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The stable isotope fingerprint of chemosymbiosis in the shell organic matrix of seep-dwelling bivalves

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ABSTRACT

Chemosymbiotic bivalves harboring endosymbiotic, chemotrophic bacteria have been investigated from a variety of hydrocarbon seeps worldwide. It has been shown that carbon, nitrogen, and sulfur isotopic compositions of the animal soft body parts are excellent indicators for evaluating energy transfer and food sources for the respective deep-sea habitats. However, recognition of chemosymbiosis has proven to be difficult for bivalves that dwelled at ancient seeps due to the lack of soft tissue. Here, we investigated $\delta^{13}C$, $\delta^{15}N$, and $\delta^{34}S$ signatures of the tissue (mantle) and the shell organic matrix (SOM) of the same specimens of three bathymodiolin mussel species with different chemotrophic symbionts (methanotrophs in *Bathymodiolus platifrons* and *B. childressi* and thiotrophs in *B. aduloides*) and one vesicomyid clam (*Calyptogena* sp.) from a variety of hydrocarbon seeps from the South China Sea and the Gulf of Mexico. The data obtained demonstrate that all seep bivalves regardless of species or locations reveal overall small differences in $\delta^{13}C$ ($\leq +4‰$), $\delta^{15}N$ ($\leq +1‰$), and $\delta^{34}S$ ($\leq +5‰$) values between SOM and mantle ($\Delta_{SOM\text{-}mantle}$) of the specimens. Relatively larger $\Delta_{SOM\text{-}mantle}$ for $\delta^{13}C$ values (as high as +10‰) in *B. platifrons* and larger $\Delta_{SOM\text{-}mantle}$ for $\delta^{34}S$ values (up to 16‰) in *B. aduloides* and *Calyptogena* sp. might be due to different symbionts in their gills. Since SOM can be extracted from fossil bivalve shells, the proxy can be used as a fingerprint of chemosynthesis-based food chains, although its utility will depend on the quality of preservation of the shell organic matter. Despite this uncertainty, the new proxy has great potential to reconstruct energy flow through different types of chemosynthesis-based ecosystems.

1. Introduction

The migration of methane-rich fluids from subsurface reservoirs to the sea floor at sites on continental slopes, referred to as hydrocarbon seeps, sustains some of the richest ecosystems on the sea bed (Boetius and Wenzhöfer, 2013; Suess, 2014). Seep ecosystems are characterized by dense accumulations of chemosymbiotic metazoans, which contain chemotrophic bacteria in their tissues that can utilize methane and/or hydrogen sulfide as energy source (Levin, 2005 and references therein). Chemosymbiotic animals have commonly been used to recognize hydrocarbon seeps on the sea floor (Suess, 2014). Although it needs to be stressed that chemosymbiosis is not confined to sites where reduced fluids emanate, hydrocarbon seeps – like hydrothermal vents – tend to exhibit the greatest densities of chemosymbiotic metazoans (Levin, 2005). Among the dominant animals at seeps are bathymodiolin mussels and vesicomyid clams, which live in symbiosis with thiotrophic and/or methanotrophic symbionts and derive the vast majority of their nutrition from their endosymbionts (e.g. Paull et al., 1985; Childress et al., 1986; Cordes et al., 2009; Duperron, 2010; Becker et al., 2010).

Numerous studies have used stable isotope compositions of tissue to analyze dietary intake and environmental settings of seep bivalves (e.g. Fisher, 1990; Kennicutt et al., 1992; Conway et al., 1994; Dattagupta et al., 2004; Yamanaka et al., 2015). For instance, it has been shown that the carbon isotopic composition of tissue reflects the isotopic

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composition of the diet, methane in case of methanotrophic endosymbionts and dissolved inorganic carbon (DIC) with a characteristic fractionation in case of thiotrophic endosymbionts (Fisher, 1990; Conway et al., 1994; Macavoy et al., 2008). Sulfur sources for seep bivalves are either hydrogen sulfide or seawater sulfate, depending on the type of symbionts in their gills (Vetter and Fry, 1998). The nitrogen sources of chemosymbiotic animals are not well constrained because of the lack of $\delta^{15}N$ signals of nitrate, nitrite, and ammonium from the environment. However, a local nitrogen origin has been hypothesized by a few studies (Paull et al., 1985; Kennicutt et al., 1992; Becker et al., 2010; Feng et al., 2015; Yamanaka et al., 2015). Furthermore, the combination of tissue $\delta^{13}C$, $\delta^{15}N$, and $\delta^{34}S$ values are useful to elucidate processes that affect the chemical and isotopic compositions of seeping fluids as well as seepage dynamics (Becker et al., 2010, 2013, 2014; Rodrigues et al., 2013; Feng et al., 2015).

A great number of ancient seep ecosystems has now been recognized in the fossil record (e.g. Campbell, 2006; Kiel et al., 2017). The geological record reveals that faunal assemblages and community structures have changed continuously, and that different taxa have dominated hydrocarbon seeps in the course of Earth's history (Kiel, 2015). Knowledge on the mode of adaptation of bivalves to seeps is scarce in the fossil record, because soft tissue is typically not preserved. Therefore, inferences on the trophic structure of ancient seep assemblages have so far been mainly based on the association of animal fossils with ^{13}C-depleted authigenic carbonates (Campbell, 2006; Peckmann et al., 2011; Jenkins et al., 2013). However, such inferences are necessarily subject to great uncertainties and ancient chemosymbiotic assemblages may even be overlooked if the carbonate rock matrix enclosing animal fossils is typified by only moderately low $\delta^{13}C_{carbonate}$ values caused by mixing processes and if diagnostic lipid biomarker information is erased during burial.

Bivalve shells are made of calcium carbonate minerals (aragonite and calcite) that precipitated onto an organic matrix (Lowenstam and Weiner, 1989). Case studies of modern seep bivalves reveal that the carbon isotopic composition of shell carbonate largely reflects the isotopic composition of dissolved inorganic carbon of ambient seawater (e.g. Paull et al., 1985; Feng et al., 2009). Because the organic matrix is secreted from the organism's mantle (Barnes, 1987), the isotopic composition of the mantle should be archived in the shell organic matrix (SOM), and such information is expected to be retained during depositional processes (Degens, 1969; O'Donnell et al., 2003; Mae et al., 2007; Dreier et al., 2012, 2014). But as yet, only one study has used such a stable isotope approach to investigate SOM in chemosymbiotic bivalves from seeps (Mae et al., 2007).

To examine if there is a systematic pattern between the isotopic composition of soft tissue and shell organic matter in the same specimens of seep bivalves, we conducted a survey of $\delta^{13}C$, $\delta^{15}N$, and $\delta^{34}S$ data pairs from two South China Sea (Haima and Site F) and two Gulf of Mexico (Bush Hill and Brine Pool) modern seep provinces (Fig. 1). Two different species with either methanotrophic or thiotrophic symbiont in their gills from the same sites (Haima and Site F) and one species from different sites (Bush Hill and Brine Pool) were available for a thorough analysis.

2. Materials and methods

Seep bivalves analyzed in this study came from two sites of the South China Sea in water depths of 1120 to 1390 m (Site F and Haima) and two sites (Bush Hill and Brine Pool) of the Gulf of Mexico in water depths of 550 to 650 m (Fig. 1). Three bathymodiolin mussel species with different chemotrophic symbionts (methanotrophs in *Bathymodiolus platifrons* and *B. childressi*, and thiotrophs in *B. aduloides*) and one vesicomyid clam species (*Calyptogena* sp. with thiotrophs) were analyzed (Table 1); the vesicomyid is a new species that is currently described (Chen et al., submitted).

Once onboard the ship, the bivalves were sampled by dissecting a piece of mantle and shell from the same organism. The mantle samples were rinsed with deionized water to remove residual seawater, freeze-dried, homogenized, and acidified to remove inorganic carbonate (Feng et al., 2015). The samples were then rinsed with deionized water and freeze-dried for isotope measurements.

The bivalve shells were used for X-ray diffraction (XRD), analysis of the stable carbon and oxygen isotope compositions, and extraction of the organic matrix. Body soft tissue attached to the shells and periostracum on the shells were removed by using fine sand paper before the treatments. Shells were then immersed in NaOCl (5%, vol/vol) for 72 h to remove organic contaminants, rinsed with Milli-Q water, dried and mechanically ground into fine powder.

Semi-quantitative mineralogy of shell carbonate was determined with a Rigaku DXR 3000 diffractometer equipped with a diffracted beam graphite monochromator and using CuKα radiation at Guangzhou Institute of Geochemistry, Chinese Academy of Sciences (GIG, CAS). The relative proportions of carbonate minerals were quantified by Rietveld analysis of the diffractograms with the program SIROQUANT.

For carbon and oxygen stable isotope measurements, powdered samples of the shell carbonate were processed with 100% phosphoric acid at 80 °C to release CO_2 for analysis using a Thermo Finnigan Delta V Advantage mass spectrometer at Louisiana State University. All isotope values are expressed using the δ notation relative to the Vienna-Pee Dee Belemnite (V-PDB) standard. Precision was on the order of 0.1‰ for both $\delta^{13}C$ and $\delta^{18}O$ values.

Extraction of organic matrix from the shells was performed following the procedure described by O'Donnell et al. (2003). Shell powders (~4.9–46.6 g) were sieved through a mesh (200 μm) to remove large particles, bleached in NaOCl solution (10 times dilution, 0.26% active chlorine) for 5 h to remove exogenous or endogenous organic contaminants that can be entrapped in the porous shell spaces, then washed with milli-Q water several times to remove NaOCl. The dried powder was decalcified overnight in acetic acid (10% vol/vol) at 25 °C on a shaker. The solution was centrifuged, and the organic pellet (acid-insoluble matrix) was rinsed several times with Milli-Q water and freeze-dried.

The $\delta^{13}C$, $\delta^{15}N$, and $\delta^{34}S$ values of soft tissues and corresponding SOM were measured via CO_2, N_2, and SO_2 generated by a Costech Elemental Analyzer (EC4010) coupled with a Thermo Finnigan Delta V Advantage in a continuous flow mode. The determination of the isotope compositions was performed at Louisiana State University. Values are reported in permil (‰) using the δ notation, relative to the standards Vienna-Pee Dee Belemnite (V-PDB) for carbon, air N_2 for nitrogen, and the Vienna-Canyon Diablo Triolite (V-CDT) for sulfur. The precision for $\delta^{13}C$, $\delta^{15}N$, and $\delta^{34}S$ determinations are ± 0.2‰, ± 0.4‰, and ± 0.3‰, respectively.

3. Results

3.1. Shell carbonate mineral composition

Mineral compositions of the shell carbonates are summarized in Table 1. *Bathymodiolus aduloides* and the *Calyptogena* sp. are composed mainly of aragonite (mean: 95.1%, sd: 4.4, n = 6), whereas *B. platifrons* and *B. childressi* show only a dominance of aragonite (mean: 71.4%, sd: 7.6, n = 16) with relatively higher contents of calcite (mean: 28.7%, sd: 7.5, n = 16).

3.2. Shell carbonate $\delta^{13}C$ and $\delta^{18}O$ values

Shell carbonate $\delta^{13}C$ values measured from the bivalves with methanotrophic symbionts reflect a slightly greater depletion in ^{13}C (by approximately 2‰) than the shells of bivalves with thiotrophic symbionts from both Site F and Haima seep sites. Average $\delta^{13}C$ values of *B. childressi* were −1.3‰ for Bush Hill and −7.1‰ for Brine Pool, but isotopic variations for individual study sites are small. Shell carbonate