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Stimulatory effect of sulphide on thiotaurine synthesis in three hydrothermal-vent species from the East Pacific Rise

Audrey M. Pruski* and Aline Fiala-Médioni

Observatoire Océanologique de Banyuls, Laboratoire Arago, Université Pierre et Marie Curie, CNRS UMR 7621, BP 44, F-66651 Banyuls-sur-mer Cedex 01, France

*Author for correspondence (e-mail: a_pruski@hotmail.com)

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Summary

Symbiotic associations between marine invertebrates and sulphur-oxidising bacteria are a common feature in communities from sulphide-rich environments, such as those flourishing in the vicinity of hydrothermal vents. While the bacterial endosymbionts provide the host with an undoubted nutritional advantage, their presence also requires specific adaptations for the transport and storage of sulphide, which is a potent toxin of aerobic respiration. Although different mechanisms such as the reversible binding of sulphide to serum binding proteins or its oxidation to less toxic forms have been described, many questions still remained unanswered. In the last decade, large amounts of thiotaurine, an unusual sulphur-amino acid, have been reported in sulphur-based symbioses from hydrothermal vents and cold seeps. Compounds such as thiotaurine are known to take part in trans-sulphuration reactions, so the involvement of thiotaurine in sulphide metabolism has been suggested. We present here an experimental study on thiotaurine biosynthesis in three sulphur-oxidising symbiont-bearing species from the East Pacific Rise: the vesicomyid *Calyptogena magnifica*, the mytilid *Bathymodiolus thermophilus* and the vestimentiferan *Riftia pachyptila*. In all three species, thiotaurine synthesis is stimulated *in vitro* by an input of sulphide, as well as by thiosulphate in *B. thermophilus*. Several distinct metabolic pathways seem to occur, however, since hypotaurine is the only precursor in the bivalves *C. magnifica* and *B. thermophilus*, whereas thiotaurine is also produced from taurine in *R. pachyptila*. Hypotaurine (NH_2-CH_2-CH_2-SO_2H) and thiotaurine (NH_2-CH_2-CH_2-SO_2SH) are two free sulphur amino acids whose chemical formulae differ by only one atom of sulphur. It appears that the extent of thiotaurine synthesis is strongly dependent on the initial equilibrium between these two amino acids, since the strongest thiotaurine synthesis rates are found in tissues with the lowest initial thiotaurine concentration. Moreover, the lack of any effect of sulphide in symbiont-free tissues and in gills of the methanotrophic mussel *Bathymodiolus childressi* reinforces the assumption that thiotaurine synthesis is a specific adaptation to the thiotrophic mode of life. While the precise function (i.e. transport and/or storage of sulphide) of hypotaurine and thiotaurine has yet to be established, our results strongly support a general role for these free amino acids in the metabolism of sulphide in hydrothermal-vent thiotrophic symbioses.

Key words: thiotaurine, hypotaurine, sulphide, sulphur-based symbiosis, hydrothermal vent, amino acid metabolism.

Introduction

In contrast to the generally bare aspect of the deep-sea bottom, dense and rich faunistic communities have flourished in the vicinity of hydrothermal vents (Hessler and Kaharl, 1995). The root of these unique ecosystems are chemoautotrophic bacteria, which oxidise the geothermal sulphide released in hydrothermal fluids (Jannasch, 1995). Many of the metazoan species that dominate these extreme habitats live in association with intracellular sulphur-oxidising symbionts. Such endosymbioses are found in mytilid and vesicomyid bivalves, provannid gastropods and vestimentiferan tubeworms (for a list of references, see Fisher, 1995). The host is provided with a regular input of organic matter produced by the symbionts, but also has to transport in its tissues the poisonous compound used by the bacteria as energetic substrate (i.e. sulphide). These symbioses therefore require structural and functional adaptations by the host for the uptake, transport and storage of sulphide (Powell and Somero, 1986). For example, in the vestimentiferan tubeworm *Riftia pachyptila*, sulphide is taken up from the surrounding seawater across the plume; the sulphide is then reversibly bound in the blood to extracellular haemoglobins and transported to the trophosome, where the bacteria are located. The high affinity of these haemoglobins ensures that internal sulphide can reach very high levels in the body fluids of vestimentiferans (e.g. up

to 12 mmol l^{-1} in the vascular blood of *R. pachyptila*; Childress et al., 1991*)*. Vesicomyid clams also concentrate sulphide from their environment (e.g. up to 1.9 mmol l^{-1} H_2S in blood of freshly collected *Calyptogena magnifica*; Arp et al., 1984). Sulphide is taken up through the highly vascularized foot, bound in the blood to a lipoprotein and transported to the gill tissue, which contains the endosymbionts (Childress et al., 1993). Bathymodiolid mussels, in contrast, are apparently less specialised and have not evolved a mechanism for the transport and concentration of sulphide (Powell and Somero, 1986). In *Bathymodiolus thermophilus*, sulphide diffuses across the gills, where it is oxidised to thiosulfate.

We previously demonstrated that sulphur-based symbioses are characterised by the presence of thiotaurine, a free sulphur amino acid, which has never been reported in high amounts in non-symbiotic species (Pruski et al., 2000b). Based on its distribution in the tissues and its chemical properties, the involvement of thiotaurine in sulphide metabolism was proposed (Pruski et al., 2000a,b). In the present study, we investigated thiotaurine biosynthesis by measuring changes in tissue free amino acid composition in response to exposure of the symbiont-bearing species to sulphide and thiosulphate.

Materials and methods

Sample collection

Three thiotrophic species were selected for this study: the vesicomyid *Calyptogena magnifica* Boss and Turner 1980, the mytilid *Bathymodiolus thermophilus* Kenk and Wilson 1985 and the vestimentiferan *Riftia pachyptila* Jones 1980. Samples were collected by the French submersible *Nautile* at the 9°N site in the East Pacific Rise (EPR) during the *HOT 96* cruise. The cold seep methanotrophic mussel *Bathymodiolus childressi* Gutafson, Turner, Lutz and Vrijenhoek 1998 was chosen as a negative control and sampled from shallow sites on the Louisiana slope of the Gulf of Mexico by the submersible *Johnson Sea Link I* in August 1995. All samples were transported to the surface in chilled seawater and then transferred in cold seawater. Experiments were initiated within 2 h of surfacing.

Preparation of stock solutions of Na_2S and $Na_2S_2O_3$

Sulphide stock solutions (0.5, 1 and 2 mmol l^{-1}, pH 7.5) were made up with $Na_2S.9H_2O$ in deoxygenated bidistilled water inside a glove bag filled with helium. The anaerobic solutions were distributed in 20 ml vials, sealed with Teflon caps and refrigerated until utilisation (storage time was ≤2 weeks). Stock solutions of thiosulphate (2 mmol l^{-1}) were freshly prepared before each experiment by dissolving crystals of $Na_2S_2O_3.5H_2O$ in deoxygenated bidistilled water.

Incubation of tissue homogenates with reduced sulphur

Prior to the experiments, small pieces of selected tissues (gill and mantle of bivalves, plume and trophosome of tubeworms) were isolated from each specimen and frozen for high performance liquid chromatography (HPLC) determination of the free amino acid (FAA) composition (see below). Since thiotaurine occurrence was restricted to the symbiont-containing tissues, only the gills of bivalves and the trophosome of vestimentiferans were used in the experiments described below.

Adult specimens of similar size were used for the experiments (clams of 15.2±1.6 cm shell length, mussels of 13.2±1.3 cm shell length, and tubeworms of approximately 80 cm length). One complete demibranch was excised from each bivalve, rinsed in filtered seawater to remove excess mucus and rapidly chopped. Because we could not weigh the tissues on board, 1 volume (10 ml) of chopped gills or trophosome was homogenized on ice in 3 volumes of a cold deoxygenated buffer (10 mmol l^{-1} Hepes, 5 mmol l^{-1} $MgCl_2$, 0.25 mmol l^{-1} saccharose, pH 7.5) using a glass homogenizer. Tissue homogenates were then distributed in incubation vials with a screw top and Teflon septum (1.5 ml of homogenate per vial). At time zero, 1.5 ml of sulphide stock solution or thiosulphate solution was injected with a hypodermic syringe through the septum of the incubation vials. Throughout the experiment, vials were gently agitated. After 0, 5, 10 and 25 min, homogenates were transferred into cryovial tubes and frozen in liquid nitrogen until FAA analysis. Controls were prepared by incubating gill and trophosome homogenates with buffer. These experiments were repeated 4–6 times, each time with a different animal.

Amino acid quantification

Samples were lyophilized in the laboratory and the free amino acid pool extracted in cold 70% ethanol (for a detailed description of the protocol, see Pruski et al., 1998). Amino acids were separated and quantified by reverse-phase HPLC as previously described (Pruski et al., 1998). All chemicals were purchased from Sigma-Aldrich (Saint-Quentin Fallavier, France) except for thiotaurine, which was prepared as described by Albéric and Boulègue (1990).

Data presentation and statistical analyses

Data are presented as means ± S.D. and values of *N* (number of repetitions) are given in the figure legends and the tables. Simple regressions were used to show linear relationships. Two-sample comparisons were made using paired one-tailed *t*-tests assuming equal variance (Microsoft Excel). Multiple comparisons were made using analysis of variance (ANOVA) with a Bonferroni *post-hoc* test (Statview). Significance was accepted at the 5% level.

Results

Synthesis of thiotaurine in Calyptogena magnifica

Thiotaurine was the second most abundant FAA in gills of *C. magnifica*, with concentrations ranging from 66 to 152 μmol g^{-1} dry mass, corresponding to 10–26% of the FAA pool (Table 1). In comparison, the hypotaurine concentration was low and only accounted for 2–9% of the FAAs. Based on the assumption that thiotaurine is formed from hypotaurine and

sulphide, Pranal et al. (1995) proposed the use of the thiotaurine:(thiotaurine+hypotaurine) ratio as an indicator of sulphide exposure; the closer this ratio is to 1, the higher the likelihood that the animal has been exposed to the vent fluid. Thiotaurine:(hypotaurine+thiotaurine) ratios were superior or equal to 0.69 in gills from *C. magnifica* (Table 2), which suggests they were exposed to relatively high levels of sulphide. These values were consistent with previous results for this species (Pruski et al., 2000a).

Gill homogenates from six clams were exposed to various concentrations of Na_2S for up to 25 min and the concentration of 26 FAAs followed over time. Apart for thiotaurine and hypotaurine concentrations, no significant changes in the FAA composition were observed (not shown). Thiotaurine concentration increased considerably in gills incubated with Na_2S (Fig. 1A), but no significant difference was observed between the different treatments (ANOVA, $P<0.05$, Fig. 1A), which suggests that the mechanism responsible for thiotaurine synthesis was saturated at concentrations above 0.25 mmol l^{-1} Na_2S.

The time courses of thiotaurine and hypotaurine variations in homogenates exposed to 1 mmol l^{-1} Na_2S are shown in Fig. 2A.

Table 1. *Initial concentrations of taurine derivatives in the symbiont-containing tissues of three species from the East Pacific Rise and thiotaurine concentration in the same tissues exposed to Na_2S and $Na_2S_2O_3$*

	Initial concentration (μmol g^{-1})			Thiotaurine concentration (μmol g^{-1}) after exposure to:			
	Thiotaurine	Hypotaurine	Taurine	Na_2S 0.25 mmol l^{-1}	Na_2S 0.5 mmol l^{-1}	Na_2S 1 mmol l^{-1}	$Na_2S_2O_3$ 1 mmol l^{-1}
Calyptogena magnifica							
1	152	21	36	154	158	167	157
2	66	30	34	92	92	93	77
3	122	43	30	157	156	152	132
4	132	16	42	140	141	143	143
5	115	25	48	127	122	123	125
6	88	9	22	91	88	89	89
Bathymodiolus thermophilus							
1	80	77	62	122	111	84	121
2	105	39	46	113	109	76	112
3	59	76	24	82	75	67	80
4	41	47	36	56	59	35	49
5	44	63	35	88	77	13	62
Riftia pachyptila							
1	5	150	266	73	258	329	10
2	113	119	143	240	90	203	116
3	92	99	27	194	197	212	118
4	125	110	41	236	227	233	147

Each line corresponds to one single specimen.

Among the 26 amino compounds measured thiotaurine, hypotaurine and taurine were the only ones to vary in concentration during the experiments.

Taurine concentration only decreased in the trophosome of *R. pachyptila* exposed to Na_2S.

Table 2. *Values of the Thiotaurine:(thiotaurine+hypotaurine) ratio in symbiont-containing tissues of three hydrothermal-vent species before and after 25 min incubation with sulphide (Na_2S) or thiosulphate ($Na_2S_2O_3$)*

	Thiotaurine:(thiotaurine+hypotaurine)		
	C. magnifica *N*=6	*B. thermophilus* *N*=5	*R. pachyptila* *N*=4
T_0	0.83 (0.69–0.90)	0.51 (0.41–0.73)	0.38 (0.03–0.53)
Na_2S			
0	0.89 (0.84–0.92)	0.52 (0.41–0.75)	0.50 (0.07–0.68)
0.25 mmol l^{-1}	0.93 (0.89–0.96)	0.73 (0.61–0.83)	0.79 (0.39–0.96)
0.5 mmol l^{-1}	0.93 (0.87–0.96)	0.68 (0.56–0.76)	0.78 (0.31–0.97)
1 mmol l^{-1}	0.94 (0.88–0.97)	0.42 (0.12–0.53)	0.89 (0.64–0.99)
$Na_2S_2O_3$ (1 mmol l^{-1})	0.88 (0.81–0.97)	0.66 (0.56–0.77)	0.51 (0.11–0.68)

T_0, before incubation.

Values are means (range in parentheses).

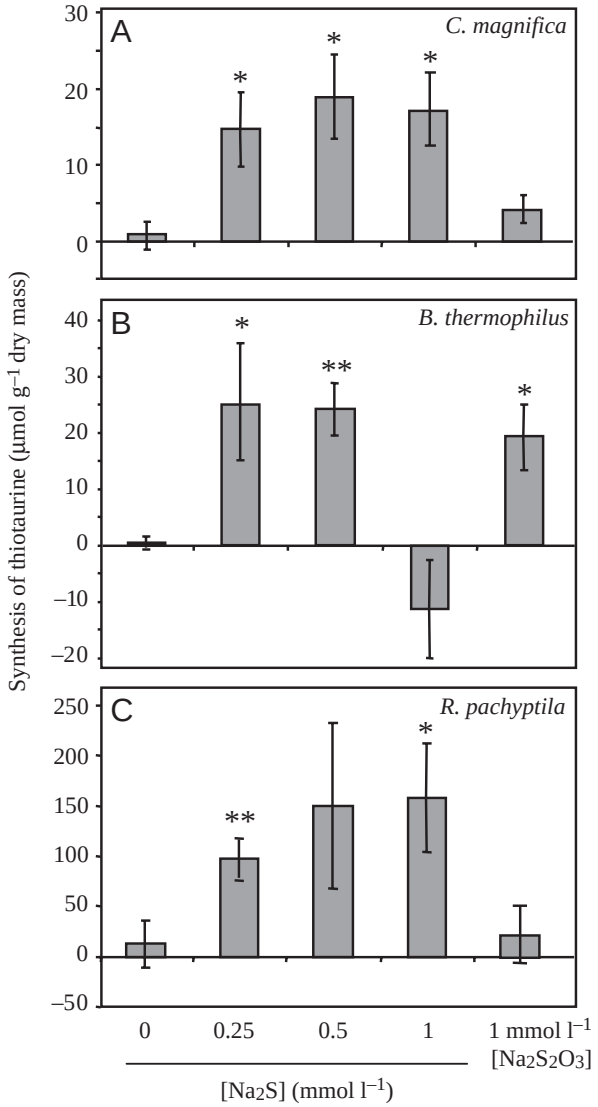

Fig. 1. Synthesis of thiotaurine in gill homogenates of (A) *Calyptogena magnifica* and (B) *Bathymodiolus thermophilus*, and (C) trophosome homogenates of *Riftia pachyptila*, incubated with various concentrations of sulphide (0.25, 0.5 and 1 mmol l^{-1} Na_2S) and thiosulfate (1 mmol l^{-1} $Na_2S_2O_3$). Values (µmol g^{-1} dry mass) are means ± S.D. (*C. magnifica*, *N*=6; *B. thermophilus*, *N*=5; *R. pachyptila*, *N*=4). Asterisks designate significant differences from the controls (Student's *t*-test, **P*≤0.05, ***P*≤0.005).

Thiotaurine concentration increased rapidly during the first 5 min of incubation, whereas hypotaurine concentration declined. Both concentrations remained relatively constant after this initial stage. Overall, an approximately 1:1 stoichiometric conversion of hypotaurine to thiotaurine was observed.

The individual response was quite variable (e.g. thiotaurine synthesis ranged from 3 to 30 µmol g^{-1} dry mass after 25 min of incubation with 1 mmol l^{-1} Na_2S; Table 1) with lower rates of thiotaurine synthesis in tissues with high initial thiotaurine content. A negative linear relationship between the initial thiotaurine:(thiotaurine+hypotaurine) ratio and the amount of thiotaurine synthesized was indeed apparent (r=0.83, N=6, P<0.01). This suggests that thiotaurine synthesis is strongly dependent on the initial equilibrium between hypotaurine and thiotaurine in the gill.

In the absence of Na_2S, a linear, but lower synthesis of thiotaurine was evident throughout the experiment, with a concomitant decrease of hypotaurine (Fig. 2A). No significant increase in synthesis of thiotaurine compared to controls was observed when gills were incubated with 1 mmol l^{-1} $Na_2S_2O_3$ (Fig. 1A).

Synthesis of thiotaurine in Bathymodiolus thermophilus

Thiotaurine concentration was lower in *B. thermophilus* than in *C. magnifica*, whereas the opposite trend was

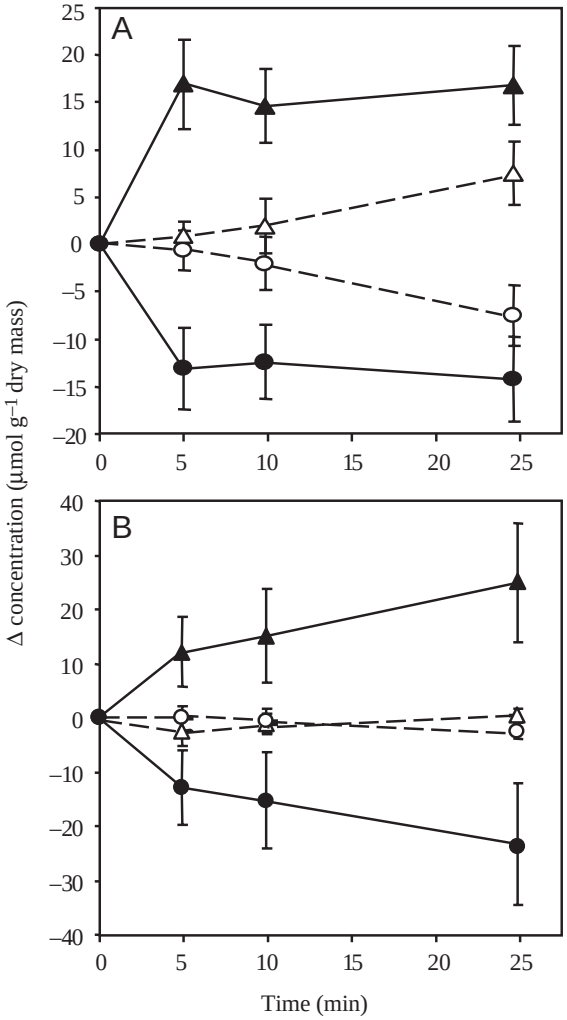

Fig. 2. The kinetics of thiotaurine production in gill homogenates of (A) *Calyptogena magnifica* and (B) *Bathymodiolus thermophilus*. Homogenates of *C. magnifica* and *B. thermophilus* were exposed, respectively, to 1 mmol l^{-1} and 0.25 mmol l^{-1} Na_2S at time zero (filled symbols). Controls were obtained by incubating the homogenates with buffer (open symbols). Thiotaurine (triangles) and hypotaurine (circles) concentrations are given as µmol g^{-1} dry mass (means ± S.D.; *C. magnifica*, *N*=6; *B. thermophilus*, *N*=5).

observed with hypotaurine (Table 1). The resulting thiotaurine:(thiotaurine+hypotaurine) ratios were therefore lower (0.41–0.73; Table 2), which suggests the mussels were exposed to a more diluted hydrothermal fluid. This is consistent with the location of these specimens at the periphery of the vent sites.

As observed with the clams, Na_2S exposure (at 0.25 and 0.5 mmol l^{-1}) induced the formation of thiotaurine in *B. thermophilus* gills (Fig. 1B). However, thiotaurine concentration did not reach a plateau after the first 5 min of incubation, but continued to increase at a lower rate (Fig. 2B). Hypotaurine concentration decreased concomitantly throughout the incubation (Fig. 2B). Although the individual response was highly variable (Table 1), a clear inhibition of thiotaurine synthesis was observed at 1 mmol l^{-1} Na_2S (Fig. 1B), the final thiotaurine concentration being even lower than in controls. A maximum thiotaurine synthesis rate of approximately 25 μmol g^{-1} dry mass was observed in homogenates incubated for 25 min with 0.25 mmol l^{-1} Na_2S (Fig. 1B). Values of the thiotaurine:(thiotaurine+hypotaurine) ratio remained lower at the end of the incubation time than in the clams, ranging from 0.61 to 0.83 after 25 min of incubation with 0.25 mmol l^{-1} Na_2S (Table 2).

Thiotaurine synthesis was also stimulated by 1 mmol l^{-1} $Na_2S_2O_3$ (Fig. 1B). The amounts of thiotaurine synthesized were of the same order of magnitude as in gills incubated with 0.25 mmol l^{-1} Na_2S (approximately19 μmol g^{-1} dry mass after 25 min of incubation; Fig. 1B).

No significant synthesis of thiotaurine was observed in the absence of Na_2S (Fig. 2B).

In order to determine if the ability to produce thiotaurine is a specific adaptation to thiotrophy, gills of the methanotrophic mussel *B. childressi* were also incubated with Na_2S (data not shown). This mussel, like all non-thiotrophic species, is characterized by very low levels of thiotaurine (<0.5 μmol g^{-1} dry mass; for a description of its FAA composition, see Pruski et al., 2000a). Hypotaurine was found in its gill tissue, but only trace amounts of thiotaurine were synthesized (<4 μmol g^{-1} dry mass after 25min of incubation with 0.5 mmol l^{-1} Na_2S).

Synthesis of thiotaurine in Riftia pachyptila

Taurine derivatives (i.e. taurine, hypotaurine and thiotaurine) were particularly abundant in the trophosome of *R. pachyptila* and accounted for up to two thirds of the FAA pool. These compounds contained approximately 14% of the total sulphur content of the trophosome (on the basis of a sulphur content of 11.1% dry mass; A. M. Pruski and A. Fiala-Médioni, unpublished data). Thiotaurine was restricted to the trophosome and its concentration was highly variable (5–140 μmol g^{-1} dry mass; Table 1), as was the thiotaurine:(thiotaurine+hypotaurine) ratio (0.03–0.53; Table 2).

A dose-dependent increase of the amount of thiotaurine synthesized was observed in trophosomes incubated with Na_2S at concentrations ranging from 0 to 1 mmol l^{-1} (Fig. 1C). Although the individual response was again quite variable, a clear displacement of the hypotaurine–thiotaurine equilibrium was observed in each sample, with values of the thiotaurine:(thiotaurine+hypotaurine) ratio reaching 0.64–0.99 at the end of the incubation time (Table 2). This indicates that vestimentiferan tubeworms have robust ability for thiotaurine synthesis. The magnitude of thiotaurine synthesis was actually far greater in *R. pachyptila* than in the two bivalve species and reached a maximum of 324 μmol g^{-1} dry mass. Assuming 80% of the vestimentiferan's mass was water, this would allow the fixation of up to 65 μmol sulphide g^{-1} fresh trophosome.

The time courses of thiotaurine, hypotaurine and taurine variations in homogenates exposed to 1 mmol l^{-1} Na_2S are shown in Fig. 3. A rapid and elevated synthesis of thiotaurine was observed during the first 5 min of incubation (mean synthesis = 150 μmol g^{-1} dry mass), while hypotaurine concentration decreased concomitantly. In contrast to the results obtained in the bivalve experiments, taurine concentration also declined remarkably within 5 min. The amount of thiotaurine synthesized was equivalent to the combined decrease of the taurine and hypotaurine concentrations. After 5 min of incubation, the initial reserves in hypotaurine and taurine were completely depleted in three of the four individuals tested, which explains the lack of further thiotaurine synthesis (data not shown).

A low synthesis of thiotaurine was also evident in the absence of Na_2S (approximately 30 μmol g^{-1} dry mass), but no significant synthesis of thiotaurine was observed in the presence of $Na_2S_2O_3$ (Fig. 1C).

Discussion

Binding of reduced sulphur to thiotaurine

The results of this study clearly demonstrate the incorporation of sulphide into thiotaurine in the three dominant

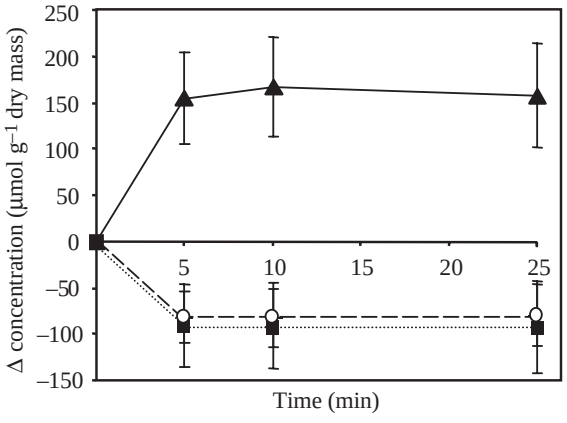

Fig. 3. The kinetics of thiotaurine production in trophosome homogenates of *Riftia pachyptila* exposed to Na_2S (1 mmol l^{-1}). Thiotaurine (filled triangles), hypotaurine (open circles) and taurine (filled squares) concentrations are given as μmol g^{-1} dry mass (means ± S.D., N=4).

symbiotic species from the EPR. The stoichiometry of the reaction is 1:1 (i.e. 1 atom of reduced sulphur incorporated per molecule of thiotaurine), with hypotaurine being the main precursor. Such trans-sulphuration reactions between a sulphinate (hypotaurine) and a thiosulphonate (thiotaurine) have been described in animals (for example, see De Marco et al., 1961, 1972). These reactions can be spontaneous or catalysed by three sulphur transferases: mercaptopyruvate sulphur transferase, thiosulphate sulphur transferase and thiosulphate sulphur reductase, also called rhodanese (Chauncey and Westley, 1983). The latter enzyme was found in hydrothermal-vent species with sulphur-oxidising symbionts (Felbeck et al., 1981; Diouris et al., 1988). In this type of reaction, an oxidant power is necessary and is often glutathione (Chauncey and Westley, 1983):

$$\underset{\text{(hypotaurine)}}{RSO_2^-} + SH^- + \underset{\text{(oxidised glutathione)}}{GSSG} + H^+ \leftrightarrows \underset{\text{(thiotaurine)}}{RS_2O_2^-} + \underset{\text{(reduced glutathione)}}{GSH}$$

Hypotaurine is the only precursor in bivalves, while taurine is also involved in the synthesis of thiotaurine in vestimentiferan tubeworms. This suggests that different enzymatic pathways are used. Although no direct reaction from taurine to thiotaurine has been described to date, one hypothesis is that taurine is first reduced to hypotaurine, which subsequently reacts with sulphide. The reverse reaction, the oxidation of hypotaurine to taurine, occurs in most animals during the catabolism of sulphur amino acids, whose last end-product is taurine (Huxtable, 1992).

Thiosulphate also stimulates thiotaurine synthesis, although only in mytilids. From the literature, two mechanisms may be proposed to explain this result: (1) the conversion of thiosulphate to sulphide before thiotaurine synthesis, and (2) a direct formation of thiotaurine with sulphite as a byproduct (De Marco et al., 1961, 1972). The symbionts of *Bathymodiolus thermophilus* are known to use thiosulphate preferentially (Nelson et al., 1995), in contrast to those of vesicomyids and vestimentiferans, which rely only on sulphide. Therefore, one may expect that specific metabolic pathways for the utilisation and storage of thiosulphate occur in *B. thermophilus*.

Potential involvement of thiotaurine in the transfer of sulphide from the blood to the bacteriocytes

One surprising finding of this work was the linear synthesis of thiotaurine observed in tissues of *C. magnifica* (Fig. 1) and *R. pachyptila* after incubation with buffer alone. Those two species share one specific adaptation to the thiotrophic mode of life, namely the occurrence of sulphide-binding proteins in the blood (i.e. the haemoglobins in *R. pachyptila* and a lipoprotein in *C. magnifica*; Arp et al., 1987; Childress et al., 1993). In the absence of any external source of sulphur, one may hypothesize that the sulphur incorporated into thiotaurine originated from these sulphide-binding proteins. Sulphide binds to the haemoglobins of *R. pachyptila* at the level of free cysteine residues and disulphide bridges, with the formation of persulphide groups (Zal et al., 1998). The transfer of reduced sulphur from protein persulphides (R-SSH) was previously shown to be rapid and spontaneous in the presence of hypotaurine (less than 10 min; Cavallini et al., 1970), and would explain such a result:

$$R_1\text{-}SSH + \underset{\text{(hypotaurine)}}{NH_2\text{-}CH_2\text{-}CH_2\text{-}SO_2H} \leftrightarrows R_1\text{-}SH + \underset{\text{(thiotaurine)}}{NH_2\text{-}CH_2\text{-}CH_2\text{-}SO_2SH}$$

where R1 = haemoglobin in vestimentiferans.

In the blood of *Calyptogena magnifica*, sulphide is bound to a transport lipoprotein *via* zinc ions (Zal et al., 2000) and could equally well be transferred to hypotaurine. As those sulphide-binding proteins are too large to penetrate the bacteriocytes, thiotaurine synthesis would indeed facilitate the transfer of sulphide from blood components to the bacteriocytes, just as myoglobin facilitates the transfer of oxygen from blood to muscular cells. This hypothesis is further supported by the fact that no synthesis of thiotaurine was observed in the absence of an external source of reduced sulphur in *B. thermophilus* (a species that is devoid of any sulphide-binding protein).

An alternative explanation for the observed synthesis of thiotaurine in controls from *C. magnifica* and *R. pachyptila* is provided by the recent results from Arndt et al. (2001). This study showed that sulphide is produced under anoxic conditions in several thiotrophic symbioses including *C. magnifica*, *B. thermophilus* and *R. pachyptila*. Elemental sulphur (S°) is proposed to be the substrate responsible for this anaerobic generation of sulphide, which started a few hours after anoxia had set in (Arndt et al., 2001). Since S° was never found in *B. thermophilus*, one expects this species to be unable to generate sulphide under anoxic conditions, which is consistent with the lack of thiotaurine synthesis that we observed in mussel gills. In contrast, the symbiont-bearing tissues of *C. magnifica* and *R. pachyptila* contain large amounts of S° (Somero et al., 1989). If the anaerobic production of sulphide is responsible for the synthesis of thiotaurine observed in the absence of an external input of reduced sulphur, large amounts of sulphide must be generated within minutes (up to 4 μmol g^{-1} and 8 μmol g^{-1} fresh mass after 25 min of incubation in *C. magnifica* and *R. pachyptila*, respectively). These rates of sulphide production are higher than the values reported by Arndt et al. (2001). For example, in *R. pachyptila*, less than 0.5 μmol g^{-1} sulphide were released in 15 min under anoxic and hypoxic conditions. This suggests that if some of the sulphide incorporated to thiotaurine in our controls was produced anaerobically, there must be another source of sulphide (i.e. sulphide bound to proteins).

Function(s) of thiotaurine in sulphur-based symbioses: thiotaurine as a carrier of reduced sulphur

Living in association with sulphur-oxidising symbionts has forced the hosts to evolve specific adaptations to survive the toxic sulphide, such as molecules that can reversibly bind sulphide until it is oxidised by the symbionts. Thiosulphonates such as thiotaurine meet the requirements for a biologically perfect carrier of reduced sulphur. They exhibit higher lipid solubility than inorganic thiosulphate and have been shown to penetrate cell membranes readily (Petrikovics et al., 1994). One may thus expect that thiotaurine can easily enter the

bacteriocytes to deliver the energy-containing substrate to the symbionts. Another requisite satisfied by thiotaurine is that it can accumulate in the cytoplasm in high concentrations without perturbing protein function. In tissues containing high amounts of thiotaurine, we have previously shown a compensatory loss of other free amino acids such as glycine, enabling maintenance of a steady intra-osmotic pressure and thus the integrity of the cell (Pruski et al., 2000a). Thiotaurine appears to have other positive effects on the cell, such as protection of DNA from damage by sulphur compounds (i.e. mustard gas; Baskin et al., 2000). Furthermore, thiotaurine has no other known metabolic function in the cell and is synthesized from non-essential compounds. The energetic cost of thiotaurine synthesis is thus relatively little.

To be efficient, a carrier has to be able to bind and release reduced sulphur rapidly. We have shown that reduced sulphur can be incorporated to thiotaurine and have provided arguments in favour of the possible involvement of this amino acid in the transfer of sulphide from the blood to the bacteriocytes. The release of reduced sulphur from thiotaurine has previously been demonstrated (Pruski et al., 2001) by a catalytic factor present in the trophosome of vestimentiferans that enables the release of reduced sulphur from thiotaurine prior to its oxidation by sulphur-oxidising bacteria (Pruski et al., 2001). Although the incorporation of reduced sulphur into thiotaurine can occur spontaneously (De Marco et al., 1961), the differences observed between the three species and the lack of significant thiotaurine synthesis in symbiont-free tissues (data not shown) and in methanotrophic mussels suggest some form of enzymatic control. Whether the host or its symbionts produce this enzyme is a key question for understanding how energy delivery (as sulphur) is controlled in the symbiosis.

Conclusion

Thiotaurine synthesis appears as a general adaptation supporting life with sulphur-oxidising symbionts (Pruski et al., 2000b). The incorporation of reduced sulphur to thiotaurine provides the host with an efficient and relatively cost-free mechanism to bind, transport and/or store sulphide in its host tissues. As thiotaurine is not the only compound involved in the transport of reduced sulphur in sulphide-based symbioses, future studies will have to determine how these different binding systems interact.

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