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Nanostructure and crystallography of aberrant columnar vaterite in *Corbicula fluminea* (Mollusca)

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ABSTRACT

Both the crystallographic and nanostructural organisation of aberrant columnar vaterite occurring in *Corbicula fluminea* were characterised in detail for the first time using electron microscopic and X-ray powder diffraction techniques. At the millimetre scale, only a confinement of the otherwise randomly oriented *c*-axis to the growth surface is observed. Domains of 100 or more individual vaterite columns with common *c*-axis orientation exist within this disordered material. Each column behaves as a single crystal on the scale of EBSD measurements, but is internally composed of smaller irregularly shaped and slightly misaligned crystalline units (0.3–1.3 μm in dimension). These are in turn partitioned by porous boundaries into rounded nanodomains, up to 600 nm in size. The geometry of the nanodomains and their respective boundaries might suggest formation by the accretion of vesicles. In addition to crystallographic textures, this observation indicates formation under significant biological control with wider implications for possible causes of the condition.

1. Introduction

The detailed characterisation of biomineralised structures on the micro- and nanometre scales is of great interest to the Materials and Earth science communities alike since it might lead to a better understanding of their mode(s) of formation (Cartwright and Checa, 2007; Erben and Watabe, 1974; Jacob et al., 2008). This might in turn allow the development of new, highly advanced materials (Noll et al., 2002; Tang et al., 2003) and has a bearing on the fractionation of stable isotopes used in palaeoclimate reconstructions (Cohen and McConnaughey, 2003; Erez, 2003; Weiner and Dove, 2003). Microstructural variations can also to some extent be used in phylogenetic analyses of, for instance, corals (Debrenne et al., 1987), hominids (Rozzi, 1998) and molluscs (Chateigner et al., 2000). Both physical organisation and crystallographic textures are of interest in these contexts (Chateigner et al., 2000; Wilmot et al., 1992).

In this study, a recently discovered pathological condition affecting the hard tissue of the invasive heterodont bivalve *Corbicula fluminea* has been investigated in more detail. The condition involves the production of large volumes of vaterite, a rare polymorph of calcium carbonate (Bentor et al., 1963; Grasby, 2003; Giralt et al., 2001; Lowenstam, 1981; McConnel, 1960), as prominent bulges in the shells of these animals which normally produce aragonite only (Frenzel and Harper, 2011; Spann et al., 2010). This is of interest not just because of the low stability of vaterite with respect to aragonite and calcite (Johnston et al., 1916; Plummer and Busenberg, 1982; Rao, 1973; Turnbull, 1973), but also because possible causes of the condition remain obscure, with recent populations only being affected in part of Egypt and south east England (Spann et al., 2010) although the originally Asian species now occurs in many more parts of the world, most notably the Americas and Europe (Aldridge and Müller, 2001; Carlton, 1992; Counts, 1986; Elliott and zuErmgassen, 2008; Howlett and Baker, 1999). A detailed microstructural and chemical characterisation of this biogenic vaterite was given by Frenzel and Harper (2011) but neither crystallographic nor nanostructural studies were conducted. The objective of this work was therefore primarily to investigate more closely:

(1) the physical organisation on submicron length scales of the vateritic material produced by *C. fluminea* and
(2) the crystallographic textures present within this material at all length scales.

This was done exemplarily for the columnar vaterite of the outer shell layers (cf. Frenzel and Harper, 2011). The collected data, in conjunction with what is already known, might shed further light on the nature of vaterite production in *Corbicula* putting constraints onto possible causes of the condition.

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2. Materials and methods

2.1. Specimens

All specimens were collected alive from the River Thames at Richmond, London, UK (51.434235°N, 0.327564°W) in September 2010. They were either shucked and immersed in ethanol in place, or later frozen. The soft tissue was removed and the shells air-dried, labelled and catalogued.

2.2. X-ray textural analyses (XRD)

Shell fragments of varying size (2–5 mm across) were cut in the desired orientation (see Fig. 2 of Frenzel and Harper (2011) for cutting modes) using a Dremel Multipro 395 multi-purpose rotary tool. They were then glued onto glass slides using Super Glue® in clusters larger than 1 cm^2, lapped flat, and the excess glass around the clusters cut off. Samples prepared in this way were mounted on a Bruker AXS D8 advance X-ray powder diffractometer (Bragg–Brentano symmetry) and diffractograms recorded (2θ from 5° to 90°) while rotating the samples about the diffraction vector. Data were collected for three radially, three longitudinally, four transversely and cut samples, and also four powders. The collection of powder data was conducted primarily to test the goodness of fit provided by the several structural models proposed for vaterite in the literature. Rietveld refinement of the collected data was performed using the General Structure Analysis System (GSAS) software package in its graphical user interface implementation (EXPGUI).

2.3. Electron microscopic techniques

Radial shell fractures were prepared for examination with a JEOL JSM 820 scanning electron microscope (SEM) at a voltage of 20 kV. Contact of the fracture surfaces with water was avoided and loose material blown off with compressed air. All samples were sputter-coated with gold.

Standard optical thin sections (one radial, one transverse) were polished (0.5 μm diamond slurry, 0.02 μm colloidal silica) and lightly carbon coated for EBSD measurements with a JEOL 6340F FEG-SEM (25 kV). The HKL CHANNEL 5 software package was used for data collection and analysis.

For transmission electron microscopy (TEM), thin sections (40–60 μm thick; radial, longitudinal and transverse) were prepared of shells embedded in epoxy resin, using a thermoplastic, acetone soluble adhesive (Crystalbond® 509) as a bonding agent. TEM-grids (Cu, 2.3 mm, 200 mesh) were then glued onto the regions of interest using epoxide glue, cut out and floated off by soaking in acetone. Further thinning was done by double-sided ion-milling using an Iontech 306 A atom mill (Ar-ions, 5.2 kV, 100 μA, 4–10 h). Finally, samples were very lightly carbon coated for examination with a JEOL 100SX EM at 100 kV. The Web Electron Microscopy Applications Software (WebEMAPS) (Zuo and Mabon, 2004) was used to simulate SAED patterns. Five grids were examined in total (two transverse, two longitudinal and one radial).

3. Results

3.1. Crystallographic textures of vaterite on the millimetre to micrometre scales

Optical examination of thinned radial and longitudinal TEM sections showed vaterite columns to have straight extinction (Fig. 1) and be length fast indicating that the vaterite crystallites within this structure are oriented with their *c*-axis preferentially parallel to growth surfaces. The size and relative orientation of domains with common *c*-axis orientation could be estimated from transverse sections and was found to range between 50 and 200 μm in diameter. *C*-axis orientation within domains seemed to be random, i.e. to show neither a preferred radial nor longitudinal alignment.

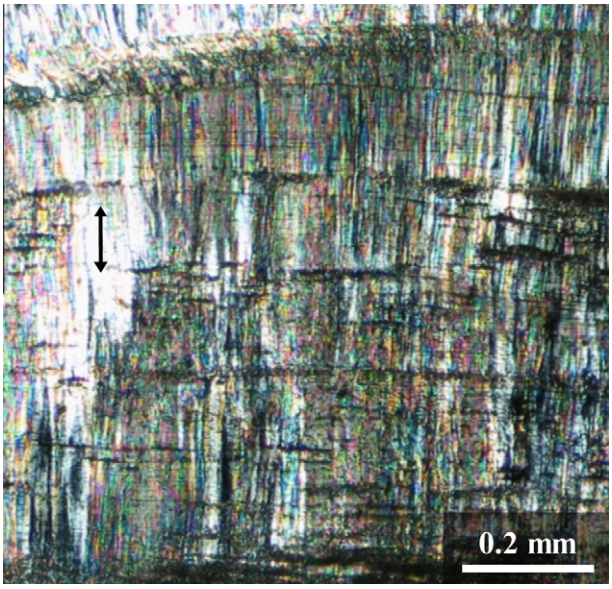

Fig.1. Optical micrograph of radially sectioned vateritic shell (crossed polarisers), illustrating straight extinction of the vaterite columns. The double headed arrow indicates the polarisation direction of the analyser.

Collected X-ray data were of generally rather mediocre quality ($1.1 < \chi^2 < 1.5$ and $0.25 < R_p < 0.40$ for fitted data), but good enough to extract some structural and textural information. The best fits for the powder data were obtained using the average carbonate disordered structure ($P6_3/mmc$) of Kamhi (1963) modified according to Meyer (1969). Very weak superlattice reflections could be detected around $2\theta = 39°$, but not fitted by any of the proposed ordered structures (Le Bail et al., 2011; Wang and Becker, 2009). Convergence of fits could be attained allowing the lattice parameters and isotropic temperature factors, but not the atomic coordinates to vary. The average lattice parameters extracted from the present analyses were $a = 4.129 \pm 0.006$ Å and $c = 8.462 \pm 0.028$ Å for the disordered cell.[1] These values are within error of those given by previous investigators (Kamhi, 1963; Meyer, 1959, 1969; Olshausen, 1925).

For textural analyses of diffraction data, the spherical harmonics (SH) preferential orientation correction within GSAS was used (Von Dreele, 1997; Whitfield, 2009). This was thought to be more suitable in the present case than the March–Dollase approach (Dollase, 1986; March, 1932) for several reasons, specifically:

(1) No prior knowledge of any existing texture is required.
(2) Correlations between different planes do not have to be separately considered and no assumptions are made concerning the nature of these correlations.
(3) No fixed shape for the orientation distribution profile is imposed.
(4) Parameterised fitting functions are mutually orthogonal giving good convergence properties.

[1] Uncertainties given equal two standard deviations, calculated from variations between measurements. Estimated standard deviations calculated by GSAS for individual datasets were smaller by one or two orders of magnitude.