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The identification of growth lines in abalone shell using a nuclear microprobe

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Abstract

Ionoluminescence (IL) combined with particle induced X-ray emission (PIXE) imaging has been employed to identify intrinsic growth bands in the spire region, and extrinsic bands at the growth edge of Australian Black-lip abalone shell (*Haliotis rubra*). Previous studies using optical flood cathodoluminescence, scanning electron microscope cathodoluminescence (SEM-CL) and Raman spectroscopy on samples from the same population suggest that the visible luminescence is due to Mn^{2+} activated calcium carbonate. In this study we confirm Mn^{2+} as the activator in both the spire and growth edge regions of the shell. The sensitivity of ionoluminescence to the co-ordination environment of the Mn^{2+} activators in the shell allows for the spatial identification of the calcium carbonate polymorph responsible for the growth lines observed optically. Furthermore the detection and mapping of trace elements such as Mn and Sr with the PIXE technique enables comparisons to be made between calcite and aragonite biomineralized in the wild and under laboratory conditions.

1. Introduction

There has been a lot of recent interest in studying biomineralization in shell fish, in particular the ability for biological systems to switch between calcium carbonate polymorphs [1–4]. Such studies could eventually lead to controlled biofabrication in the laboratory. The characterisation of the structure and impurity trace element distribution in abalone shell could also assist in deriving methods for determining the age of abalone populations, help to further our knowledge on the effect of environment and physiology on the shell microstructure and to understand the biological control of biomineralization.

The development of an efficient method for aging abalone has important implications for the management of fisheries [5]. A method for aging mollusc populations suggested by Munoz–Lopez [6] involved counting the growth rings laid down in the spire of Mexican abalone which were thought

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to be deposited annually. Prince et al. [7] found that rings in *Haliotis Rubra* were laid down annually corresponding to sea water temperature minima. Shepherd however, while studying *Haliotis fulgens* measured an annual deposition of three rings corresponding to sea temperature maxima, minima and the spawning season [8]. Further studies since by a number of workers have shown the technique to be problematic [9,10]. Its failure has been attributed to the fact that the frequency of growth line deposition varies between populations and also depends on the habitat and environment of the abalone. Furthermore the presence of boring predators and the effect of erosion was also found to impact on the shell ring structure [11]. In order to try to resolve some of these issues it was deemed important to characterize the ring structure at the microstructural level.

Scanning electron microscopy has been used to great effect in a number of studies to characterise the nacreous and prismatic layers in mollusc shells [8,12]. Using backscattered and secondary electrons and cathodoluminescence imaging, the size of the ring structure has been shown to vary between 10–100 μm. The presumed activator of the luminescence in biogenic carbonates Mn^{2+}, can activate luminescence at concentrations as low as 20 ppm [13]. Trace elements such as Fe and Sr which are quite common in sea water, are known to quench luminescence or in the case of Sr, shift the peak of the characteristic luminescence to longer wavelengths. This was shown by Sommer [14] who studied the aragonite–strontianite and calcite–magnesite solid-solution series with X-ray induced luminescence, and CL performed with an electron microprobe.

Concentrations of Mn in the range 10–100 ppm is below the practical detection limit of electron microprobe techniques. The keV ion microprobe (SIMS-secondary ion mass spectrometry) on the other hand has the required trace element sensitivity and spatial resolution, however, problems with molecular interference and surface sputtering restrict the accuracy of the technique. Clearly nuclear microprobe imaging techniques such as PIXE and IL offer advantages to studying both the micron scale structure and the trace element distribution in such samples.

Previous nuclear microprobe studies by Fraser et al. [15] using PIXE showed the technique to be useful for measuring micron sized zonation of Mn, Fe and Zn in dolomite samples. The chemical

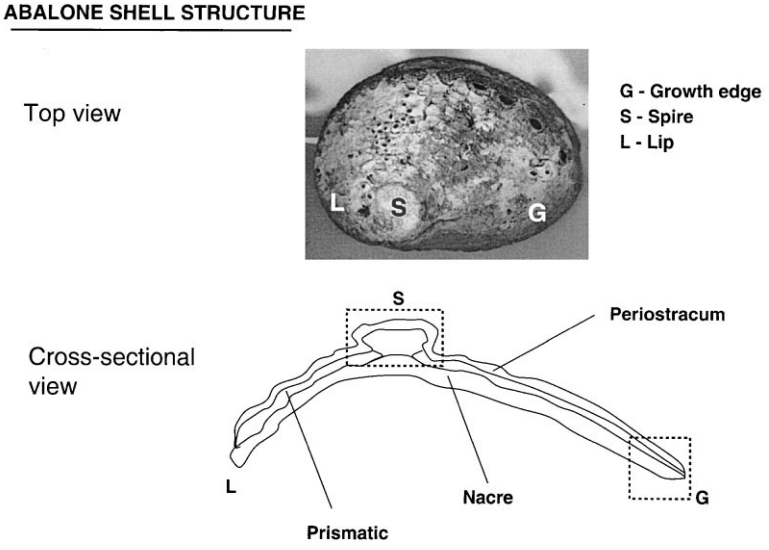

Fig. 1. Photograph of the Abalone shell structure as viewed from the top and a schematic cross-sectional diagram identifying the regions studied by a dotted box.

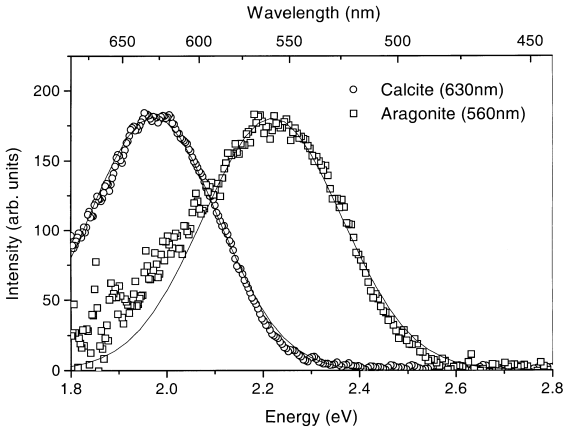

Fig. 2. Ionoluminescence spectra taken from red and green regions of the luminescent mark at the growth edge confirm that the shell is made up of calcite and aragonite, respectively. The spectra were collected at Melbourne and excited by 3 MeV protons.

information was however obtained by using CL. Combined PIXE and IL has been used in a number of nuclear microprobe studies to give chemical information on zonation in geological samples [16,17]. These combined techniques are used in this study to identify both the trace element distribution and the chemical environment of impurities in abalone shell.

2. Experiment

Cross-sections taken form the spire and growth edge regions of abalone shell were analysed using proton induced X-ray emission (PIXE) and ionoluminescence (IL) imaging and spectroscopy. The details of sample preparation, SEM and Raman analysis on these samples are published elsewhere [18].

Growth edge specimens were analysed with the Melbourne Nuclear microprobe in Australia using

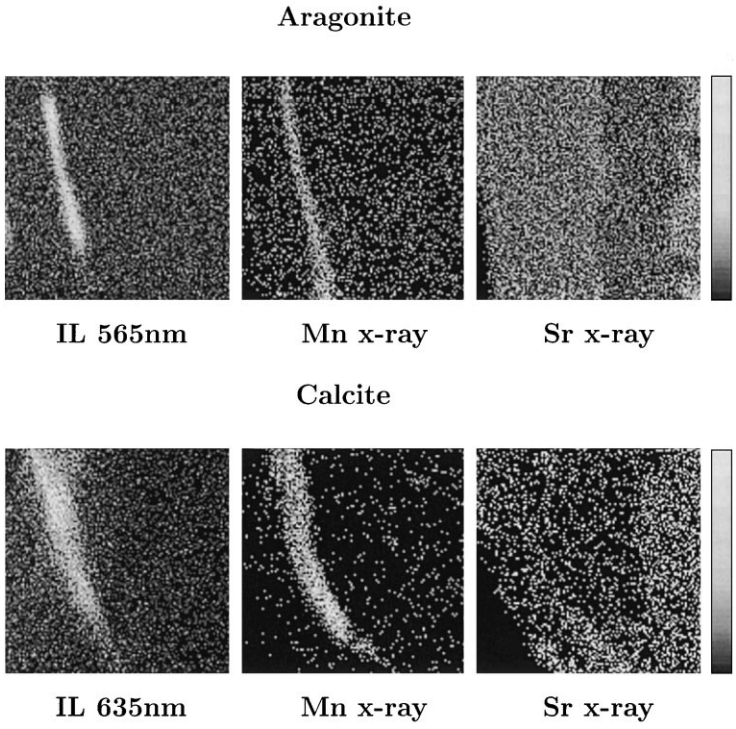

Fig. 3. Monochromatic IL and X-ray maps (400×400 μm) from the red (calcite) and green (aragonite) regions of the growth edge mark.

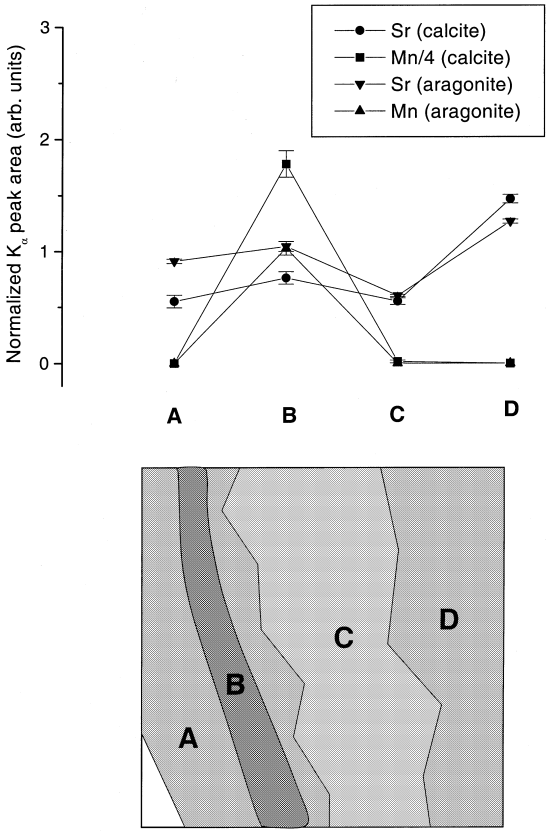

Fig. 4. Mn and Sr K_α peak areas extracted from four regions corresponding to (A) the inner surface of the shell, (B) the extrinsic luminescent mark, (C) a region adjacent to the mark and (D) the outer shell of the growth edge sample studied at Melbourne. Peak areas are normalized to the extracted area and charge. Note the area of the Mn K_α peak in the calcite region is divided by four in order to display the data on the same scale.

3 MeV protons. IL images were collected using a 943-2 Hamamatsu photomultiplier tube coupled to a monochromator with a bandpass set to 5 nm. IL spectra were acquired with an Ocean Optic SD1000 CCD spectrometer and corrected for the spectral response of the detector using a method described elsewhere [19].

Specimens from the spire region and the growth edge were analysed with the Lund nuclear microprobe in Sweden using 2.55 MeV protons. IL images were collected with a Hamamatsu R585 photomultiplier tube and a filter monochromator [20]. Spectra were collected with an Acton 1/4 m monochromator and a Hamamatsu PMA-50 cooled photodiode array detector. IL spectra collected at Lund were not corrected for the spectral response of the detector as efficiency curves were not available at the time of publication.

The term intrinsic in this study refers to a naturally occurring luminescent mark or growth line found in a shell. Luminescent bands introduced artificially by a marking technique are called extrinsic.

3. Results and discussion

The abalone shell consists of an outer prismatic layer made up of both calcite and aragonite and an inner nacreous layer containing dark growth rings under the spire of the shell which are deposited periodically [12,21,22].

Results from three abalone samples, two from the growth edge and one from the spire region are presented in this study. These regions are depicted schematically and represented by a dashed box in Fig. 1. The part of the shell denoted by G represents the growth edge of the shell which corresponds to the most recent shell deposited by the abalone. Immersing abalone in a non-toxic solution containing Mn^{2+} results in a luminescent marker being incorporated into the shell microstructure. These markers can be used to time stamp mollusc populations and serve as references for determining shell growth rates. Flood–CL photographs of the growth edge which have been published in a previous study [18] revealed the presence of two distinct regions in the marker luminescing in the green and red part of the spectrum. IL point spectra taken from these regions (Fig. 2) show broad Gaussian bands with peak wavelengths at 560 and 630 nm corresponding to Mn^{2+} activated trigonal aragonite and orthorhombic calcite, respectively. Monochromatic IL images from the aragonite and calcite regions were taken by setting the monochromator at wavelengths of 565 and 635 nm. These images in addition to images formed by mapping the Mn and Sr K_α and K_β X-rays are shown in Fig. 3. The Mn and Sr K_α X-ray peaks extracted from the four regions (shown in Fig. 4) were fitted and normalized to the extraction area and charge. Sr was

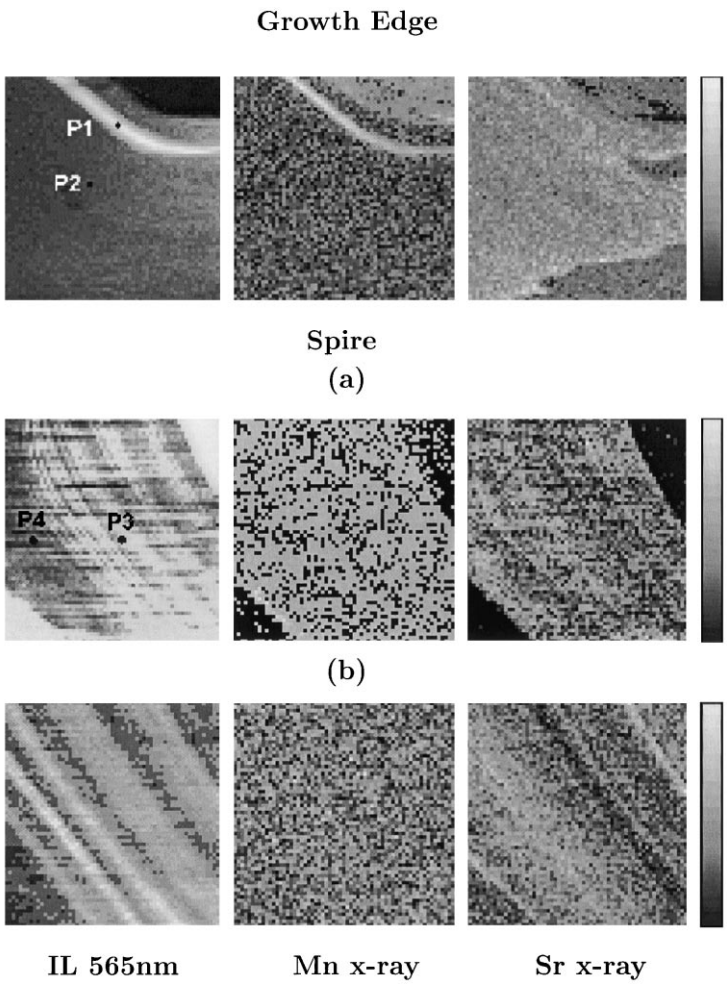

Fig. 5. Monochromatic IL and X-ray maps from the growth edge (500×500 μm) and the spire (a) 1×1 mm and (b) 500×500 μm showing points where IL and PIXE spectra were measured. No detectable Mn and very little luminescence was observed at points P2 and P4.

found in all parts of the shell at roughly the same concentration with a slight relative increase in region D which corresponds to the nacreous layer. Mn was entirely found in the mark (region B) in both the calcite and aragonite parts of the sample however in much higher concentration in the calcite structure.

The aragonite extrinsic mark was studied on a second sample. The images formed by mapping the 565 nm luminescent peak and the Mn and Sr K_α X-rays are shown in Fig. 5. Mn and Sr were again found to be the dominant trace impurities along with Br which is commonly found in sea water. The bright luminescent mark was found to correlate with the Mn impurities. The IL spectra taken from bright regions denoted by P1 and P3 on the IL map are shown in Fig. 6. The region of bright luminescence at the growth edge is comprised of two Gaussian bands, the most intense having a peak position at 570 nm. A second component with peak position at 615 nm seems to suggest that the region may also contain calcite. The relative amount of calcite and aragonite cannot be determined from this spectrum since the

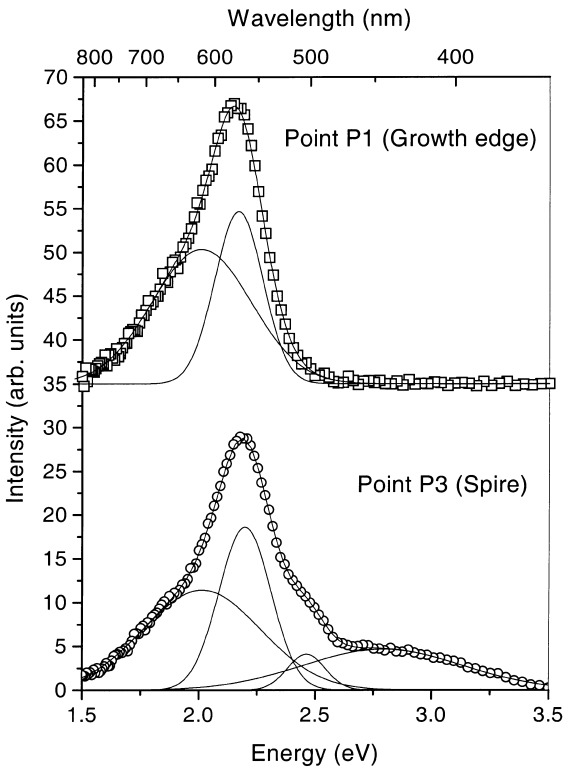

Fig. 6. IL spectra measured at points P1 in the growth edge sample and P3 in the spire region of the shell (shown in Fig. 5). Spectra were collected at Lund and excited by 2.55 MeV protons. Gaussian fits are also shown.

response curve for the spectrometer was not available at the time when experiment was performed. The PIXE spectra which were collected from the same points reveal the presence of Mn in the mark along with some Br and Sr.

The spire region of abalone is the oldest part of the shell. Under CL excitation a naturally occurring concentric blue–green luminescent ring structure is observed. IL monochromatic maps at 565 nm and X-ray maps of Mn and Sr of the spire at two different magnifications are shown in Fig. 5. For comparison with growth edge data, IL and X-ray spectra were measured at two different points denoted on the IL map as P3 and P4. In addition to the familiar Mn activated calcite and aragonite bands, two further bands of unknown origin were found in the spectrum (Fig. 6). The blue luminescence in shells is sometimes attributed to the organic material which is found in the shell structure. No conclusion about the nature of this luminescent band can be made at this point. The dominant luminescent band is due to aragonite and evidence for Mn being present in trace amounts in the spire was found in the corresponding X-ray spectrum.

4. Conclusion

From this study there is some evidence to suggest that Mn may be found in higher concentrations in regions of the shell which are made of calcite. A strong positive correlation between luminescence intensity and Mn trace element concentration suggests that Mn is indeed the activator of luminescence. Similarities between the IL spectrum from the extrinsic growth edge band which was artificially incorporated into the shell, and the intrinsic spire rings shows that the bands are composed of aragonite and calcite, and the mechanism for the luminescence activation is the same. Two more luminescent bands were observed in the spire region of the shell which could possibly be attributed to luminescent organic material.

The combination of IL and PIXE analysis with high resolution spatial imaging has provided a powerful tool for the identification and characterization of the structural properties of biogenic carbonates. The ability to simultaneously identify the calcium carbonate polymorph and the trace element distribution in samples could be extended to include the study of zonation in other natural carbonate minerals.

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