



Molecular signatures of biomacromolecules at micron and submicron scales in *Arctica islandica* shells

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ABSTRACT

Arctica islandica (Linnaeus, 1767) is a bivalve mollusk with a lifespan of up to 500 years. Its shell is a complex inorganic-organic nanocomposite, which is still not fully characterized. Here, we used Optical Photothermal Infrared (O-PTIR) microspectroscopy to characterize its organic phase, i.e. the hydrophobic insoluble extracts of both modern and fossil *A. islandica* shells. We compared the spectra from O-PTIR technique with those obtained from microRaman, microFTIR and ATR-FTIR spectroscopies. The structure of the shells was also studied using scanning electron microscope imaging. The spectroscopic techniques confirmed the presence of β -chitin and chitin-binding protein in the hydrophobic insoluble organic matrix. The results of element and light stable isotope analyses ($\delta^{13}\text{C}$, $\delta^{15}\text{N}$) from the hydrophobic insoluble extracts of both the modern and fossil shells were consistent with a shallow marine environment. The results demonstrate how O-PTIR can be applied in-situ for the study of biomaterials in shells, and points towards how it can be applied for the elucidation of intricate structure-function relationships. Furthermore, the approach of using the hydrophobic insoluble extracts to probe conditions and the nature of organic remains in fossil shells demonstrate an alternative to the use of soft tissues in isotopic studies and hence opens new possibilities for environmental reconstruction using fossil material when soft tissues are unavailable.

1. Introduction

Bivalves have intricate shell ultrastructures, which are nanocomposite biominerals, arranged in distinct shell layers (Skinner and Jahren, 2003). The shell itself is made of calcium carbonate (typically aragonite and/or calcite in different layers) and an organic matrix (Marin et al., 2007). The unique composition and structure of the organic matrix and mineral phase enable bivalve shells to perform their protective functions effectively (Kennedy et al., 1969) while also serving as unique objects for biomineralization studies and potential inspirations for advanced materials design. The organic phase in bivalve shells typically does not exceed 5 % of the total shell weight (Agbaje et al., 2017), with a substantial portion comprising hydrophobic insoluble biomacromolecules (Agbaje et al., 2018; Furuhashi et al., 2009a). Organic matrix has been extensively studied in several species,

especially those with nacreprismatic shell structures, such as *Pinctada* and *Atrina* (Nudelman et al., 2008; Furuhashi et al., 2009b). Based on the characterization of nacreprismatic shells, several models have been proposed to explain the organization and composition of the organic phase (Levi-Kalisman et al., 2001; Furuhashi et al., 2009b). The insoluble fraction in such shells is proposed to consist of relatively stiff and strong β -chitin fibers and a hydrophobic silk protein, coated with a complex assemblage of hydrophilic proteins (Weiner and Traub, 1980). Characterization of the acid insoluble fractions has been limited and so far, mainly focused on deriving histochemical data and biochemical analyses of biomacromolecules between (inter-crystalline) and within (intra-crystalline) biominerals of nacreprismatic shells (Bezares et al., 2012; Nudelman et al., 2008; Weiss et al., 2006). However, the nacreprismatic architecture is just one among several shell structures.

The ocean quahog *Arctica islandica* (Linnaeus, 1767) (Bivalvia;

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