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Research paper

Molecular and isotopic composition of foraminiferal organic linings

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ABSTRACT

Fossil remnants of benthic foraminifera consist of carbonate tests and their organic linings. The macromolecular and stable isotopic composition of these benthic foraminiferal organic linings was characterized to evaluate their potential use as paleoclimate proxies. Using Curie point pyrolysis–GC–MS (Py–GC–MS) we show that benthic foraminiferal organic linings consist of protein and polysaccharides, bound together in a complex macromolecular structure. Both chitin derivatives and traces of guaiacols and syringols, usually assigned to lignin are found. Although the five species of benthic foraminifera all contain chitin derivatives and proteins, the relative contribution of these compounds tends to vary considerably. Oxygen stable isotopic analyses of the organic linings of the benthic foraminiferal species *Ammonia tepida* indicates that $\partial^{18}O_{OL}$ values are in line with fractionation between seawater and organic matter. In contrast a $\partial^{13}C$ deliberate tracer experiment showed that metabolic carbon is the main source for the carbon fixed in the organic lining. The different pathways of carbon and oxygen stable isotopes into the foraminiferal linings have important implications for future proxy development as they reflect different components of the environment compared to the carbonate bound stable isotopes. Still, the future application of benthic foraminiferal organic linings and their isotopic values critically relies on improvements in calibration and sample size required for isotopic analyses.

1. Introduction

Since the 1970's foraminiferal tests have successfully been exploited in terms of their stable isotopic composition in order to reconstruct past climate conditions (Shackleton, 1974; Erez and Luz, 1983; Bemis et al., 1998). Oxygen isotopes measured on foraminiferal tests record changes in ice volume, seawater temperature and salinity, whereas the carbon isotopes provide information on carbon cycling. Over the last two decades the trace metal composition of foraminiferal tests has become an important extra indicator of past oceanic conditions (Lea, 1993; Rosenthal et al., 1997; Reichart et al., 2003; Elderfield et al., 2010). For example, the Mg–Ca ratio is used to reconstruct past temperatures and B–Ca ratios reveal past changes in seawater carbonate chemistry. Whereas the proxy potential of the inorganic part of foraminifer tests has thus been extensively explored (Lea, 1993; Rosenthal et al., 1997; Reichart et al., 2003; Elderfield et al., 2010), relatively few studies exist on the proxy potential of the organic component of foraminiferal tests.

The organic component of benthic foraminifera can be divided into two parts, the cytoplasm and the organic matrix of the test. The organic matrix can further be divided into two operationally defined components, (1) the soluble organic matrix and (2) the insoluble organic matrix (hereafter known as the organic lining (OL)) (Fig. 1). While the cytoplasm degrades quickly upon death of the foraminifer, the soluble organic matrix and the OL remain intact (Stancliffe, 1989; Robbins and Brew, 1990). Studies of the soluble organic matrix show that it consists of proteins (Weiner and Erez, 1984; Robbins and Brew, 1990), while OLs are composed predominantly of polysaccharides with traces of proteins (Angell, 1967; Weiner and Erez, 1984), known collectively as 'glycosaminoglycans'. This compound is found in agglutinating, perforate and imperforate foraminifera (see Langer, 1992 and references therein). Most work on benthic foraminiferal OLs has focussed on the morphology of their remains in the fossil record (Stancliffe, 1989; Winchester-Seeto and Bell, 1999).

Benthic foraminiferal organic linings are often found preserved alongside pollen, spores and dinoflagellate cysts in palynological preparations, and at least partially maintain the internal test morphology of the original foraminifer (Fig. 1). To date, the macromolecular composition of OLs has not been studied systematically. Knowing the composition of OLs yields insight into their function, structure and susceptibility to diagenesis. Furthermore, the macromolecular composition of OLs can be compared to other resistant-membrane bearing organisms (Van Bergen et al., 2004; De Leeuw et al., 2006; Versteegh et al., 2007; Verbruggen et al., 2010), as a tool to gain insight into any evolutionary linkages between their biochemical pathways. The susceptibility of OLs to diagenesis can be constrained by comparing the macromolecular composition of extant and fossil foraminiferal tests. Doing so sheds light on the molecular preservation potential of OLs and their applicability as a paleoceanographic proxy. Constraining the macromolecular

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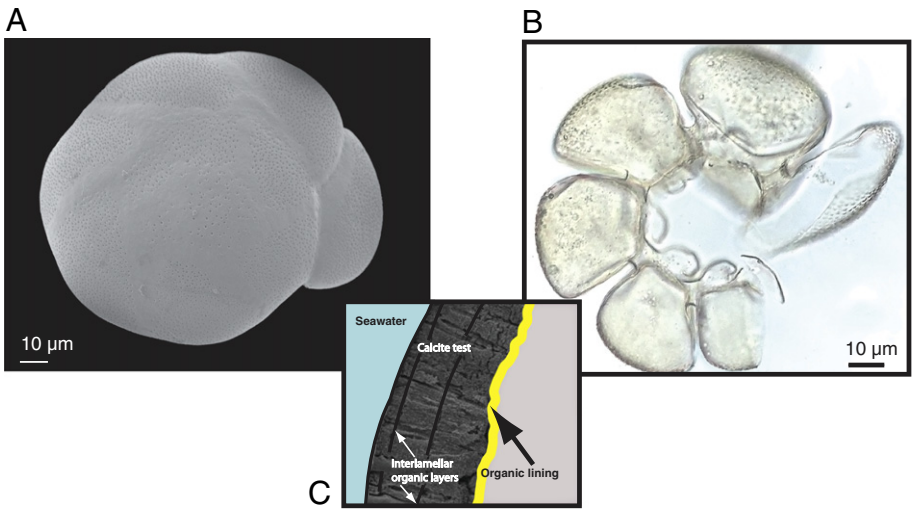

Fig. 1. Overview of organic lining structure in *Ammonia* sp. A. SEM image of *Ammonia* sp. B. Image of organic lining of *Ammonia* sp. C. Schematic cross section of *Ammonia* test (SEM courtesy of L. de Nooijer).

composition is also a prerequisite for testing the application of OLs as carriers of oxygen and carbon stable isotopic signals and susceptibility to isotopic alteration.

To date, carbon and oxygen isotope values of benthic foraminiferal OLs are unreported. Carbon and oxygen stable isotopes measured on decay-resistant organic matter are currently being developed for application in both marine (Sluijs et al., 2007) and lacustrine (Verbruggen et al., 2010) environments. In environments where carbonate is absent (due to calcite undersaturation), such information can provide insight into past environmental conditions. Whereas the oxygen stable isotopes of the organic matter can be related to the seawater the foraminifera grew in, carbon isotopes could reflect both food source and/or the isotopic values of seawater DIC. Additionally, by coupling oxygen isotope values from foraminiferal OLs and their carbonate tests, the first step could be taken towards the development of an independent proxy for past sea water $\partial^{18}O$ values.

In this study, the macromolecular and isotopic composition of foraminiferal OLs is constrained, giving insight into their potential as paleoceanographic proxies. The organic linings of four species of extant benthic foraminifer (*Ammonia* sp., *Sorites* sp., *Calcarina* sp., *Cycloclypeus* sp.) and one fossil foraminifer (*Amphistegina* sp.) are isolated and their macromolecular structure characterized using pyrolysis GC–MS. The organic test of *Gromia sphaerica*, a 'distant cousin' of foraminifera is also characterized, to investigate any biochemical evolutionary linkages and to cover the widest possible range in OL compositions. For the first time, carbon and oxygen isotopes are measured in isolated foraminiferal OLs and a carbon tracer study carried out to unravel the influence of food source on the carbon isotopic composition of OLs.

2. Methods

2.1. Qualitative survey of OL in benthic foraminifera

Cytoplasm-free individuals belonging to twenty-nine different benthic foraminiferal species (Table 1) were picked and placed in a solution of 0.1 M hydrochloric acid (HCl) to dissolve the calcitic test. The remaining organic lining was described based on completeness compared to the original test morphology. Although this approach is qualitative only, this comparison yields a first order overview of species that are potentially suited for an OL approach. Based on this first inventory species were selected for further investigation.

2.2. Isolation of organic linings for pyrolysis GC–MS

Specimens of *Ammonia tepida* were picked from surface sediments collected at an intertidal flat of the Wadden Sea (near Den Oever, the Netherlands). Specimens of *Calcarina* sp. and *Cycloclypeus* sp. were recovered from a boxcore from a coastal shelf off East Kalimantan, Indonesia. Live *Sorites* sp. specimens were recovered by divers off the coast of Maratua, Indonesia.

Individuals of *G. sphaerica* were picked from the surface sediments of a multicore, recovered during the PASOM cruise (2009) in the Arabian Sea. Fossil *Amphistegina* specimens were Late Oligocene in age and recovered from the Aquitaine Basin in Bordeaux, France. Individuals of *Ammonia* sp., *Calcarina* sp., *Sorites* sp., *Cycloclypeus* sp., and *Amphistegina* sp. were picked after visually inspecting their test. All selected tests were clean and free of infill. The samples were subsequently rinsed in UHQ water and placed in a sonic bath for 20 s to remove small particles possibly still adhering to the test. This was repeated five times. Isolation of OLs was achieved by placing the foraminifera into dialysis tubing (CelluSEP T1 Dialysis Membrane, MW cutoff 3500), which was placed in a cylinder containing UHQ water and ion exchange resin (Dowex cation-exchange resin 50 × 8, mesh 50–100) (Gotliv et al., 2003). The cylinders containing both sample and resin were placed on a roller bench and regularly flushed with new UHQ water until the carbonate test was fully removed. The remaining OL was extracted with an ultrasonic needle using organic solvents, methanol (MeOH) and dichloromethane (DCM), following a sonification/centrifugation scheme of 1 × 1:1 MeOH:DCM and 4 × 1:9 MeOH:DCM, after which the sample was dried under a stream of N_2. For *G. sphaerica*, the cell was pierced and the contents removed under a stream of UHQ water. After visual inspection, the remaining membrane was extracted following the same scheme as described above for the foraminifera.

2.3. Pyrolysis GC–MS

The isolated and extracted OL was pressed onto a flattened ferromagnetic wire with a Curie point temperature of 610 °C. The wire was subsequently inserted into a glass liner, which was placed into a RF coil in a He flow and heated for 10 s at its Curie point. The Curie point pyrolyzer (FOM-5XL) was coupled to a gas chromatograph (Thermo Finnigan Trace GC Ultra), which was interfaced to a mass spectrometer (Thermo Finnigan Trace DSQ). Separation of the compounds released during