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ORIGINAL ARTICLE

Cysteine dioxygenase and cysteine sulfinate decarboxylase genes of the deep-sea mussel *Bathymodiolus septemdierum*: possible involvement in hypotaurine synthesis and adaptation to hydrogen sulfide

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Abstract It has been suggested that invertebrates inhabiting deep-sea hydrothermal vent areas use the sulfinic acid hypotaurine, a precursor of taurine, to protect against the toxicity of hydrogen sulfide contained in the seawater from the vent. In this protective system, hypotaurine is accumulated in the gill, the primary site of sulfide exposure. However, the pathway for hypotaurine synthesis in mollusks has not been identified. In this study, we screened for the mRNAs of enzymes involved in hypotaurine synthesis in the deep-sea mussel *Bathymodiolus septemdierum* and cloned cDNAs encoding cysteine dioxygenase and cysteine sulfinate decarboxylase. As mRNAs encoding cysteamine dioxygenase and cysteine lyase were not detected, the cysteine sulfinate pathway is suggested to be the major pathway of hypotaurine and taurine synthesis. The two genes were found to be expressed in all the tissues examined, but the gill exhibited the highest expression. The mRNA level in the gill was not significantly changed by exposure to sulfides or thiosulfate. These results suggests that the gill of *B. septemdierum* maintains high levels of expression of the two genes regardless of ambient sulfide level and accumulates hypotaurine continuously to protect against sudden exposure to high level of sulfide.

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Introduction

Rich communities of organisms live around deep-sea hydrothermal vents (Van Dover and Lutz 2004; Rogers et al. 2012; Sen et al. 2013). These communities nutritionally depend on organic matter produced by chemosynthetic microorganisms such as sulfur-oxidizing bacteria, which synthesize carbohydrates using the chemical energy of hydrogen sulfide contained in the vent water. It is also known that various invertebrates in the hydrothermal vent communities have symbiotic chemosynthetic bacteria within their bodies (Cavanaugh 1983; Dubilier et al. 2008; Duperron et al. 2013). For example, the deep-sea mussels of the genus *Bathymodiolus* and the clams of the family vesicomyidae harbor sulfur-oxidizing bacteria within their gill cells. For the host animals, such endosymbiosis increases the efficiency of the ingestion of organic matter. However, the hosts are required to expose themselves to vent water, absorb hydrogen sulfide, and supply it to the symbiont in the gill cells. As hydrogen sulfide is a toxic substance inhibiting the respiratory process (Wang 2012), the hosts must have special mechanisms to cope with its toxicity.

The use of hypotaurine and thiotaurine has been suggested to be one of the major mechanisms for adaptation to sulfide exposure (Pruski and Fiala-Médioni 2003; Rosenberg et al. 2006; Brand et al. 2007; Ortega et al. 2008; Yancey et al. 2009). Hypotaurine is a precursor of taurine and is contained in tissues of various mollusks (Ouchi 1959). Thiotaurine is a substance generated by the reaction of sulfide and hypotaurine (Yancey et al. 2009).

Both hypotaurine and thiotaurine are essentially non-toxic substances. Thus, the animals residing near vents can avoid the toxicity of sulfide by converting it to thiotaurine when they are exposed to sulfide-rich water. In this detoxification system, it is important for the hosts to accumulate hypotaurine in the gill tissue, which is the primary site of the contact with the vent water. We have already identified a membrane transporter involved in the uptake of hypotaurine into cells (Inoue et al. 2008). The transporter has been called "taurine transporter (TAUT)" as it has ability to transport taurine, but later it was shown to be orthologous to the mammalian creatine transporter (CT1) (Kinjo et al. 2013). It was also demonstrated that mRNA level of the transporter is influenced by the ambient sulfide level (Koito et al. 2010a, b).

Another possible strategy to accumulate hypotaurine within cells is the enzymatic synthesis of hypotaurine. The pathways of hypotaurine synthesis have been identified in mammals (Stipanuk 2004; Ubuka et al. 2008; Ueki and Stipanuk 2009; Vitvitsky et al. 2011; Ueki et al. 2012); it is synthesized from cysteine through two pathways, "cysteine sulfinate (CSA) pathway" and "cysteamine pathway". The CSA pathway is catalyzed by two enzymes, cysteine dioxygenase (CDO) that converts cysteine to CSA and cysteine sulfinate decarboxylase (CSAD) that converts CSA to hypotaurine. As for the cysteamine pathway, cysteamine dioxygenase (ADO), converting cysteamine to hypotaurine, is involved. There is one more pathway for taurine synthesis in mammals; cysteine lyase (CL) catalyzes the reaction from cysteine to taurine through L-cysteate (cysteate pathway), but the reaction pathway does not produce hypotaurine. Despite hypotaurine/taurine being major components of mollusk tissues, enzymes catalyzing hypotaurine synthesis in mollusks have not been identified. However, some papers reported enzymatic activities related to hypotaurine/taurine synthesis (Allen and Awapara 1960; Yoneda 1968), and there is evidence by way of annotation on the oyster genome and transcriptome (Zhang et al. 2012; Meng et al. 2013).

In this study, we tried to identify genes involved in hypotaurine/taurine synthesis in the gill tissue of the deep-sea mussel *Bathymodiolus septemdierum*, a dominant species in hydrothermal vent areas of Izu-Ogasawara Arc, utilizing the RNA-seq data obtained by pyrosequencing technology. As CDO- and CSAD-like sequences were detected, we cloned cDNAs containing whole coding regions. We also found that mRNA expression levels of both genes are highest in the gill. Subsequently, we examined changes in mRNA level of the CDO and CSAD genes in the gill after exposure to sulfide in order to examine whether expression of the two genes responds to ambient sulfide. On the basis of the results of these analyses, we discuss the roles of the two genes in hypotaurine synthesis and adaptation to ambient sulfide.

Materials and methods

Collection of mussels

B. septemdierum was obtained at hydrothermal vent field in Myojin Knoll (depth, 1,228 m) during the cruise NT10-08 (May 11–18, Bettencourt et al. 2010) and that in Suiyo Seamount (depth, 1,381 m) during the cruise NT11-09 (June 15–26, Stipanuk et al. 2011) of the research vessel (RV) Natsushima/remotely operated vehicle (ROV) Hyper-Dolphin of Japan Agency of Marine Science and Technology (JAMSTEC).

Dissection

Gill, mantle, foot, and adductor muscle of mussels were dissected out immediately after the ROV returned from depth to the research vessel, or after rearing under the conditions described below. Isolated tissues were frozen immediately using liquid nitrogen and stored at −80 °C until use.

RNA extraction

Total RNA of the isolated tissues was extracted using Trizol (Life Technologies, Carlsbad, CA).

Screening for the CDO and CSAD sequences in pyrosequencing data

The RNA-seq data, based on pyrosequencing technology using Roche 454 sequencer, of the gill tissue of *B. septemdierum* (manuscript in preparation) was screened by TBLASTN analyses using human and rat CDO, CSAD, ADO and CL as queries.

cDNA cloning of BsCDO and BsCSAD

Whole coding regions of CDO and CSAD cDNA were cloned utilizing the partial cDNA sequences detected in RNA-seq data as follows. A cDNA pool was prepared using SMART cDNA Library Construction Kit (Clontech, Mountain View, CA) from 2 μg of gill total RNA of the mussel obtained on the NT10-08 research cruise. In order to obtain complete coding sequences of CDO, the forward primer CDO-utrF1 (CAATTCCAGAG ATGTCAAAATGT) was designed using the 5′-untranslated region (UTR) sequence from the RNA-seq data. As the region corresponding to the C-terminal 10 residues were not detected in the RNA-seq data, the reverse primer CDO-utrR1 (CTTTCCCATTGCTCTTCTTTTAT) was designed from the sequence obtained from *Bathymodiolus azoricus* transcriptome database (DeepSeaVent, http://transcriptomics.biocant.pt:8080/deepSeaVent/). PCR amplification was performed using ExTaq (Takara bio Inc., Kyoto, Japan) using 35 thermal cycles of 30 s at 96 °C, 30 s at 56 °C and 60 s at 72 °C.

Concerning CSAD, the partial coding region was amplified with CSAD-F3 (GCACACGTACATACGCTAACAG) and CSAD-5GSP3 (ATCAGAATAAACACTGGGGCAACC), both of which are designed using the RNA-seq data, under the same PCR condition as above except that extension at 72 °C was elongated to 100 s. Upstream and downstream sequences were obtained by RACE (Rapid Amplification of cDNA Ends) method. For upstream, PCR was conducted with the 5′-primer supplied with the library construction kit and CSAD-5GSP2 (GGAAATGATGTGACGATTCTGGAC) using the same thermal cycles as that for the partial coding region. For downstream, PCR was performed using the primers CSAD-F4 (AAATACAGCGTGAAAACAGGTC) and CDSIII supplied with the kit and the same thermal cycles as above. The RACE for downstream was repeated replacing the CSAD-F4 primer with CSAD-F6 (TTCAGAGAAGGGTCATTACTCG) and then with CSAD-F7 (GTTGATGCTCTAAAGGTGTGG) until whole coding region was obtained. All DNA sequences were determined using 3130 Genetic Analyzer (Applied Biosystems, Foster City, CA).

Sequence analyses

The TBLASTN search was performed using the encoded peptide sequences as query at the DDBJ BLAST site (http://blast.ddbj.nig.ac.jp/tblastn). The characterization of peptide sequences based on motif structure was conducted using Pfam (http://pfam.xfam.org/) and Prosite (http://prosite.expasy.org/).

Molecular phylogenetic analyses

The peptide sequences for molecular phylogenetic analyses were obtained from the KEGG database (http://www.genome.jp/kegg/). Alignment of the sequences and construction of maximum likelihood trees on the basis of the LG+G (CDO) and LG+G+I (CSAD) models with 1,000 bootstrap replications were performed using the MEGA software program ver. 6.06. The model selection for tree building was according to the result of "find best DNA/protein" model [ML] by MEGA 6.06 (Tamura et al. 2013). The bootstrap consensus trees with the highest log likelihood (CDO, −4600.89; CSAD, −4881.81) inferred from 1,000 replicates were generated. The CDO and CSAD trees were rooted by evolutionary related enzymes, i.e., ADO and HDC (histidine decarboxylase), of human, respectively.

Reverse-transcription PCR

The cDNA for RT-PCR was prepared using a Superscript II Reverse Transcriptase 1st Strand cDNA Kit (Invitrogen) from 2 μg total RNA extracted from the mussels obtained in NT10-08. The PCR program for BsCDO was 31 cycles of 30 s at 94 °C, 30 s at 55 °C, and 40 s at 72 °C using the primers CDO-cdsF1 (ATAGACTACGTGAAAACTTTA ATGAC) and CDO-cdsR1 (TGCTCCAGAACGTAACCT TG), whereas BsCSAD was 30 cycles of 30 s at 94 °C, 30 s at 55 °C, and 60 s at 72 °C using the primers CSAD-F3 and CSAD-5GSP2. The 18S rRNA was used as an internal control and the PCR program for this target was 25 cycles of 30 s at 94 °C, 30 s at 55 °C, and 50 s at 72 °C.

Exposure to sulfide and thiosulfate

Experimental exposure to sulfide and thiosulfate were conducted in a cold room (4 °C) on the research vessel. Surface seawater of the sampling area chilled to 4 °C was used for all the experiments. Mussels obtained in the NT11-09 cruise were kept in a stock tank until experiment (approximately 96 h duration). The only a few individual died during this period, suggesting that the mussels did not suffer greatly upon retrieval to the ship. Seventy-two mussels, whose shells were not damaged, were selected for the experiment. Four plastic tanks each containing 10 L surface seawater with sealable lids were prepared. After sampling of 8 mussels as the initial specimens (0 h), 16 mussels were put into each of the four tanks. To each tank, 1 mg or 10 mg of $Na_2S{\cdot}9H_2O$ (Wako Pure Chemicals, Tokyo, Japan), or 10 mg of $Na_2S_2O_3{\cdot}5H_2O$ (Wako Pure Chemicals) were directly added and dissolved immediately. The concentration of hydrogen sulfide, determined hourly using the methylene blue method (Fogo and Popowsky 1949), in the tank to which 10 mg $Na_2S{\cdot}9H_2O$ was added was 0.63 μM (0–3 h), 0.31 μM (4–7 h) and became undetectable before 8 h. Hydrogen sulfide was undetectable throughout the experiment in the tank to which 1 mg $Na_2S{\cdot}9H_2O$ was added. The hydrogen sulfide concentration around wild mussel colonies is usually less than 0.3 μM (our unpublished observation). Thus, hydrogen sulfide concentration in the tank with 10 mg $Na_2S{\cdot}9H_2O$ is higher than that in their natural habitat. As for thiosulfate, the concentration in the natural habitat of the mussels has not been reported, the same amount of $Na_2S_2O_3{\cdot}5H_2O$ as sodium sulfide was added to the tank. The concentration of thiosulfate in the tank was not measured. Control treatment tanks received no additives. Eight specimens were sampled from each group at 24 and 48 h after the start of exposure. These specimens were dissected and frozen as described above.

Quantitative real-time PCR analysis of BsCDO and BsCSAD mRNA expression

The first-strand cDNAs were synthesized using the High Capacity cDNA Reverse Transcription kit, as described by the manufacturer (Applied Biosystems), from the total RNA extracted from the gill using Trizol (Life Technologies).

Fig. 1 Comparison of the peptide sequences, around the cupin motifs 1 and 2, of cysteine dioxygenases of the deep-sea mussel, sea urchin, fruit fly, and human. Amino acid residues that are conserved among 4 and 3 species are indicated with *black* and *gray* background, respectively. Consensus of cupin motifs are shown above the sequences. *Asterisk* and *hash* indicate conserved histidine and cysteine residues, respectively

Quantitative real-time PCR for the relative quantitation of BsCDO, BsCSAD and 18S rRNA gene expression were performed using the 7900HT Sequence Detection System (Applied Biosystems). The 10 µl PCR mixture contained 5 µl of TaqMan Universal PCR Master Mix (Applied Biosystems), 0.2 µl of 45 µM forward primer, 0.2 µl of 45 µM reverse primer, 0.2 µl of 10 µM fluorescent-labeled probe, 2.9 µl of DEPC treated water and 1.5 µl of cDNA template. The forward primer, reverse primer, and probe used for BsCDO mRNA were BsCDO-F (GGCGCAGGCTAGTACGATACA), BsCDO-R (TCCGTCCAATATCTTCATAAAACAGT) and BsCDO-MGB (TCCCATGCTAATTCT), respectively. Those for BsCSAD were BsCSAD2-F (GGCAGGAAGGTTGATG CTCTAA), BsCSAD2-R (GTCTGCAGCCATTCCGGTAT) and BsCSAD2-MGB (CATGTGGAAAGCAAAGG), respectively. As for 18S rRNA detection, 5 µl of TaqMan Universal PCR Master Mix (Applied Biosystems), 0.5 µl of 18S primer probe mix (Applied Biosystems), 3 µl of DEPC treated water and 1.5 µl of cDNA template were mixed.

The real-time PCR cycling conditions were 50 °C for 2 min and 95 °C for 10 min, followed by 40 cycles of 95 °C for 15 s and 60 °C for 1 min. The data for CDO and CSAD mRNA were normalized using 18S rRNA level. A standard curve was generated each time by serial dilutions of cDNA transcribed from gill RNA of an individual. Data are presented as mean ± standard error (SE). Statistical analyses were performed using analysis of variance (ANOVA) and the Bonferroni's post hoc test.

Results

Screening of CDO and CSAD in RNA-seq data

Contigs encoding peptides exhibiting high similarity to mammalian CDO and CSAD were detected. However, sequences similar to mammalian ADO and CL gene were not detected.

cDNA cloning and phylogenetic analyses

Using the sequences obtained from the screening described above, a 651-bp cDNA fragment was obtained; it encodes a peptide of 216 residues showing highest similarity to mammalian CDOs in BLAST analysis against DDBJ/EMBL/Genbank databases. For example, 107 residues (50 %) were found identical to the rat CDO sequence, and additional 62 positions (30 %) exhibited similarity. The Pfam analysis also showed high expectation to be CDO (*E* value, 4.8e-52). In addition, the predicted amino acid sequence contained a domain that is highly conserved among known CDOs (Fig. 1). In this domain, the sequences similar to the consensus motif of the cupin family, in which all known CDOs are included, were also detected (Fig. 1). In the molecular phylogenetic analysis rooted with ADO, the peptide encoded by the cloned cDNA formed a clade with CDOs of vertebrates, sea urchin, and insects (Fig. 2). Thus, we named the encoded peptide as BsCDO. Within the clade, vertebrate CDOs and insect CDOs clustered independently with significant bootstrap support. BsCDO and the sea urchin CDO were, unexpectedly, clustered although it was without significant bootstrap support. Another unexpected point is that CDOs of a bacteria and a protist formed a cluster, which diverged at the basal position.

Likewise, a 1,551-bp cDNA fragment encoding 516 amino acid residues showing highest similarity to mammalian CSADs was also obtained. The identity with the rat CSAD was 243 residues (47 %) and another 170 residues (33 %) showed similarity. The Pfam analysis classified it as a member of group II pyridoxal-dependent decarboxylase family, which contains known CSADs of vertebrates and invertebrates, with an *E* value of 7.2e-97. Prosite analysis identified the shared motif of type II pyridoxal phosphate (PLP)-dependent amino acid carboxylases (Sandmeier et al. 1994), 22 residues from

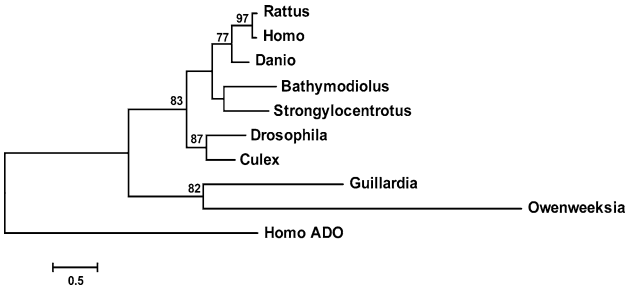

Fig. 2 Maximum likelihood tree of cysteine dioxygenase (CDO). All the sequences were obtained through the KEGG database. The tree was constructed using MEGA 6.06 with the analytical parameters described in the text. The human cysteamine dioxygenase (ADO) was used as outgroup. Bootstrap values of 70 or higher values are indicated at each node. Name of organisms are indicated by genus names: *Bathymodiolus B. septemdierum* (deep-sea mussel), *Owenweeksia O. hongkongensis* (bacteria), *Culex C. quinquefasciatus* (mosquito), *Danio D. rerio* (zebrafish), *Drosophila D. melanogaster* (fruit fly), *Guillardia G. theta* (protist), *Homo H. sapiens* (human), *Rattu R. norvegicus* (rat), *Strongylocentrotus S. purpuratus* (sea urchin)

Fig. 3 Comparison of the consensus domain of the type II pyridoxal phosphate (PLP)-dependent amino acid decarboxylase family in cysteine sulfinate decarboxylases of the deep-sea mussel, sea urchin, fruit fly, and human. Amino acid residues that are conserved among 4 and 3 species are indicated with *black* and *gray* background, respectively. Putative PLP-binding lysine and the adjacent histidine are indicated by "*closed triangle*" and "*asterisk*", respectively

S-322 (Fig. 3). In the molecular phylogenetic analysis, the obtained sequence was clustered with known CSADs of vertebrates and invertebrates (Fig. 4). Thus, we named the encoded peptide BsCSAD. Although the close relationship between BsCSAD and sea urchin CSAD, which is similar to the result in the CDO tree, was unexpected, the order of branching of bacterial and protist CSADs was consistent with the standard phylogeny of organisms. cDNA sequences encoding BsCDO and BcCSAD were submitted to DDBJ/EMBL/GenBank under accession numbers, AB970771 and AB970772, respectively.

Tissue specificity of mRNA expression

The transcripts of BsCDO and BsCSAD genes were detected in all tissues examined (Fig. 5). As for the BsCDO gene, the transcript level was higher in the gill followed by the mantle, whereas that of BsCSAD was higher in the gill, followed by the gonad, foot and mantle. In the

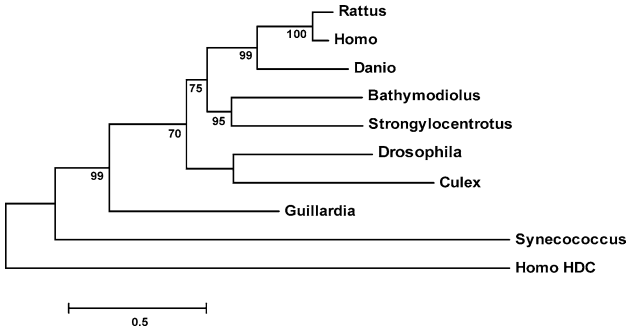

Fig. 4 Maximum likelihood tree of cysteine sulfinate decarboxylase (CSAD). All the sequences were obtained through the KEGG database. The tree was constructed using MEGA 6.06 with the analytical parameters described in the text. The human histidine decarboxylase (HDC) was used as outgroup. Bootstrap values of 70 or higher values are indicated at each node. Name of organisms are indicated by genus names: *Bathymodiolus B. septemdierum* (deep-sea mussel), *Culex C. quinquefasciatus* (mosquito), *Danio D. rerio* (zebrafish), *Drosophila D. melanogaster* (fruit fly), *Guillardia G. theta* (protist), *Homo H. sapiens* (human), *Rattus R. norvegicus* (rat), *Strongylocentrotus S. purpuratus* (sea urchin), *Synechococcus Synechococcus* sp. CC9311 (bacteria)

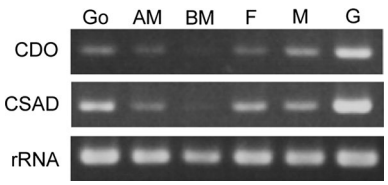

Fig. 5 Tissue specificity of the expression of the cysteine dioxygenase (CDO) and cysteine sulfinate decarboxylase (CSAD) genes of the deep-sea mussel *Bathymodiolus septemdierum* examined by reverse-transcription (RT)-PCR. The 18S ribosomal RNA was shown as internal control. *AM* adductor muscle, *BM* byssus retractor muscle, *F* foot, *G* gill, *Go* Gonad, *M* Mantle

byssus retractor muscle, both BsCDO and BsCSAD exhibited lower levels of expression. The same patterns were obtained from two different individuals.

Effects of sulfide and thiosulfate exposure

The relative mRNA level of the BsCDO gene in the gill after the exposure to sodium sulfide and sodium thiosulfate is shown in Fig. 6. After 24-h exposure, the average for sulfide-exposed groups tended to be higher than that for control. After 48-h exposure, the average mRNA level of the control was higher than sulfide- or thiosulfate-exposed groups. Overall, the differences among the experimental groups and exposure time were not significantly different due to the variation among individuals.

The relative mRNA levels of the BsCSAD gene exhibited similar tendencies to those of the BsCDO gene (Fig. 7),

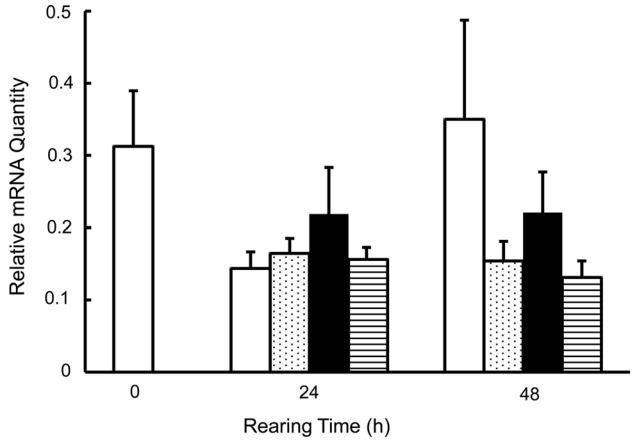

Fig. 6 Relative levels of the cysteine dioxygenase (CDO) mRNA in the gill of the deep-sea mussel *Bathymodiolus septemdierum* exposed to sodium sulfide or sodium thiosulfate. The mRNA quantity was measured by real-time PCR and shown as the relative amount to 18S rRNA. *White columns*, *control*, *doted* and *black columns* exposure to 1 mg/L and 10 mg/L sodium sulfide, respectively; *striped columns* exposure to 10 mg/L sodium thiosulfate

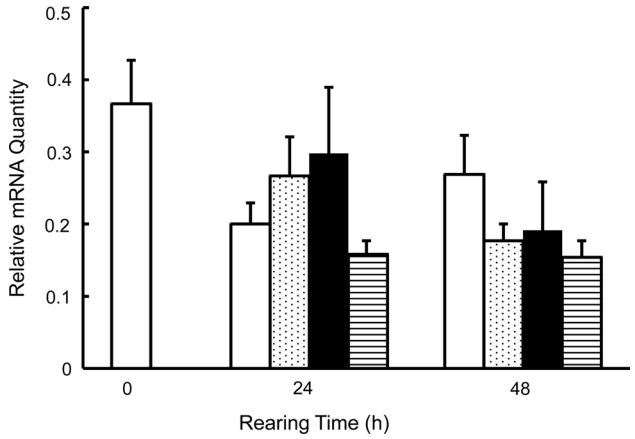

Fig. 7 Relative levels of the cysteine sulfinate decarboxylase (CSAD) mRNA in the gill of the deep-sea mussel *Bathymodiolus septemdierum* exposed to sodium sulfide or sodium thiosulfate. The mRNA quantity was measured by real-time PCR and shown as the relative amount to 18S rRNA. *White columns, control*, *doted* and *black columns* exposure to 1 mg/L and 10 mg/L sodium sulfide, respectively; *striped columns* exposure to 10 mg/L sodium thiosulfate

and no significant differences were observed among the experimental groups and sampling time.

Discussion

Domain structure of CDO and CSAD

In this study, we report the cloning of the cDNA encoding CDO and CSAD from the deep-sea mussel *B. septemdierum*, a species of the genus *Bathymodiolus*, one of the representative genera in the hydrothermal vent ecosystem (Lorion et al. 2013; Thubaut et al. 2013). Although these two genes are recognized in the oyster genome and transcriptome (Zhang et al. 2012; Meng et al. 2013), cDNAs of these genes have not been cloned and characterized in mollusks.

The CDO enzyme belongs to the cupin superfamily. In early studies of this superfamily, cupin motifs 1 (Gx5HxHx3-6Ex6G) and 2 (Gx5-7PxGx2Hx3 N) were identified, but later it was recognized that these motifs are not so strictly conserved (Dunwell et al. 2004; Stipanuk et al. 2011). BsCDO contains the motif, Ax5HxHx4Cx6G, corresponding to motif 1 (Fig. 1) with substitution of G to A, and E to C. Although motif 2 is not obvious in BsCDO, there are three histidine residues at the downstream of motif 1. Among the four, the H-159 (in *Bathymodiolus* numbering) is likely to function as part of motif 2 because it accompanies asparagine (N-163) residue (Fig. 1).

CSAD is a member of the type II PLP-dependent amino acid decarboxylase carboxylase family (Sandmeier et al. 1994). We confirmed, using the Pfam analysis, that BsCSAD is also a member of this family. The Prosite program also identified the motif shared by type II PLP-dependent amino acid carboxylases (Fig. 3), containing the PLP-binding site reported previously (Jackson 1990). Thus, BsCSAD is suggested to be functional.

Molecular phylogenetic analysis

In phylogenetic analysis, BsCDO and BsCSAD clustered with known vertebrate and invertebrate CDOs and CSADs, respectively, and the clades were supported by bootstrap analysis (Figs. 2, 4). These results suggest that the functions of BsCDO and BsCSAD are common with known CDOs and CSADs, respectively. However, the clustering patterns within the clade were not robust and not consistent with the phylogeny of animals in both trees. For example, both BsCDO and BsCSAD clustered with the sea urchin CDO and CSAD, respectively, and did not cluster with insect CDOs although mollusks and insects are protostomes and sea urchins are deuterostomes. These results may be attributed to the restriction of amino acid substitutions that is common in functional sequences but also to unique diversification in each animal lineage. In addition, the clustering of bacterial and protist CDOs is also an unexpected result. CDO sequence is relatively short so that they may not have enough information to resolve distant relationship. It is also possible that ADO emerged in the early stage of the eukaryote lineage.

Hypotaurine biosynthetic pathway in the deep-sea mussel

The discovery of CDO and CSAD from *B. septemdierum* suggests the existence of the CSA pathway in this species.

As mRNA for ADO was not detected in RNA-seq data, the CSA pathway is likely to be the major route for hypotaurine synthesis. In addition, mRNA encoding CL was not detected, which suggests that the CSA pathway is also the major route for taurine synthesis. The absence of CL might not be detrimental, and may ever be advantageous, for *B. septemdierum* because hypotaurine is more important than taurine to adapt to hydrogen sulfide.

Since CDO and CSAD gene expressions were observed in all the tissues examined, it is likely that hypotaurine is synthesized extensively in mussel tissues. Among the tissues, the gill exhibited higher levels of expression of the two genes, suggesting active hypotaurine synthesis in the gill. This result is consistent with the gill being the primary site of the exposure to ambient sulfide.

Expression level after sulfide exposure

We exposed mussels experimentally to two different levels of sodium sulfide, which has been used as a sulfide source in laboratory studies (Bettencourt et al. 2010; Koito et al. 2010b; Fujinoki et al. 2012; Martins et al. 2013), and to thiosulfate, which is detected in the water of hydrothermal vent areas (Waite et al. 2008), and examined the mRNA level of the CDO and CSAD genes (Figs. 4, 5). Although the vent fluid of Suiyo Seamount contains 1.2–1.6 mM hydrogen sulfide (Gamo et al. 2006), the hydrogen sulfide concentration around most mussel colonies are less than 0.3 μM because *B. septemdierum* colonizes avoiding direct exposure to vent fluid (our unpublished observation). Thus, the tank with 10 mg $Na_2S{\cdot}9H_2O$ contains higher concentration of hydrogen sulfide than their natural habitat. However, the expression levels of both genes varied among individuals and thus neither CDO nor CSAD mRNA levels exhibited significant differences among experimental groups. These results suggest that the expression levels of the CDO and CSAD genes were not influenced remarkably by environmental sulfide and thiosulfate, at least under the conditions in this study. It was reported that hypotaurine levels in the gill of bathymodiolin mussels tends to be higher than those in shallow-sea mussels *Mytilus galloprovincialis* (Pruski et al. 2000). In bathymodiolin mussels inhabiting hydrothermal vent areas, it is supposed that hypotaurine is most needed in the gill tissues that are directly exposed to ambient sulfide. To meet the demand, the mussels may express CDO and CSAD at high levels in the gill (at least at higher levels than other tissues), regardless of ambient sulfide levels, and accumulate hypotaurine as much as possible to prepare for sudden increase of ambient sulfide level. To confirm this, it is necessary to measure enzyme activities or concentrations of the proteins because mRNA level does not always correlate to protein level. Comparisons between hydrothermal vent- and shallow sea-residing mussels in the expression levels and/or activities of the enzymes involved in hypotaurine/taurine synthesis is of interest, and will advance understanding of life in the challenging deep ocean environment.

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Conflict of interest The authors declare that they have no conflict of interest with respect to this manuscript.

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