JOURNAL OF EXPERIMENTAL ZOOLOGY 311A:439–447 (2009)

A Journal of Integrative Biology

Thiotaurine and Hypotaurine Contents in Hydrothermal-Vent Polychaetes Without Thiotrophic Endosymbionts: Correlation With Sulfide Exposure

PAUL H. YANCEY[1]*, JOANNE ISHIKAWA[1], BRIGITTE MEYER[1], PETER R. GIRGUIS[2], AND RAYMOND W. LEE[3]
[1]*Biology Department, Whitman College, Walla Walla, Washington*
[2]*Department of Organismic and Evolutionary Biology, Harvard University, Cambridge, Massachusetts*
[3]*School of Biological Sciences, Washington State University, Pullman, Washington*

ABSTRACT Invertebrates at hydrothermal vents and cold seeps must cope with toxic H_2S. One proposed protection mechanism involves taurine derivatives: At vents and seeps, many animals have high levels of hypotaurine and thiotaurine (a product of hypotaurine and HS), originally found in animals with thiotrophic endosymbionts. To further test the role of these compounds, we analyzed them in vent polychaetes without endosymbionts: *Paralvinella sulfincola*, *P. palmiformis* and *P. pandorae* (paralvinellids) and *Nicomache venticola* (maldanid). *P. sulfincola* were collected from a high temperature (42–68°C) and a warm site (21–35°C). *P. palmiformis* and *pandorae* were from cool sites (12–18°C) and *N. venticola* were from a cold site (4°C). H_2S concentrations in vent effluent largely correlate with temperature. Some specimens were frozen; other ones were kept alive in laboratory chambers, with and without sulfide. Tissues were analyzed for taurine derivatives and other solutes that serve as organic osmolytes. The major osmolyte of all species was glycine. Thiotaurine contents were significantly different among all species, in the order *P. sulfincola* hot > *P. sulfincola* warm > *P. pandorae* > *P. palmiformis* > *N. venticola*. *P. sulfincola* also had high levels of sarcosine; others species had none. Sarcosine and hypotaurine contents of *P. sulfincola*'s branchiae were higher, while glycine contents were lower, than in main body. In *P. palmiformis* kept in pressure chambers with sulfide, thiotaurine contents were higher and hypotaurine lower than in those kept without sulfide. These results support the hypothesis that conversion of hypotaurine to thiotaurine detoxifies sulfide in vent animals without endosymbionts. *J. Exp. Zool. 311A:439–447, 2009.*

How to cite this article: Yancey PH, Ishikawa J, Meyer B, Girguis PR, Lee RW. 2009. Thiotaurine and hypotaurine contents in hydrothermal-vent polychaetes without thiotrophic endosymbionts: correlation with sulfide exposure. J. Exp. Zool. 311A:439–447.

Marine invertebrates at hydrothermal vents and cold seeps must cope with potentially toxic levels of hydrogen sulfide (Somero et al., '89), which can bind to iron and disrupt various processes including mitochondrial function (Bagarinao, '92; Arp et al., '95; Fisher, '98). Some of these animals—vestimentiferan (siboglinid) polychaetes, vesicomyid clams, and bathymodiolin mussels—cannot avoid sulfide exposure because they need it for their sulfide-oxidizing (thiotrophic) bacterial endosymbionts. Other vent and seep animals such as other polychaetes and gastropods have no endosymbionts, but feed on external bacteria and detritus in sulfide-laden waters, and may therefore also be exposed to toxic sulfide (Juniper and Martineu, '95). In addition, animals without thiotrophic endosymbionts do not benefit from the sulfide-oxidizing capacity of symbionts, which can detoxify sulfide. Such

Grant sponsors: Perry Grant, Whitman College; National Science Foundation; Grant number: OCE-0623554.
*Correspondence to: Paul H. Yancey, Biology Department, Whitman College, Walla Walla, WA 99362. E-mail: yancey@whitman.edu
Received 12 January 2009; Revised 20 March 2009; Accepted 23 March 2009
Published online 28 April 2009 in Wiley InterScience (www.interscience.wiley.com). DOI: 10.1002/jez.541

animals include the paralvinellid polychaetes (Terebellida, Alvinellidae), *Paralvinella sulfincola* (sulfide worms), *P. palmiformis* (palm worms), and *P. pandorae*, which are among the most abundant animals at vents of the Juan de Fuca Ridge (Levesque et al., 2003). These congeners form a model system for testing adaptations to temperature and sulfide, because *P. sulfincola* lives in much warmer and more sulfide-laden waters than the other two species (Sarrazin et al., '99).

For protection against sulfide toxicity, various mechanisms have been proposed and tested. These include the following: sulfide-binding proteins (e.g., modified hemoglobins) in body fluids of endosymbiont-bearing animals, for transporting sulfide nontoxically (e.g., Arp et al., '87; Childress et al., '93; Kraus, '95); external tubes and mucus of some polychaetes, which may reduce diffusion of sulfide into the animal; microbes living on the tubes, which oxidize sulfide before it can enter the animal (Juniper and Martineu, '95); specialized sulfide-oxidizing organelles in epidermal tissue in some polychaetes from sulfide-rich habitats (Arp et al., '95; Menon et al., 2003); conversion to thiosulfate or elemental sulfur (Vetter, '85; Powell and Somero, '86; Arp et al., '95; Arndt et al., 2001); and binding to intracellular metals, protein, and glutathione (Vismann, '91). Among vent animals without endosymbionts, *P. palmiformis* has been reported to have high (but variable) sulfide levels in the blood along with a sulfide-binding hemoglobin-like protein (Martineu et al., '97). Both *P. palmiformis* and *P. sulfincola* have enzymatic sulfide-oxidizing activity in their tissues, which is considerably higher in *P. sulfincola* (Juniper and Martineu, '95), the worm from warmer, higher-sulfide vents.

In recent years, two compounds called thiotaurine and hypotaurine have been proposed to serve in protection from and/or transport of sulfide intracellularly. Hypotaurine reacts with sulfide to produce thiotaurine (Pruski et al., 2000b):

$$(\text{hypotaurine})^{+}\text{NH}_3-\text{CH}_2-\text{CH}_2-\text{SO}_2^{-}+\text{SH} \rightarrow \text{NH}_3-\text{CH}_2-\text{CH}_2-\text{SO}_2^{-}-\text{SH}(\text{thiotaurine})$$

These unusual amino acids were first reported in seep and vent vestimentiferans and bivalves with thiotrophic endosymbionts (Alberic, '86; Pranal et al., '95; Pruski et al., 2000a). The source of SH could be a free radical, a ligand donated by a protein R as R-SSH, or a moiety donated by glutathione-SSH. Synthesis of thiotaurine from hypotaurine appears to be enzymatic and is reversible (Pruski and Fiala-Médioni, 2003). Thus, thiotaurine might serve to store sulfide nontoxically within cells, and release it as the endosymbionts deplete free sulfide, thus acting as a sulfide ''buffer''. Laboratory studies show that thiotaurine contents increase during sulfide exposure in symbiont-bearing tissues of vesicomyids, bathymodiolins, vestimentiferans (Pruski and Fiala-Médioni, 2003), and shallow-living solemyid clams (Joyner et al., 2003).

Thiotaurine was originally found at high levels in tissues with endosymbionts (trophosome in vestimentiferans, gills in bivalves), but only at low levels in other tissues, hemolymph or blood. These findings suggest that the solutes are located primarily intracellularly (Yin et al., 2000), and led to a proposal that thiotaurine is a general marker of thiotrophic endosymbiosis (Pruski et al., 2000b). However, we have found that two species of gastropods (snail and limpet) from hydrothermal vents of the Juan de Fuca Ridge contain substantial contents of both thiotaurine and hypotaurine (Rosenberg et al., 2006), even though they lack endosymbionts. Recently, hypotaurine has been found to protect erythrocytes of a polychaete without thiotrophic symbionts from sulfide toxicity because of the solute's sulfide-scavenging ability (Ortega et al., 2008). These findings suggest that the two solutes are used in sulfide detoxification but not strictly for symbiosis.

The ratio of thiotaurine to total hypotaurine plus thiotaurine (hereafter called the ''exposure indicator ratio'') has been proposed to be an indicator of the level of sulfide exposure (Pranal et al., '95). Indeed, we and others have found that the ratio tends to be higher in animals with higher exposures in situ and in the laboratory in seep and vent species (Pruski et al., 2000a; Brand et al., 2007), including the vent gastropods without endosymbionts (Rosenberg et al., 2006). We also found evidence that, regardless of differing exposure indicator ratios between populations of a species, the total of hypotaurine plus thiotaurine tends to be consistent in and to correlate with a species' maximum sulfide exposure (Brand et al., 2007).

Thiotaurine and hypotaurine are concentrated enough in these animals to serve another function, namely that of an organic osmolyte. Like most other marine invertebrates, vent and seep invertebrates are osmoconformers that must balance their osmotic pressure with that of the ocean. In cells of most marine invertebrates, this is done with organic osmolytes, primarily free

amino acids and methylamines such as sarcosine (methylglycine) and betaine (trimethylglycine). Organic osmolytes are often called ''compatible solutes'', because (unlike salt ions) they usually do not disrupt protein function at high concentrations and thus can be safely accumulated to raise cellular osmotic pressure. Other organic osmolytes are called ''counteracting'' because they can offset the effects of some factors that perturb proteins such as temperature and hydrostatic pressure; other organic osmolytes may be metabolic cytoprotectants such as antioxidants (Yancey et al., '82; Yancey, 2005). In various invertebrates from shallow waters, taurine, betaine, and glycine are the dominant organic osmolytes. However, in deep-sea invertebrates studied to date, there is less taurine and more methylamines plus inositols and organic phosphates. These may counteract the effects of pressure (Fiess et al., 2002; Yancey et al., 2002). And of course there are high levels of thiotaurine and hypotaurine in vent and seep species that contribute to osmotic balance, but these may primarily be for sulfide detoxification.

To further test the possible roles of hypotaurine and thiotaurine in adaptation to sulfide stress in vent animals without symbionts, and to continue investigating other osmolytes that might relate to habitat stresses other than osmotic, we have analyzed the three *Paralvinella* species from cool to hot hydrothermal diffuse flows among the vents of the Juan de Fuca Ridge. *P. sulfincola* live in soft mucous tubes having a high content of elemental sulfur (Juniper et al., '86), are found at the warmest sites (Sarrazin et al., '99), tolerate temperatures of 50–56°C in laboratory pressure chambers (Lee, 2003; Girguis and Lee, 2006), and have the most thermostable enzymes of the three paralvinellids (Jollivet et al., '95). *P. palmiformis* have a continuously produced mucus sheath also having a high content of elemental sulfur (Juniper et al., '86), are found in cooler waters (Sarrazin et al., '99), and were active between 5 and 37°C in laboratory pressure chambers (Lee, 2003). *P. pandorae* (specifically *P. pandorae pandorae*) also have sheaths, and were found by us in cool sites (see Results). We also analyzed the bamboo worm *Nicomache venticola* (a maldanid), which we found around vents but only in cold diffuse-flow areas. Specimens were collected from a number of vent sites with temperatures ranging from 4 to 68°C, and which likely exhibited various sulfide concentrations (Juniper and Martineu, '95; Sarrazin et al., '99).

MATERIALS AND METHODS

Sample collection and temperature measurements

Species used in this study were collected at hydrothermal vents of the Juan de Fuca Ridge, Endeavour Segment, using the R/V *Atlantis* and DSRV *Alvin*. Sites of collection (all around 2,200 m depth) included the Dante edifice at the Main Endeavour field, Clam Bed field, the Faulty Towers complex in the Mothra field, and a new small field found 700 m north of the Dante edifice. Temperatures of vent fluids around the worms were taken for periods greater than 10 sec with an external probe using the *Alvin* manipulator, before the worms were removed with a suction device. Upon recovery, most specimens were rapidly frozen and stored aboard the ship at −70°C, and then transported on dry ice for storage at −80°C to the home laboratory.

A subset of freshly collected *P. palmformis* worms collected from the Roane (Faulty Towers) chimney in the Mothra field were kept alive on board ship. Upon recovery, these worms were rapidly placed into ice-cold seawater, and then pressurized to 3,500 PSI (238 atm or 24 MPa) in a 1.1 L titanium pressure vessel. Thirty minutes later, the vessel was flushed with seawater containing 95 μM oxygen, 235 μM sulfide, pH 7.0, at 30°C as described in Girguis and Childress (2006). Worms were maintained at these conditions for 52 hr. They were then rapidly depressurized and frozen at −70°C. Another group of worms were placed into an identical vessel and were treated as described here, but were maintained in flowing pressurized seawater devoid of hydrogen sulfide, with 120 μM oxygen and pH 8.1 for 70 hr. All animals were alive before freezing. Because of other experiments on the ship, there were insufficient *P. sulfincola* and *P. pandorae* specimens and pressure chambers available for similar experiments on those species.

Solute analysis

Whole semi-thawed animals were weighed on an electronic balance (Mettler AE100, Columbus, OH; 0.0001 g accuracy) after gentle blotting to remove surface seawater. For some *P. sulfincola* and *P. palmformis*, branchiae were cut from the main body and analyzed separately. Samples were then homogenized in 1,000–1,500 μL of 70% cold ethanol, and kept at 4°C overnight before centrifugation at 15,000*g* for 20 min to remove proteins and cellular

debris. Ethanol rather than acid extraction is used to preserve thiotaurine (which breaks down to hypotaurine and sulfide gas in acid; Pruski et al., 2000b). Supernatants were dried overnight in a vacuum centrifuge and then resuspended in 600–1,500 μL of purified water. All samples were passed through with C-18 cartridges to remove lipids (Varian, Inc., Palo Alto, CA) and 0.45 micron filters (Millipore, Inc., Billerica, MA) as described by Wolff et al. ('89). Samples were analyzed for amino acids, sugars, and methylamines using high performance liquid chromatography as previously described (Wolff et al., '89; Yin et al., 2000). Thiotaurine standard was synthesized according to Cavallini et al. ('63).

Statistical analysis

Data are presented as means ± SD. Statistical significance was determined using *t*-tests or ANOVA with Student–Newmann–Keuls post-tests (Instat, GraphPad Software, Inc., La Jolla, CA). Arcsin transformations were used for statistics of ratios.

RESULTS

In situ temperatures

The *Alvin* temperature probe revealed fluctuating temperatures within most of the worm aggregations, with *P. sulfincola* (sulfide worms) experiencing the widest range. Based on temperatures, specimens were designated as follows (Table 1): *P. sulfincola*, hot group with temperatures measured at 42–68°C; *P. sulfincola*, warm group at 21–35°C; *P. palmiformis* (palm worms), cool at 14–18°C; *P. pandorae*, cool at 12–14°C; and *N. venticola*, cold at 4°C. For analysis of branchiae, another group of sulfide worms was collected from a warm site at 18–31°C.

Body mass

Specimens of *P. palmiformis* weighed the most at 0.40 ± 0.17 g. *P. sulfincola* were intermediate at 0.24 ± 0.07 g and *P. pandorae* averaged one-tenth the size of palm worms at 0.041 ± 0.002 g.

Glycine and other nonsulfur amino acids (Table 1)

Of the organic solutes, the most abundant osmolyte in paralvinellids was glycine, ranging from 96 to 119 mmol/kg wet mass (with no significant difference in concentrations between species). This was true for *N. venticola* as well,

TABLE 1. Amino acid, methylamine, and polyol contents of hydrothermal vent polychaetes

Species, temperature	*n*	Thiotaurine	Hypotaurine	Taurine	Glycine	Sarcosine	Betaine	Other AAs	Scyllo-inositol
P. sulfincola, hot (42–68°C)	4	$6.59^{a} \pm 1.15$	$8.40^{a} \pm 2.33$	$6.19^{a} \pm 1.21$	$119^{a} \pm 16.9$	$43.0^{a} \pm 8.2$	$19.0^{a} \pm 4.5$	$18.2^{a} \pm 4.9$	$4.2^{a} \pm 1.04$
P. sulfincola, warm (21–35°C)	6	$4.36^{b} \pm 0.43$	$8.31^{a} \pm 1.49$	$7.78^{a} \pm 2.36$	$109^{a} \pm 17.6$	$35.5^{a} \pm 8.8$	$15.3^{a} \pm 6.7$	$20.8^{a} \pm 4.2$	$5.36^{a} \pm 2.5$
P. palmiformis, cold (14–18°C)	5	$0.37^{c} \pm 0.27$	$2.78^{b} \pm 0.59$	$9.78^{a} \pm 4.17$	$99.7^{a} \pm 21.0$	0^{b}	$14.8^{a} \pm 2.0$	$16.5^{a} \pm 5.1$	0^{b}
P. pandorae, cold (12–14°C)	9	$1.85^{d} \pm 0.29$	$7.52^{a} \pm 1.21$	$6.38^{a} \pm 0.95$	$96.3^{a} \pm 16.1$	0^{b}	$37.6^{b} \pm 5.4$	$20.9^{a} \pm 2.2$	0^{b}
N. venticola, cold (4°C)	3	0^{e}	$0.74^{c} \pm 0.65$	$0.76^{b} \pm 0.36$	$207^{b} \pm 19$	0^{b}	$4.12^{c} \pm 1.94$	$14.7^{a} \pm 2.6$	0^{b}

Values are mmol/kg wet mass ± SD. AAs, amino acids, primarily alanine, proline, and glutaminea. [a,b,c,d,e]Statistically different from others in the same column with a different letter ($P \leq 0.02$, ANOVA, SNK post-test).

which had glycine concentrations of 207 mmol/kg (Table 1). The total of other amino acids (primarily alanine, glutamine, and proline) ranged from 15 to 21 mmol/kg wet mass and did not differ among the species. *P. palmiformis* also contained substantial amounts (estimated 30–50 mmol/kg wet mass) of a compound that did not match any amino acid, methylamine, or carbohydrate standard.

Sarcosine, betaine, and scyllo-inositol (Table 1)

Sarcosine at 36–43 mmol/kg wet mass and scyllo-inositol at 4–5 mmol/kg wet mass were found only in the two *P. sulfincola* groups (Table 1). Betaine contents were the same in two species and different in the other two species, ranging from 38 to 4 mmol/kg wet mass in the order *P. pandorae* > *P. sulfincola* hot = *P. sulfincola* warm = *P. palmiformis* > *N. venticola.*

Thiotaurine, hypotaurine, and taurine

Thiotaurine contents were significantly different among all species and the two *P. sulfincola* groups, ranging from 6.6 to 0 mmol/kg wet mass in the order *P. sulfincola* hot > *P. sulfincola* warm > *P. pandorae* > *P. palmiformis* > *N. venticola* (Table 1). Hypotaurine contents were the same in two species and higher than in the other two species, ranging from 8.8 to 0.7 mmol/kg wet mass in the order *P. sulfincola* hot = *P. sulfincola* warm = *P. pandorae* > *P. palmiformis* > *N. venticola* (Table 1). Taurine contents were low and statistically identical in all species, except for *N. venticola*, which had almost none.

Branchiae vs. main body of P. sulfincola

The branchiae and main body of sulfide worms had statistically identical contents of thiotaurine, taurine, betaine, other amino acids, and scyllo-inositol (Table 2). This batch of sulfide worms were collected from a warm site (18–31°C), and had thiotaurine contents similar to or slightly lower than those of the other warm group (Table 1). However, the branchiae had much higher contents of hypotaurine and sarcosine and much lower contents of glycine. A similar analysis of *P. palmiformis* found no differences between branchiae and main body (data not shown).

TABLE 2. Amino acid, methylamine, and polyol contents of sulfide worm (P. sulfincola) main body vs. branchiae

P. sulfincola body section	*n*	Thiotaurine	Hypotaurine	Taurine	Glycine	Sarcosine	Betaine	Other Aas	Scyllo-Inositol
Main body	3	3.65±0.46	11.3±1.9	8.78±1.90	115±9.9	34.1±3.6	17.0±3.9	32.2±2.4	2.00±0.50
Branchiae	3	3.08±0.74	18.1†±1.52	12.5±3.5	54.4†±10.1	61.0†±11.4	15.0±3.6	29.9±7.2	1.93±2.21

Values are mmol/kg wet mass±SD. AAs, amino acids, primarily alanine, proline, and glutamine. These specimens were from a warm site, 18–31°C.
†Statistically different than in main body ($P \leq 0.02$, *t*-test).

Hypotaurine and thiotaurine totals and exposure indicator ratios in fresh animals

The sum of hypotaurine and thiotaurine differed significantly among the species, but not between the two *P. sulfincola* groups, ranging from 15 to 0.7 mmol/kg wet mass in the order *P. sulfincola* hot = *P. sulfincola* warm > *P. pandorae* > *P. palmiformis* > *N. venticola* (Table 3; Fig. 1). The exposure indicator ratio [thiotaurine:(thiotaurine+hypotaurine)] differed between the two *P. sulfincola* groups and was higher than in the other species, ranging from 0.4 to 0 in the order *P. sulfincola* hot > *P. sulfincola* warm > *P. pandorae* = *P. palmiformis* > *N. venticola* (Table 3; Fig. 1). In the sulfide worm's branchiae, the sum of the two solutes was significantly higher than in the main body (18 vs. 11 mmol/kg wet mass), though the exposure indicator ratio was lower (0.14 vs. 0.24; Table 3; Fig. 1).

TABLE 3. The sum of hypotaurine, and thiotaurine, and the exposure indicator ratio (see text) in hydrothermal-vent polychaetes

Species, temperature	Total Th+Hy	Th/[Th+Hy]
P. sulfincola, hot (42–68°C)	$15.0^{a} \pm 1.0$	$0.435^{a} \pm 0.057$
P. sulfincola, warm (21–35°C)	$12.7^{a} \pm 1.7$	$0.349^{b} \pm 0.038$
P. palmiformis, cool (14–18°C)	$3.15^{b} \pm 0.46$	$0.123^{c} \pm 0.090$
P. pandorae, cool (12–14°C)	$9.36^{c} \pm 0.92$	$0.200^{c} \pm 0.036$
N. venticola, cold (4°C)	$0.74^{d} \pm 0.65$	0^{d}
P. sulfincola, body section		
Main body	14.9 ± 2.7	0.244 ± 0.022
Branchiae	$21.2^{\dagger} \pm 1.2$	$0.146^{\dagger} \pm 0.037$

Values for Total are mmol/kg wet mass ± SD. Th, thiotaurine; Hy, hypotaurine.
[a,b,c,d]Statistically different from other groups in the top section of the table with a different letter ($P \leq 0.02$, ANOVA, SNK post-test).
[†]Statistically different than in main body ($P < 0.01$, *t*-test).

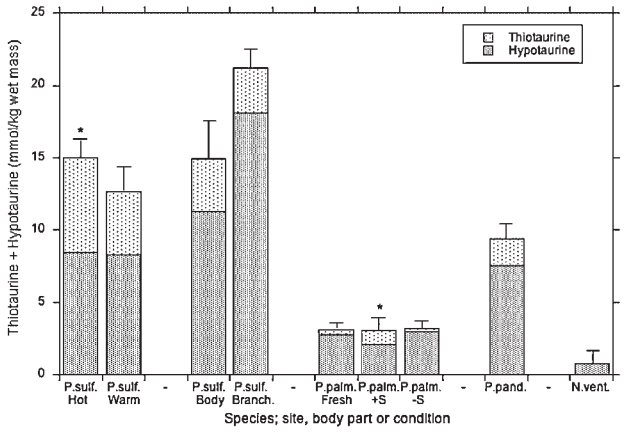

Fig. 1. Total of hypotaurine and thiotaurine in polychaetes analyzed in this study, from Tables 3 and 4. P. sulf., *Paralvinella sulfincola*; P. palm., *P. palmiformis*; P. pand., *P. pandorae*; N. vent., *Nicomache venticola*; Branch., branchiae; +S, −S: kept with sulfide or without sulfide, respectively, in laboratory chambers. *Significantly higher thiotaurine than in other group(s) of the same species.

Hypotaurine and thiotaurine and exposure indicator ratios in experimental animals

In the palm worms kept in laboratory pressure chambers, those held 235 μM sulfide had much higher levels of thiotaurine (0.97 vs. 0.16 mmol/kg wet mass; $P < 0.001$) and lower levels of hypotaurine (2 vs. 3 mmol/kg wet mass; $P = 0.02$) compared with those held with no sulfide (Table 4; Fig. 1). The exposure indicator ratio was considerably higher in the sulfide-treated group (0.32 vs. 0.05; $P < 0.001$). The total of the two solutes, however, was the same in the two groups at about 3 mmol/kg wet mass.

DISCUSSION

This study on closely related polychaetes from different vent habitats greatly extends our previous finding (Rosenberg et al., 2006) that some vent animals without thiotrophic endosymbionts probably use hypotaurine to detoxify sulfide by conversion to thiotaurine. Presumably, sulfide would be removed from thiotaurine (regenerating hypotaurine) and released to the environment during periods of low sulfide exposure, or the animals may expend energy in the active transport (elimination) of the compound. Although in situ

TABLE 4. The sum of hypotaurine and thiotaurine, and the exposure indicator ratio (see text) in palm worms kept in shipboard pressure chambers with and without sulfide

Species, condition	Thiotaurine	Hypotaurine	Total Th+Hy	Th/[Th+Hy]
P. palmiformis, Chamber, no S	0.16 ± 0.12	3.00 ± 0.54	3.16 ± 0.65	0.048 ± 0.033
P. palmiformis, Chamber, 235 μM S	$0.97^{\dagger} \pm 0.31$	$2.08^{\dagger} \pm 0.32$	3.04 ± 0.40	$0.317^{\dagger} \pm 0.091$

Values ($n = 4$ each) are mmol/kg wet mass ± SD (except for the ratios). Th, thiotaurine; Hy, hypotaurine.
[†]Statistically different from chamber, no S ($P \leq 0.02$, *t*-test).

sulfide data were not available in this study, previous work has shown that sulfide levels correlate with vent fluid temperatures (Sarrazin et al., '99; work done on Juan de Fuca sites with these paralvinellids). The detoxification hypothesis is supported by the higher contents of thiotaurine (Tables 1 and 4) and higher exposure indicator ratios (Tables 3 and 4) in (1) *P. sulfincola* compared with its congeners from cooler sites, (2) all paralvinellids compared with the maldanid worm from the coldest site, (3) the *P. sulfincola* from hot waters compared with those from warm waters, and (4) the *P. palmiformis* in laboratory chambers exposed to high sulfide compared with those kept with no sulfide.

The total of hypotaurine plus thiotaurine (Tables 2 and 4; Fig. 1) is consistent with our hypothesis that this is relatively consistent within a species and indicates the maximum likely sulfide exposure for a species (Brand et al., 2007). The sulfide worms had a higher total than the other species, but did not differ among those from the hot and the warm sites (including Main Body; Fig. 1). Also, the palm worms from different sulfide exposures in laboratory chambers had the same total (Fig. 1; Table 4), since hypotaurine apparently declined while thiotaurine apparently increased during sulfide exposure.

The only pattern that might not fit the sulfide-exposure hypothesis is that seen for *P. palmiformis* compared with *P. pandorae*. Both are found in similar thermal (and presumably sulfide) habitats (Tsurumi and Tunnicliffe, 2001), yet the latter species had more thiotaurine and hypotaurine than the former (Table 1 and the sum in Table 3). However, it is also possible that the two species differ in their strategies for sulfide tolerance. *P. pandorae* in this study were much smaller, averaging 10% of the body mass of *P. palmiformis* (possibly a result of competition between the two species; Tsurumi and Tunnicliffe, 2001; Levesque et al., 2003). Thus, *P. pandorae* have a much higher surface-area:volume ratio. As a consequence, they may find it more difficult to exclude sulfide diffusion into their bodies and may therefore need more hypotaurine. The palm worms also have external bacteria in their mucus sheaths that might oxidize sulfide (Juniper et al., '86; Juniper and Martineu, '95), although *P. pandorae* also have a mucus sheath that presumably provides some protection. Transient temperatures (and thus sulfide exposures) could also be higher for *P. pandorae*: of three enzymes examined in paralvinelleds by Jollivet et al. ('95), two were more thermostable in *P. pandorae* than in the palm worms (though less so than in sulfide worms). Finally, *P. pandorae* tend to colonize open vent sites earlier than *P. palmiformis* (Levesque et al., 2003), which could subject them to warmer fluids.

Hypotaurine may play another protective role in vent habitats. Oxygen radicals may be generated during the spontaneous oxidation of sulfide (Tapley et al., '99), and hypotaurine can scavenge some oxygen radicals, becoming taurine (Aruoma et al., '88). This might explain the pattern of much higher taurine contents in the paralvinellids compared with the maldanid.

The occurrence of sarcosine only in the hot-adapted species (*P. sulfincola*) (Table 1) and the considerably higher contents of sarcosine and hypotaurine in branchiae compared with main body of these worms (Table 2) are intriguing. One or both of these differences could be due to specialized metabolic reactions in sulfide worms (especially in the branchiae) that are not related to environmental stresses. However, one or both might also be adaptations to exposure to hotter, more sulfide-laden waters. In particular, branchiae probably have a greater exposure than the main body because they have a much higher surface area:volume ratio and also frequently protrude from the tube. If their exposure is higher, they might need more hypotaurine to react with any surges in sulfide diffusing into them (although we did not find a difference in thiotaurine in this particular group). As evidence of a higher environmental exposure for branchiae, Marie et al. (2006) found that, in oxygenated water in a laboratory pressure chamber, the branchiae of *P. grasslei* exhibited greater oxidative damage than the body wall. In addition, the aerobic enzyme citrate synthase in the branchiae is more thermostable than the same enzyme from the body wall (Rinke and Lee, 2009).

Scyllo-inositol may also relate to habitat conditions, since it was only found in the sulfide worms (Tables 1 and 2). However, it was relatively low in content, and did not differ between branchiae and main body. Also, it has been reported at moderate levels in other deep-sea (but not shallow) marine invertebrates from cold sites (Yancey et al., 2002, 2004). This polyol, but not other inositols, has been found to prevent β-amyloid formation associated with Alzheimer's disease (McLaurin et al., 2006). Thus, it may affect protein structure in useful ways. However, the role of this solute in deep-sea animals remains unknown.

Sarcosine could also be hypothesized to relate to greater exposure factors in sulfide worms (again, especially in branchiae). A possible factor is temperature, since there is no known relationship between sarcosine and sulfide metabolism. Sarcosine has been used in a number of biochemical studies as a thermostabilizer for various prokaryotic and eukaryotic proteins. In one study on bovine RNaseA, it was a slightly better thermostabilizer than glycine and betaine (Santoro et al., '92), and in another study on RNases, it was a better thermostabilizer than glycine and trimethylamine oxide (Ratnaparkhi and Varadarajan, 2001). However, those studies used concentrations of 1 M and higher, outside the range found in sulfide worms, so it is not clear how relevant the findings are. Future studies with sulfide worms in pressure chambers at different temperatures and sulfide levels, and on thermostabilization of proteins with sarcosine in vitro, may shed light on the role of sarcosine.

ACKNOWLEDGMENT

This research was supported by a National Science Foundation grant (NSF OCE- 0623554) to R. W. L. and P. R. G., and a Perry research grant of Whitman College to P. H. Y. and J. I. We thank the crews of the R/V *Atlantis* and the DSRV *Alvin* for their excellent work on behalf of this project.

LITERATURE CITED

Alberic P. 1986. Occurrence of thiotaurine and hypotaurine in the tissues of *Riftia pachyptila*. C R Acad Sci Paris 302:503–508.

Arndt C, Gaill F, Felbeck H. 2001. Anaerobic sulfur metabolism in thiotrophic symbioses. J Exp Biol 204: 741–750.

Arp AJ, Childress JJ, Vetter RD. 1987. The sulfide-binding protein in the blood of the vestimentiferan tube-worm, *Riftia pachyptila*, is the extracellular haemoglobin. J Exp Biol 128:139–158.

Arp AJ, Menon JG, Julian D. 1995. Multiple mechanisms provide tolerance to environmental sulfide in *Urechis caupo*. Am Zool 35:132–144.

Aruoma OI, Halliwell B, Hoey BM, Butler J. 1988. The antioxidant action of taurine, hypotaurine, and their metabolic precursors. Biochem J 256:251–255.

Bagarinao T. 1992. Sulfide as an environmental factor and toxicant: tolerance and adaptations in aquatic organisms. Aquat Toxicol 24:21–62.

Brand GL, Horak RV, LeBris N, Goffredi SK, Carney SL, Govenar B, Yancey PH. 2007. Hypotaurine and thiotaurine as indicators of sulfide exposure in bivalves and vestimentiferans from hydrothermal vents and cold seeps. Mar Ecol 28:208–218.

Cavallini D, Mondovi B, DeMarco C. 1963. Hypotaurine and thiotaurine. Biochem Prep 10:72–75.

Childress JJ, Fisher CR, Favuzzi JA, Arp AJ, Oros DR. 1993. The role of a zinc-based, serum-borne sulfide-binding component in the uptake and transport of dissolved sulfide by the chemoautotrophic symbiont-containing clam *Calyptogena elongata*. J Exp Biol 179:131–158.

Fiess JC, Hom JR, Hudson HA, Kato C, Yancey PH. 2002. Phosphodiester amine, taurine and derivatives, and other osmolytes in vesicomyid bivalves: correlations with depth and symbiont metabolism. Cah Biol Mar 43:337–340.

Fisher CR. 1998. Temperature and sulfide tolerance of hydrothermal vent fauna. Cah Biol Mar 38:283–286.

Girguis PR, Childress JJ. 2006. Metabolic uptake, stoichiometry and chemoautotrophic function of the hydrothermal vent worm *Riftia pachyptila*. J Exp Biol 209:3516–3528.

Girguis PR, Lee RW. 2006. Thermal preference and tolerance of alvinellids. Science 312:231.

Jollivet D, Desbruyeres D, Ladrat C, Laubier L. 1995. Evidence for differences in the allozyme thermostability of deep-sea hydrothermal vent polychaetes (Alvinellidae): a possible selection by habitat. Mar Ecol Prog Ser 123: 125–136.

Joyner JL, Peyer SM, Lee RW. 2003. The possible role of sulfur-containing amino acids in sulfide detoxification in a chemoautotrophic bacteria/mollusc symbiosis. Biol Bull 205:331–338.

Juniper SK, Martineu P. 1995. Alvinellids and sulfides at hydrothermal vents of the eastern pacific: a review. Am Zool 35:174–185.

Juniper SK, Thompson JAJ, Calvert SE. 1986. Accumulation of minerals and trace elements in biogenic mucus at hydrothermal vents. Deep-Sea Res 33:401–412.

Kraus DW. 1995. Heme proteins in sulfide-oxidizing bacteria/mollusc symbioses. Am Zool 35:112–120.

Lee RW. 2003. Thermal tolerances of deep-sea hydrothermal vent animals from the Northeast Pacific. Biol Bull 205: 98–101.

Levesque C, Juniper SK, Marcus J. 2003. Food resource partitioning and competition among alvinellid polychaetes of Juan de Fuca Ridge hydrothermal vents. Mar Ecol Prog Ser 246:173–182.

Marie B, Genard B, Rees J-F, Zai F. 2006. Effect of ambient oxygen concentration on activities of enzymatic antioxidant defences and aerobic metabolism in the hydrothermal vent worm, *Paralvinella grasslei*. Mar Biol 150:273–284.

Martineu P, Juniper SK, Fisher C, Massoth GJ. 1997. Sulfide binding in the body fluids of hydrothermal vent alvinellid polychaetes. Physiol Biochem Zool 70:578–588.

McLaurin J, Kierstead ME, Brown ME, Hawkes CA, Lambermon MH, Phinney AL, Darabie AA, Cousins JE, French JE, Lan MF, Chen F, Wong SS, Mount HT, Fraser PE, Westaway D, St George-Hyslop P. 2006. Cyclohexanehexol inhibitors of A-beta aggregation prevent and reverse Alzheimer phenotype in a mouse model. Nature Med 12:801–808.

Menon J, Willsie JK, Tauscher A, Arp AJ. 2003. Epidermal ultrastructure and implications for sulfide tolerance in six species of deep-sea polychaetes. Invert Biol 122: 334–346.

Ortega JA, Ortega JM, Julian D. 2008. Hypotaurine and sulfhydryl-containing antioxidants reduce H_2S toxicity in erythrocytes from a marine invertebrate. J Exp Biol 211: 3816–3825.

Powell MA, Somero GN. 1986. Adaptations to sulfide by hydrothermal vent animals: sites and mechanisms of detoxification and metabolism. Biol Bull 171:274–290.

Pranal V, Fiala-Médioni A, Colomines JC. 1995. Amino acid and related compound composition in two symbiotic mytilid species from hydrothermal vents. Mar Ecol Prog Ser 119:155–166.

Pruski AM, Fiala-Médioni A. 2003. Stimulatory effect of sulfide on thiotaurine synthesis in three hydrothermal-vent species from the East Pacific Rise. J Exp Biol 206:2923–2930.

Pruski AM, Fiala-Médioni A, Fisher CR, Colomines JC. 2000a. Composition of free amino acids and related compounds in invertebrates with symbiotic bacteria at hydrocarbon seeps in the Gulf of Mexico. Mar Biol 136:411–420.

Pruski AM, Fiala-Médioni A, Prodon R, Colomines JC. 2000b. Thiotaurine is a biomarker of sulfide-based symbiosis in deep-sea bivalves. Limnol Oceanogr 45:1860–1867.

Ratnaparkhi GS, Varadarajan R. 2001. Osmolytes stabilize ribonuclease s by stabilizing its fragments s protein and s peptide to compact folding-competent states. J Biol Chem 276:28789–28798.

Rinke C, Lee RW. 2009. Pathways, activities and thermal stability of anaerobic and aerobic enzymes in thermophilic vent paralvinellid worms. Mar Ecol Prog Ser, in press.

Rosenberg NK, Lee RW, Yancey PH. 2006. High contents of hypotaurine and thiotaurine in hydrothermal-vent gastropods without thiotrophic endosymbionts. J Exp Zool 305A: 655–662.

Santoro MM, Liu Y, Khan SMA, Hou L-X, Bolen DW. 1992. Increased thermostability of proteins in the presence of naturally occurring osmolytes. Biochemistry 31:5278–5283.

Sarrazin J, Juniper SK, Massoth G, Legendre P. 1999. Physical and chemical factors influencing species distributions on hydrothermal sulfide edifices of the Juan de Fuca Ridge, northeast Pacific. Mar Ecol Prog Ser 190:89–112.

Somero GN, Anderson AE, Childress JJ. 1989. Transport, metabolism and detoxification of hydrogen sulfide in animals from sulfide-rich environments. Rev Aquat Sci 1:591–614.

Tapley DW, Buettner GR, Shick JM. 1999. Free radicals and chemiluminescence as products of the spontaneous oxidation of sulfide in seawater, and their biological implications. Biol Bull 196:52–56.

Tsurumi M, Tunnicliffe V. 2001. Characteristics of a hydrothermal vent assemblage on a volcanically active segment of Juan de Fuca Ridge, northeast Pacific. Can J Fish Aquat Sci 58:530–542.

Vetter RD. 1985. Elemental sulfur in gills of three species of clams containing chemoautotrophic bacteria: a possible inorganic energy storage compound. Mar Biol 88:33–42.

Vismann B. 1991. Sulfide tolerance: physiological mechanisms and ecological implications. Ophelia 34:1–27.

Wolff S, Yancey PH, Stanton TS, Balaban R. 1989. A simple HPLC method for quantitating the major organic solutes of the renal medulla. Am J Physiol 256:F954–F956.

Yancey PH. 2005. Organic osmolytes as compatible, metabolic, and counteracting cytoprotectants in high osmolarity and other stresses. J Exp Biol 208:2819–2830.

Yancey PH, Clark ME, Hand SC, Bowlus RD, Somero GN. 1982. Living with water stress: evolution of osmolyte systems. Science 217:1214–1222.

Yancey PH, Blake W, Conley J. 2002. Unusual organic osmolytes in deep-sea animals: adaptations to hydrostatic pressure and other perturbants. Comp Biochem Physiol 133A:667–676.

Yancey PH, Rhea MD, Bailey D, Kemp K. 2004. Trimethylamine oxide, betaine and other osmolytes in deep-sea animals: depth trends and effects on enzymes under hydrostatic pressure. Cell Mol Biol 50: 371–376.

Yin M, Palmer HR, Fyfe-Johnson AL, Bedford JJ, Smith RA, Yancey PH. 2000. Hypotaurine, N-methyltaurine, taurine, and glycine betaine as dominant osmolytes of vestimentiferan tubeworms from hydrothermal vents and cold seeps. Physiol Biochem Zool 73:629–637.