Overcoming the problems of high-resolution transmission electron microscopy of biogenic aragonite

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Abstract

High-resolution transmission electron microscopy of biogenic carbonate minerals is hampered by a lack of stability during exposure to the electron beam. However, aragonite in bivalve shells may be successfully imaged using a modification of the 'minimal-exposure technique' of Williams and Fisher (1970). Diffraction patterns taken before and after beam exposure indicate that the aragonite remained stable during imaging. The procedure described here should prove useful for further studies of the ultrastructure and/or the diagenesis of biogenic carbonate minerals.

Keywords: transmission electron microscopy, carbonate minerals, aragonite.

Introduction

High-resolution transmission electron microscopy (HRTEM) is a valuable tool with which to study the ultrastructure of both inorganic and organic compounds. Application of HRTEM techniques to the study of carbonate shells and other biogenic components can provide important insights into their biological building processes. In addition, HRTEM can reveal exceptionally fine-scaled features which could otherwise be overlooked during normal petrographic analysis. Such techniques may prove invaluable in achieving an understanding of diagenetic processes, including the transformation of aragonite to calcite ('neomorphism'; Bathurst, 1975).

To date, most HRTEM studies of carbonate minerals have been conducted on synthetic or inorganically precipitated crystals: for example, reference is made to Barber *et al.* (1981), Wenk and Zhang (1985), and Mieke *et al.* (1988) for their work on dolomite and to Gunderson and Wenk (1981), Frisia Bruni and Wenk (1985) and McTigue and Wenk (1985) for their work on aragonite and calcite. Mann *et al.* (1983) however, have studied organically precipitated aragonite by HRTEM, specifically looking at otoliths and octonia (balance organs within the inner ears of, respectively, fish and frogs). Inorganic aragonite was found to be quite stable under usual imaging conditions (i.e. 200 kV electron beam from a high-brightness LaB_6 filament). By comparison, 'biological' aragonite was found to be unstable under these conditions when exposed for a prolonged, but unspecified, period of time. Frog octonia were found to be the least stable, disintegrating completely, whilst fish otoliths displayed an apparent lowering of symmetry. Lattice fringes of the aragonite in these two samples were resolved at 0.5 to 0.6 nm.

Here, the preliminary results of a study examining the diagenetic alteration of aragonitic bivalves are reported. Specifically, we present a method by which biogenic carbonates can be studied by HRTEM, and explore the viability of HRTEM techniques for studying the diagenetic alteration of aragonite.

Description of samples

Aragonite and calcite samples were prepared from bivalve specimens of the genus *Eumarcia* (*E. plana* Marwick) and *Glycymeris* (*G. (Glycymerula) modesta* (Angas) and *G. (Veletuceta) shrimptoni* Marwick) collected from Pleistocene sediments (*ca.* 1 million years BP) exposed in the Tangoio block near Napier, New Zealand (Haywick, *et al.*, in press). Both these taxa are large bivalves, measuring up to 5 cm in size and 0.7 cm in thickness. Petrographic analysis shows these taxa to have a dominantly cross-lamellar shell-structure (cf. Taylor *et al.*, 1969). As in all Mollusca, individual aragonite crystallites are held

Mineralogical Magazine, December 1990, Vol. 54, pp. 589–592
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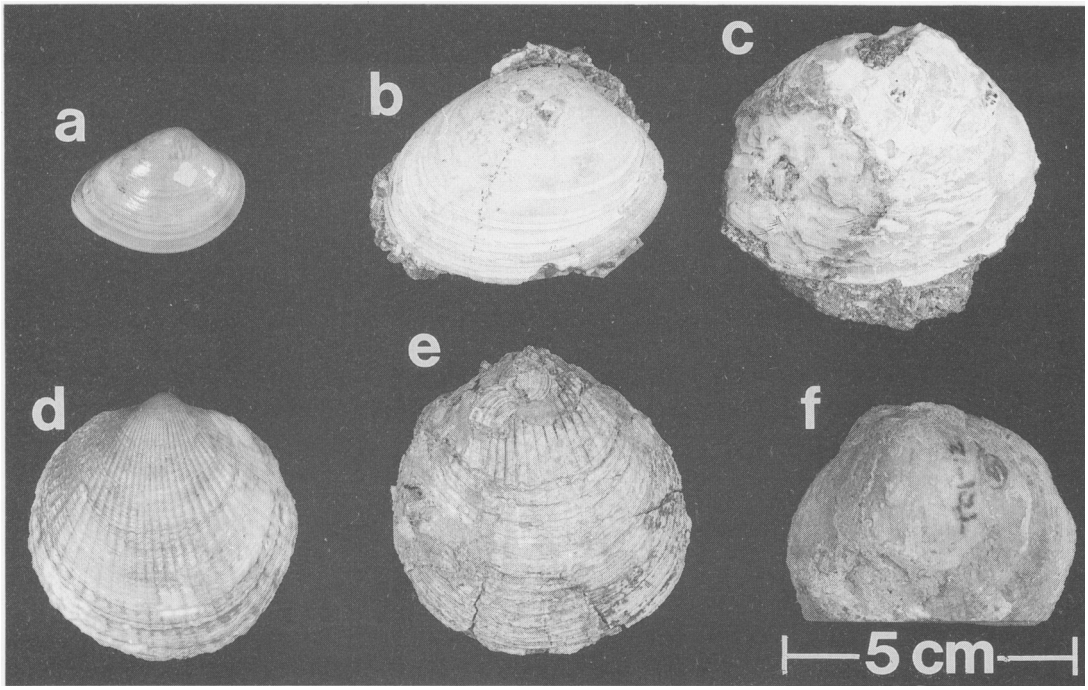

FIG. 1. Stages of diagenetic alteration within aragonitic bivalves of the genera *Eumarcia* (a—pristine, b—chalky, c—recrystallized) and *Glycymeris* (d—pristine, e—chalky, f—recrystallized).

together by an intimate matrix of organic conchiolin (Bathurst, 1975).

The bivalve specimens studied were variously altered, primarily because of meteoric diagenesis (Fig 1.). Some shells analysed were only slightly altered (near-pristine), with only minimal loss of some conchiolin in their outer layers, which is reflected in a 'chalky' surficial coating. Other specimens were completely replaced by secondary calcite. In total, the *Eumarcia* and *Glycymeris* samples examined in this study represent a complete spectrum of aragonite diagenesis, as fully discussed in Haywick and Ness (in prep.).

HRTEM preparation

Suitably-sized crystal fragments of chalky aragonite were prepared for examination in the transmission electron microscope (TEM) by grinding samples under acetone in an agate mortar with a pestle. A small portion of the resultant acetone-powder suspension was floated onto a holey-carbon support film on a 3 mm copper grid and allowed to dry.

A JEOL 2000FX TEM operating at a gun voltage of 200 kV with a conventional tungsten filament was used for both preliminary electron diffraction studies and for the subsequent high-resolution work. To facilitate the examination of crystals in various orientations, a side-entry, double-tilt (+/−25°) holder was used.

To obtain the lattice fringe images of aragonite, a modification of the 'minimal-exposure' technique of Williams and Fisher (1970) was developed. The beam current was reduced to a low intensity (approximately 110 μA, 5μA above the resting or 'dark' current): a suitable diffraction pattern was correctly orientated using standard selected-area procedures and photographed (see for example, Fig 2*b*). An objective aperture was then selected which would include only the diffracted beams required for the image, inserted, and the area of interest was moved out of the beam. Voltage centre alignment, correction of the objective astigmatism and focusing were all performed on an adjacent patch of carbon film. The area of interest was then returned to under the beam, focusing checked and a through-focal series of photographs was taken. The objective aperture was removed and another diffraction pattern was then recorded. Total beam exposure on the area

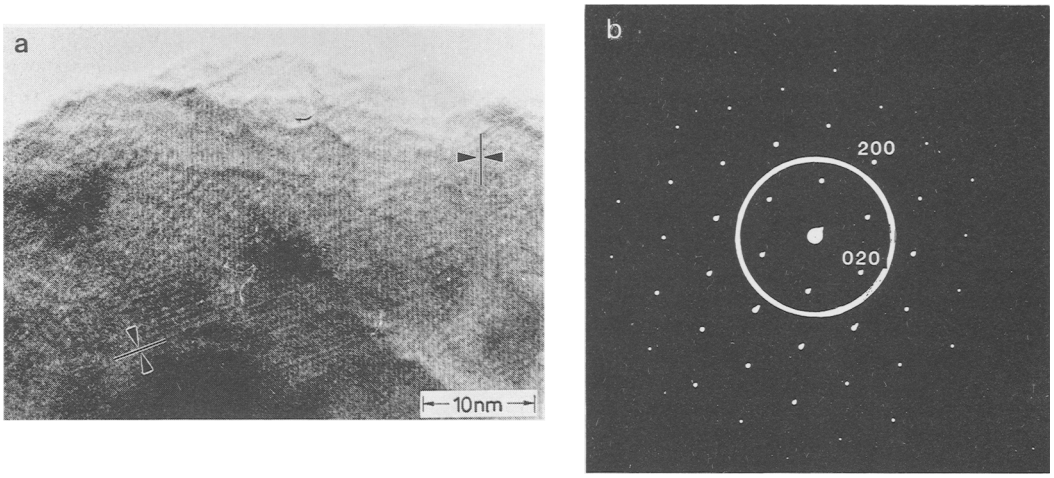

FIG. 2. (*a*) Lattice fringes from a portion of an aragonite crystal. (*b*) Corresponding electron diffraction pattern, [001] zone, of aragonite crystal shown in (*a*). The circle indicates the position of the objective aperture.

of interest during this procedure was a maximum of five minutes.

Results and discussion

Samples considered to be worthy of examination by HRTEM were initially identified through a combination of petrographic and X-ray diffraction analysis. During HRTEM, areas of interest were identified by their diffraction patterns. This necessitated obtaining a series of reference patterns of aragonite and other carbonate mineral species prior to the study of the bivalves. These were indexed based on parameters obtained from the J.C.P.D.S.X.R.D. data file for aragonite (card no. 5–0453).

The experimental procedure outlined above provided high-resolution lattice fringes of biogenic aragonite at a resolution never before obtained (0.40–0.42 nm spacing; Fig. 2*a*). Comparisons of the diffraction patterns before and after HRTEM imaging showed no signs of disintegration, and we conclude that the aragonite remained stable during exposure to the electron beam. In addition, resultant micrographs show no apparent beam damage to the surface of the crystals. In particular, there was no apparent lowering of symmetry as shown by Mann *et al.* (1983), possibly due to shorter exposure times used in our procedure. However, this reasoning must remain speculative because Mann and his coworkers do not specify over what period of time the damage to their aragonite occurred.

Conclusions

Preliminary results demonstrate that at least some biogenic carbonate minerals can be examined by HRTEM without stability problems. This has important connotations for both biological and geological studies of carbonate secreting organisms.

Application of our HRTEM technique to a variety of biogenic materials will allow subtle differences to be defined between them, and may thereby lead to new insights into calcification.

Carbonate minerals, especially aragonite, are prone to transformation to other carbonate species depending upon their inherent stability within particular diagenetic fluids. Aragonite, although metastable in seawater, is unstable in meteoric or freshwater, in which it will quickly dissolve or alter to a more stable carbonate phase (i.e. calcite). This latter transformation has been well documented in limestones of various ages around the world (e.g. Bathurst, 1975; James, 1974; Pingitore, 1976; James and Choquette, 1984), but there is little hard data relevant to the mechanisms by which this transformation is actually accomplished. Applications of the HRTEM procedure that we outline here to the aragonite–calcite transformation have the potential to clarify processes operating at an extremely fine scale. Such work is presently in progress utilizing Molluscan skeletal material obtained from the Tangoio block in New Zealand (Haywick and Ness, in prep.).

Acknowledgments

This study was supported in part by grants provided by the Great Barrier Reef Marine Park Authority (GBRMPA grant no. 125431 to C. Cuff) and the Australian Research Grant Scheme (ARGS grant no. 128732 to C. Cuff and R. A. Henderson). DWH acknowledges a postgraduate scholarship administered by the Australian Commonwealth Scholarship and Fellowship Plan. We thank Prof. R. M. Carter and Dr R. A. Henderson for comments regarding the draft manuscript, Mr H. Simmons for his assistance with the TEM and Mr G. McNamara for his help with word processing.

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[*Manuscript received 26 March 1990: revised 14 May 1990*]