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Effect of sulfide, osmotic, and thermal stresses on taurine transporter mRNA levels in the gills of the hydrothermal vent-specific mussel *Bathymodiolus septemdierum*

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ABSTRACT

Hydrothermal vent environmental conditions are characterized by high sulfide concentrations, fluctuating osmolality, and irregular temperature elevations caused by vent effluents. These parameters represent potential stressors for organisms that inhabit the area around hydrothermal vents. Here, we aimed to obtain a better understanding of the adaptation mechanisms of marine species to hydrothermal vent environments. Specifically, we examined the effect of sulfide, osmolality, and thermal stress on the expression of taurine transporter (TAUT) mRNA in the gill of the deep-sea mussel *Bathymodiolus septemdierum*, which is a dominant species around hydrothermal vent sites. We analyzed TAUT mRNA levels by quantitative real-time polymerase chain reaction (PCR) in the gill of mussels exposed to sulfide (0.1 or 1 mg/L $Na_2S \cdot 9H_2O$), hyper- (115% seawater) and hypo- (97.5%, 95.5%, and 85% seawater) osmotic conditions, and thermal stresses (12 °C and 20 °C) for 24 and 48 h. The results showed that mussels exposed to relatively low levels of sulfide (0.1 mg/L) and moderate heat stress (12 °C) exhibited higher TAUT mRNA levels than the control. Although hyper- and hypo-osmotic stress did not significantly change TAUT mRNA levels, slight induction was observed in mussels exposed to low osmolality. Our results indicate that TAUT is involved in the coping mechanism of mussels to various hydrothermal vent stresses.

1. Introduction

Deep-sea hydrothermal vents are one of the most unique environments on Earth, spouting high-temperature fluid heated by underlying magma chambers (Van Dover, 2000). In addition to extraordinary temperature, vent fluids are enriched by sulfide, hydrogen, methane, manganese, and other transition metals, some of which are toxic to organisms. For instance, hydrogen sulfide is known to inhibit the respiratory process (Wang, 2012). In contrast, low concentrations of certain components, such as magnesium and sulfate ions, have been reported in vent fluids (Van Dover, 2000; Gamo et al., 2006). Differences in the ion and mineral components of vent fluids to ambient seawater may result in differing osmolality. For example, the osmolality of fluids from some hydrothermal vents in the Izu–Ogasawara Arc is lower than that of the ambient seawater (our unpublished observation). Thus, the environments around hydrothermal vents are characterized by high hydrogen sulfide concentrations and possible fluctuating osmolality, in addition to irregular temperature elevations caused by vent flow. These conditions are considered too harsh for most animals. Yet, dense communities of animals are found at vent sites. Thus, it is important to understand the mechanisms used by these animals to adapt to such conditions.

Bathymodiolin mussels are excellent models for elucidating the adaptation mechanisms of animals to hydrothermal vent environments. Bathymodiolin mussels are one of the most common species found in vents and cold seeps, and often represent the dominant invertebrate biomass (Desbruyères et al., 2006; Duperron et al., 2013). Unlike other deep-sea animals, these mussels can be maintained in aquaria under atmospheric pressure, enabling them to be used in laboratory experiments. Comprehensive gene analysis of the genus *Bathymodiolus* identified several genes known to be involved in metallic and oxidative stress responses (Tanguy et al., 2008), in addition to immune and inflammatory reactions (Bettencourt et al., 2010). Thermal stress also

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depresses a large number of genes in bathymodiolin mussels (Boutet et al., 2009a,2009b). However, information about the molecular response of animals inhabiting vent sites to stressors remains limited.

A number of bathymodiolin species mostly rely on sulfur-oxidizing endosymbionts in the gill for nutrition. These mussels transport the toxic sulfide to the gill to supply their endosymbionts (Powell and Soremo, 1986); thus, these mussels must have evolved mechanisms to adapt to the toxicity of sulfides. Previous studies have suggested that thiotaurine and hypotaurine are involved in the adaptation of various hydrothermal vent-specific species to sulfides (Alberic and Boulegue, 1990; Pranal et al., 1995; Pruski et al., 2000b; Brand et al., 2007; Ortega et al., 2008; Yancey et al., 2009). Hypotaurine is the precursor of taurine, an abundant component in the tissues of marine mollusks and crustaceans. Hypotaurine is thought to protect the cells by binding to toxic sulfide to produce non-toxic thiotaurine in vent animals (Pruski et al., 2000a; Pruski and Fiala-Médioni, 2003). The gill of the bathymodiolin mussel *Bathymodiolus septemdierum* is the primary site exposed to ambient sulfide. Hence, we previously examined the mechanisms used for hypotaurine accumulation in the gill of this species. We proposed that a taurine transporter (BsTAUT) is involved in importing hypotaurine into the gill cells, because the BsTAUT gene is expressed in the gill and has as high an affinity for hypotaurine as it does for taurine (Inoue et al., 2008).

TAUT was originally identified as the transporter of taurine in several marine invertebrates, including the shallow-sea mussel *Mytilus galloprovincialis* (Hosoi et al., 2005, 2007; Koito et al., 2010b; Kinjo et al., 2013). Taurine is known to be a major osmolyte in marine invertebrates (Huxtable, 1992). In *M. galloprovincialis*, TAUT gene expression is influenced by ambient osmolality (Hosoi et al., 2005). As bathymodiolin mussels and *M. galloprovincialis* belong to the same family, Mytilidae, TAUT gene expression may also respond to osmotic stress in bathymodiolin mussels. Although the vent environment is assumed to be subject to fluctuations in salinity due to the irregular mixture of ambient seawater and hypotonic vent effluent, there have been no reports available about osmoregulation in vent animals.

In this study, we examined the influence of sulfide exposure and osmotic stress on the mRNA expression of the TAUT gene in *B. septemdierum*. In addition, the effect of high temperature was also examined, because it has been suggested that taurine is involved in the thermal acclimation of several organisms (Frosini, 2007; Morsy et al., 2010; Costas et al., 2012). Based on our results, we discuss the functions of BsTAUT in the adaptation of animals to environmental stresses that are common in hydrothermal vent areas.

2. Materials and methods

2.1. Sample collection

B. septemdierum was collected from the hydrothermal vent field in the Suiyo Seamount and Myojin Knoll in the Izu–Ogasawara Arc using the remotely operated vehicle Hyper-Dolphin aboard the research vessel Natsushima during research cruises NT11-09, NT13-05, and NT14-06. Specimens were maintained in a stock tank with circulated surface seawater in a cold room (4 °C) until use. All of the rearing experiments were performed in the same cold room of the vessel, using surface seawater chilled to 4 °C. Sulfide exposure and hypo-osmotic stress experiments were carried out during the NT11-09 cruise. The thermal stress experiment was performed during the NT13-05 cruise. The hyperosmotic stress experiment was carried out during the NT14-06 cruise. The location, depth, temperature, sulfide level, and osmolality of ambient seawater at the sampling points of the mussels subjected to each experiment are shown in Table 1. The temperature of the mussel colonies was measured using RMT-DTDR-1 (Rigo, Tokyo, Japan). Sulfide levels were measured using the methylene blue method (Fogo and Popowsky, 1949). The osmolality of ambient seawater was measured with a vapor pressure osmometer (Wescor 5520, Logan, UT, USA).

2.2. Sulfide exposure

Forty-eight mussels were divided into 3 groups and maintained in separate 20 L tanks containing 10 L surface seawater covered by lids. Sulfide was supplied every 8 h by dissolving $Na_2S \cdot 9H_2O$ crystals (Wako Pure Chemicals, Tokyo, Japan) to a final concentration of either 0.1 mg/L or 1 mg/L for each experimental group. Sulfide levels were checked hourly by using the methylene blue method mentioned in the previous section. After dispensing 1 mg/L sodium sulfide, the hydrogen sulfide concentration was 0.63 μM for the first 3 h. The sulfide level was 0.31 μM from 4 to 7 h after dispensing the sulfide, and became undetectable by 8 h. Hydrogen sulfide was undetectable throughout the experiment in the control and 0.1 mg/L $Na_2S \cdot 9H_2O$ tanks. Eight mussels from each group were sampled at 24 h and 48 h following sulfide exposure. No mortality was observed during the exposure experiment.

2.3. Hypo-osmotic stress

Sixty-four mussels maintained in 100% seawater (SW) were divided into 4 groups and placed in 4 separate 20 L tanks with 100% SW (control) or diluted seawater (97.5, 95.5, and 85%) prepared using deionized water. Eight mussels from each group were sampled at 24 h and 48 h after low osmolality stress. The osmolality of the tank water at the end of the experiment was 1025 mOsm/kg (control), 1001 mOsm/kg (97.5% SW), 969 mOsm/kg (95.5% SW), and 885 mOsm/kg (85% SW). No mortality was observed during the experimental period.

2.4. Hyper-osmotic stress

Thirty-two mussels kept in 100% SW were divided into 2 groups and placed in separate 20 L tanks with 100% SW (control) and 115% SW (experimental group), which was adjusted by adding artificial sea salt (Aqua Salz, Nissei Sangyo, Tokyo, Japan). Eight mussels from each group were sampled at 24 h and 48 h after hyper-osmotic stress. The osmolality of the tank water at the end of the experiment was 1039 mOsm/kg (control) and 1193 mOsm/kg (115% SW). No mortality was observed during the experimental period.

2.5. Thermal stress

Three groups of 18 mussels in separate 20 L tanks were placed in the cold room (4 °C). Each tank was equipped with an aquarium heater and a water circulator to ensure uniform water temperature. The tank water

Table 1
Location, depth, average temperature, sulfide level, and osmolality at the points where mussels were sampled for each experiment. Maximum temperature detected during sampling mussels is shown in parentheses. NA, not available. ND, not detected.

Cruise	Date	Location	Depth (m)	Temperature (°C)	Sulfide (mM)	Osmolality (mOsm/kg)	Experiment
NT11-09	Jun., 2011	Suiyo Seamount (28°34.268′N, 140°38.668′E)	1381	5.4 (~6.1)	11	981	Sulfide exposure Hypoosmotic stress
NT13-05	Mar., 2013	Myojin Knoll (32°06.213′N, 139°52.172′E)	1224	4.6 (~5.3)	NA	NA	Thermal stress
NT14-06	Apr., 2014	Myojin Knoll (32°06.250′N, 139°52.157′E)	1243	4.9 (~5.4)	ND	NA	Hyperosmotic stress