lamellae are themselves comprised of a stacked series of lath-shaped second-order lamellae (e.g., at C in Figure 2b). These laths exist with a wide range of dimensions but examination of fracture surfaces suggests that most have thicknesses of $1\text{--}10\mu\text{m}$ and are $20\text{--}50\mu\text{m}$ wide. These laths usually extend entirely through the macroscopic layer and are, therefore, typically 1 mm in length. It has not, as yet been possible to obtain suitable specimens to determine whether a crystallographic relationship exists between second order lamellae. High magnification SEM images such as Figure 2c show that the second order lamellae are comprised of a "brickwork" structure of third-order lamellae, $100\text{--}500\text{nm}$ wide and less than 50nm thick. These dimensions are consistent with the observations of Bevelander and Nakahara [7], who used TEM to show that the organic matrix of growing *Strombus* shell contains envelopes of approximately these dimensions. The length of the third-order lamellae is still under question;

although crushed grain mounts often contain laths which are 0.5-1.0 μm long, SEM images suggest that this may be a severe underestimate of the true extent. Diffraction contrast TEM images from fragments of second-order lamellae indicate that the long axes of second and third-order lamellae are approximately parallel. The third order lamellae seem to alternate between two distinct orientations within a second-order lamella, but the exact relationship between adjacent third order lamellae is still under question.

Figure 2. Arrangement of microstructural units in the shell; (a) macroscopic layers, (b) first and second order lamellae, (c) third order lamellae.

A crystallographic analysis was performed on a number of isolated third order lamellae using selected area diffraction and stereo imaging experiments in the TEM. The lamellae were found to be single crystals with a habit plane approximately perpendicular to $[112]$. The minor and major axes in this plane are parallel to the (110) normal and $[33\bar{4}]$ direction, respectively (Figure 3).

Figure 3. Schematic diagrams showing the relative orientations of crystallographic axes with respect to (a) a third order lamella, and (b) the orthorhombic unit cell.

Defect Microstructure

The microstructure of individual third order lamellae was investigated using diffraction contrast imaging. Figure 4a is a bright field image of a single third order lamella obtained with the beam direction parallel to the habit plane normal (i.e. $[112]$). Continuous bands of contrast can be observed extending through the whole length of the lamella parallel to the major axis (i.e. $[334]$). The corresponding selected area diffraction pattern (SADP) is shown in Figure 4b, and Figure 4c is an indexed schematic. The pattern is comprised of two $[112]$ zone axis patterns which are related by a reflection in (110) indicating the presence of extensive twinning on this plane. Whilst the patterns are labelled matrix (M) and twin (T), the distinction is arbitrary as approximately equal proportions of the two orientations are present in the crystal. Dark field imaging and microdiffraction experiments were used to show that the contrast bands in images such as Figure 4a correspond to alternate regions of matrix and twin related material. These features represent yet another tier in the hierarchy of the cross-lamellar microstructure and it may be appropriate to consider them as fourth-order lamellae. Figure 5 is a HREM image of a different third order lamella obtained with the beam direction parallel to the $[112]$ zone axis. The twinning is clearly revealed by reflection of the (111) and (021) planes across the twin boundaries. The boundaries lie in the $[112]$ zone axis, parallel to the (110) twin plane. These boundaries are atomically flat and defect free; no steps or dislocations have been observed in any of our lattice images. These planar boundaries are coherent with a one-to-one correspondance of lattice points across the boundary plane. The width of these fourth-order lamellae in the direction parallel to the (110) plane normal is usually in the range 1-20 nm, with an average width of 5 nm. No systematic variation in this dimension was observed.

Figure 4 Twinning in third-order lamellae: (a) bright field image at the $[112]$ zone axis, (b) selected area diffraction pattern, (c) indexed schematic of (b).

Three mechanisms of twin formation are known in crystalline materials; due to annealing, growth and deformation. Annealing twins are a phenomenon peculiar to high symmetry polycrystalline metals and are therefore not considered further here. Growth twins are formed when twin-oriented nuclei coalesce during growth or twin-oriented material is formed on matrix

material. Deformation twinning, however, requires that partial dislocations glide under applied stresses in a pole or pole-plus-ratchet configuration. In geological aragonite, deformation twinning is not known to occur below $\approx 300^\circ\text{C}$, i.e. well above the ambient temperatures at which the Strombus shell is formed [8]. Furthermore, deformation twins are usually lenticular twins with arrays of partial dislocations in the boundaries. As the observed boundaries are flat and defect-free, we conclude that they are probably growth twins.

Figure 5. High resolution lattice image from a third order lamella with coherent twin boundaries.

In the present work we were not able to unambiguously identify defects of any other type. Some evidence was obtained for the presence of dislocations with line directions approximately parallel to the (110) plane normal. Attempts to resolve these features clearly using the weak-beam imaging geometry were thwarted, as the extinction distances for low order reflections in aragonite are rather large, typically on the order of 0.2-0.5 micron at the accelerating voltages available, and hence it was not possible to achieve a sufficiently large deviation parameter.

Possible Mechanisms for Microstructural Development

Although these studies were performed on a single example of the dried shell, well after the animal had died, the distinctive defect structure of the mineral phase does allow some deductions to be made about possible formation mechanisms. The twin distribution depicted in Figure 4a closely resembles that observed in cross sectional images of certain heteroepitaxial deposits e.g. Hails et al. [9], which show arrays of planar twins with a random distribution of thicknesses. These features are usually explained as resulting from "double positioning", a growth twinning

mechanism whereby twin-related material can nucleate on an advancing planar growth front if it is oriented such that the twin boundaries formed are coherent [10]. It is possible that an analogous mechanism is responsible for the formation of growth twins in *Strombus*. This would require that the third order lamellae grow by "epitaxial" deposition of CaCO_3 from solution onto a planar growth front parallel to (110).

Twinning in aragonite is of type I and occurs on (110) and (110) [11]. It is significant that twinning is only observed on (110) in third-order lamellae. This plane is parallel to [112], and hence the habit plane of the third-order lamellae will remain invariant across a twin boundary. This would not be the case for twinning on the (110) plane. Geological aragonite, on the other hand, is known to manifest "pseudo-hexagonal" morphologies owing to repeated twinning on both (110) planes [11]. The absence of this phenomenon in the shell strongly suggests that the orientation, morphology and defect structure of the grains is controlled by the organic matrix on which they precipitate. The uniformity of the orientation and morphology of third-order lamellae, and the systematic way in which they are assembled to form second-order lamellae, suggest that the protein phase acts as a template for precipitation in a manner analogous to epitaxial substrates.

The considerations discussed above are consistent with what is known about the organic matrix of crossed-lamellar shell in general, and with *Strombus* in particular. The morphology of the organic matrix in crossed-lamellar shells has been investigated by Uozumi et al. [12]. They found that first-order and second-order lamellae are enveloped by a continuous matrix phase. Weiner [13] showed that the *Strombus* matrix is very rich in acidic proteins. In fact, the acid-insoluble fraction that comprises the largest part of the matrix in most other mollusk shells was altogether absent. In other mollusk shells, and in some other hard tissues, highly acidic proteins have been suggested as important in regulating various aspects of mineralization [5], e.g., influencing the kinetics and orientation of mineral nucleation. These observations highlight the need for more information about the structure of the macromolecules at the mineral-matrix interface.

Electron beam irradiation effects

The main factor which limited these studies was pronounced electron radiation damage. Exposure to intense electron beams lead to the disappearance of some microstructural features and the generation of many artifacts. The nature of these effects was investigated by diffraction contrast imaging and selected area diffraction, and compared to the processes which have been observed in mineralogical aragonite [14]. Information was obtained from small clusters or individual particles after various irradiation times with no specimen tilting.

One such experiment, performed using an accelerating voltage of 200kV, is shown in Figure 6. Figure 6a is a bright field image of a cluster after less than 30 second exposure to the electron beam. Prominent twin boundaries can be observed in certain particles. In the corresponding SADP, most of the spots have spacings consistent with allowed reflections from aragonite. A few additional weak spots are present, e.g. at A. These exhibit spacings which correspond to diffraction from either vaterite (a metastable hexagonal polymorph of CaCO_3), or aragonite II, which is the designation given to the effective crystal structure in the immediate vicinity of (110) twin boundaries in aragonite [15]. Further work is required in order to clarify this issue. Figure 6b was obtained after about five minutes exposure. Twin boundaries which were present in Figure 6a have disappeared. The transition between these two states is extremely rapid and is accompanied by the motion of bend contours through individual particles suggesting that substantial distortion occurs. The SADP is markedly different; the most intense diffraction spots now correspond to reflections from the stable rhombohedral polymorph of CaCO_3 , calcite. As calcite is the equilibrium room temperature form of CaCO_3 , it would appear that the electron beam stimulates the reconstructive transformation from aragonite to calcite. Additional weak spots such as at B and C arise from Aragonite. Also present are three distinct continuous rings whose spacing corresponds to the first three allowed reflections from CaO indicating that there is some evolution of CO_2 . Subsequent irradiation gives a mottled appearance to the particles (Figure 6c) which becomes more pronounced after prolonged exposure. Continuous rings are now the dominant feature in the diffraction pattern and all of these correspond to CaO. The presence of rings as opposed to discrete spots indicates that the particles have now transformed to fine grained polycrystals. Intensity variations in certain rings suggests that there may be some texture to the polycrystalline oxide. HREM images from such particles indicate that the average oxide grain size is between 5 and 10 nm. We note that a few discrete spots from aragonite are retained in Figure 6c, which be due to restricted evolution of CO_2 from particles in the center of

thicker portions of the cluster.

Similar experiments were performed in other microscopes at 120kV and 400kV; the effects described above occurred after much shorter exposures at higher voltages. Whilst this suggests some dependence of specimen sensitivity on accelerating voltage, there may be various other factors involved such as beam convergence and source brightness. However, the practical consequences of these effects was to limit the maximum time for which a particular particle could be studied to 3, 15 and 30 minutes for voltages of 400, 200, and 120kV respectively. In this context we have considered this limit as the average exposure time before the onset of the aragonite - calcite transformation.

Further experiments were performed at 120kV using a liquid nitrogen cold stage to cool the specimen to $\approx -180^{\circ}\text{C}$. Under these conditions no particles transformed even after exposures of >200 minutes, suggesting that the observed changes are due to localized specimen heating under the electron beam.

Figure 6. The effect of exposure to the electron beam on a cluster of third order lamellae. Bright field images and corresponding diffraction patterns after (a) less than one minute, (b) approximately five minutes and (c) over ten minutes, irradiation under a focussed 200kV electron beam.

There are several distinct differences between the observations presented here and those of Burrage and Pitkethly [14] in geological aragonite. While they also found that aragonite transforms to calcite which subsequently decomposes to CaO, in many cases the first effect of electron beam heating was the removal of defects by the migration of subgrain boundaries in the aragonite. Although these boundaries are not present in the shell microstructure, the disappearance of *twin* boundaries is observed early in the damage process. Burrage and Pitkethly also found a definite orientation relationship between the CaO and the calcite from which it forms, this is clearly not the case in our studies. This difference may be related to the fact that our aragonite specimens are small grains dispersed on a carbon grid, and such specimens would be expected to heat up more quickly under the electron beam than a conventional perforated bulk TEM specimen (a monolithic sample can conduct some heat away from the exposed region). This argument is consistent with Burrage and Pitkethly's observation of an advancing interface between oxide and carbonate, while we observe the development of a fine grained dispersion of oxide over the entire grain.

CONCLUSIONS

Our studies have shown that the shell from the adult mollusk *Strombus gigas* has a highly ordered form of the crossed-lamellar microstructure. Each level of this hierarchical microstructure is composed of a well oriented arrangement of smaller lamellae; from macroscopic layers through first- and second- to third-order lamellae. The third-order lamellae are single crystal laths of aragonite with a fine organic grain boundary phase surrounding them. The axes of these lamellae are well aligned with prominent crystallographic directions and arrays of planar, coherent twin boundaries are present on the (110) planes, which lie perpendicular to the habit plane. These boundaries are atomically flat and dislocation-free, suggesting that they form by a process analogous to double positioning in epitaxial growth. These observations imply that the morphology, orientation and defect microstructure of the mineral component are determined by the organic matrix on which they precipitate. The effect of electron beam irradiation on our specimens is to stimulate first the reconstructive transformation from aragonite to calcite, then the evolution of CO₂ to give CaO. These phenomena are consistent with being the effects of electron beam heating.

ACKNOWLEDGEMENTS

The authors would like to thank S. Weiner and A.I. Caplan for useful discussions. This work was supported by the DOE through a grant awarded to the Pacific Northwest Laboratory of the Batelle Memorial Institute.

REFERENCES

- [1] J.D. Taylor and M. Layman, *Paleontology* **15**, 73 (1972).
- [2] J.D. Currey and A.J. Kohn, *J. Mater. Sci.* **11**, 1615 (1976).
- [3] V.J. Laraia and A.H. Heuer, these proceedings.
- [4] O.B. Bøggild, *K. danske Vidensk. Selsk. Skr. Copenhagen* **2**, 232 (1930).
- [5] H.A. Lowenstam and S. Weiner, *On Biomineralization*, (Oxford University Press, Oxford, 1989) p.29.
- [6] W.J. Kennedy, J.D. Taylor and A. Hall, *Biol. Rev.* **44**, 499 (1969).
- [7] G. Bevelander and H. Nakahara, *The Mechanisms of Biomineralization in Animals and Plants*, Ed. M. Omori and N. Watanabe, (Tokai Univ. Press, Tokyo, 1980) p.19.
- [8] R.W. Cahn, *Adv. Phys.* **3**, 363 (1954).
- [9] J.E. Hails, G.J. Russell, P.D. Brown, A.W. Brinkham and J. Woods, *J. Crystal Growth* **86**, 516 (1988).
- [10] M.J. Stowell, in *Epitaxial Growth*, Ed. J.W. Matthews, (Academic Press, New York, 1975) p.458.
- [11] W.L. Bragg, *Proc. Roy. Soc. Lond.* **A105**, 16 (1924).
- [12] S. Uozumi, K. Iwata and Y. Togo, *Journal of The Faculty of Science Hokkaido University*, Series VI, *Geology and Mineralogy* **XV**, 447 (1972).
- [13] S. Weiner, *Calcif. Tissue Int.* **29**, 163 (1979).
- [14] J. Burrage and D.R. Pitkethly, *Phys. Stat. Sol.* **32** 399 (1969).
- [15] Y. Hiragi, S. Kachi, T. Takada and N. Nakanishi, *Bull. Chem. Soc. Japan* **39**, 2361 (1966).

END

DATE FILMED

12 / 31 / 90