# Sulphur-amino acids in symbiotic species from hydrothermal vents and cold seeps

Audrey M. PRUSKI[1]*, Aline FIALA-MEDIONI[1], Jacques BOULÈGUE[2] and Jean-Charles COLOMINES[1]

[1] *Observatoire Océanologique de Banyuls, Laboratoire Arago, Université Pierre et Marie Curie,*
*CNRS UMR 7621, BP 44, 66651 Banyuls-sur-Mer Cedex 01, France*
[2] *UPMC, département de Géochimie et Métallogénie, 4 place Jussieu, 75252 Paris Cedex 05, France*
*\* Corresponding author, Fax: (33) 4 68 88 73 95 - e-mail : audrey@arago.obs-banyuls.fr*

## Introduction

Symbioses between chemoautotrophic bacteria and benthic invertebrates are widespread in high-sulphide environments where sulphide concentrations may go up to 300-400 µM in the mixing zone (Johnson et al., 1988) and to 10 mM in reduced sediments (Martens et al., 1991). All organisms from these environments have to protect themselves from sulphide toxicity, and species harbouring sulphur-oxidizing symbionts have furthermore to supply their symbionts with sulphide. Some species have developed specialized mechanisms for the uptake and transport of sulphide. Thus, in vesicomyids and vestimentiferans, blood components are involved in the uptake and the transport of sulphide, while no sulphide-binding component was found in *Bathymodiolus*-like mussels (for review see Nelson & Fisher, 1995). Free amino acids are abundant in marine invertebrate tissues where they are primarily involved in osmoregulation processes. But certain amino acids, like taurine (Huxtable, 1992), also occur in energetic pathways (via the production of opines during anaerobiosis in marine invertebrates) or detoxification processes (for examples, $OCl^-$ ions detoxification by taurine in human neutrophils and sulphur-amino acids protection against free radicals in mammalians). It was previously suggested that sulphur-amino compounds could be involved in sulphide metabolism in the vestimentiferan *Riftia pachyptila* Jones, 1981 (see Albéric, 1986) and the vesicomyid *Calyptogena phaseoliformis* Métivier, Okutani & Ohta, 1986 (see Albéric & Boulègue, 1990).

In this study, the free amino acid composition of 21 deep-sea symbiotic species from different ecological and geological contexts were compared in order to determine specific features related to symbiosis or to environmental conditions. Three sulphur-amino acids were found in high amounts in the species studied: taurine, hypotaurine and thiotaurine. The possible involvement of these sulphur-amino acids in the metabolism of sulphur was also investigated by measuring their concentrations during sulphide exposure.

## Material and methods

### I. Sample collection

Mytilids, vesicomyids and vestimentiferan tube-worms were collected from several hydrothermal vent and cold seep sites: the East Pacific Ridge (HERO 94 and HOT 96 cruises), the junction zone between the East Pacific Ridge and the Rivera fracture (NAUTIMATE cruise), the Mid-Atlantic Ridge (LUCKY STRIKE 95, MAR 93 and DIVA2 cruises), the Fiji and Manus basins (YOKOSUKA 91 and BIOACCESS 96 cruises), the Accretionary prism of Barbados (DIAPISUB cruise), and the Gulf of Mexico (1995). On board, animals were rapidly dissected, and tissues were frozen and stored in liquid nitrogen until lyophilization and analysis at the laboratory.

### II. Extraction and quantification of the free amino acid pool

Measurements of free amino acids in samples were made by extracting 30 mg of dry tissue in a 70:30 (v/v) ethanol-water mixture for 2 hours. A solution of homoserine was added to account for global losses of amino acids during the extraction. The supernatant was evaporated to dryness, resuspended in milli-Q water and filtered on a 0.2 µm pore size membrane. Primary amino acids were separated by reverse-phase high performance liquid chromatography on a MICROSORB C-18 column after pre-column derivatization with ortho-phtaldialdehyde (Mopper & Lindroth, 1982), and detected by fluorescence (335 nm for excitation and 450 nm

for emission). Non-linear binary gradients of acetate-methanol buffer were used with a flow rate of 1 ml min^{-1} (solvent A: 80% sodium acetate/19.2% methanol/ 0.8% tetrahydrofuran, and solvent B: 20% sodium acetate/ 80% methanol). The elution gradient is given in Table 1. The derivatization procedure used by Pranal et al. (1995) was modified to allow the detection of cysteine, homocysteine and cysteamine. Briefly, 100 µl of samples or standard solution were mixed with 400 µl of iodoacetic acid 0.8M pH 10.5, 10 µl of an external standard (1µM S-methyl cysteine) and 5 µl of the derivatization reagent (ortho-phtaldialdehyde/mercapto-ethanol) for exactly 2 minutes. Primary amines were identified and quantified by comparison with standard solution of known concentration (Sigma Chemical Co.). Thiotaurine standard was prepared separately as described by Albéric & Boulègue (1990).

**Table 1.** Elution gradient of the binary phase system.

| Time (min) | 0 | 2.1 | 5.1 | 17 | 20 | 25 | 30 | 34 | 52 |
|---|---|---|---|---|---|---|---|---|---|
| Solvent A % | 100 | 100 | 90 | 90 | 86 | 86 | 50 | 50 | 0 |

### III. Sulphide incubations of crushed gill

For the experiments, gill tissue of freshly recovered animals was isolated, washed in filtered sea water and crushed in a de-oxygenated HEPES-MgCl$_2$-saccharose buffer (10 mM-5 mM-0.25 mM). The gill homogenate was distributed in vials and incubated with the same volume of an anoxic solution of Na$_2$S (final molarity: 0.25 mM, 0.5 mM, or 1 mM). After exposure to sulphide (0 to 25 minutes), the samples were transferred to cryovials and frozen in liquid nitrogen.

## Results and discussion

### I. Specific features of the free amino acid pool in deep sea symbiotic species

The free amino acid composition of the 21 species studied was dominated by three sulphur-amino acids: taurine, which is also abundant in the shallow non-symbiotic mussel *Mytilus galloprovincialis* Lamarck, 1819 and its sulphinic and thiosulphonic analogues, hypotaurine and thiotaurine. The occurrence of the two latter compounds is of particular interest because, to our knowledge, they were never found in high amounts in non-symbiotic marine invertebrates. The free sulphur-amino acid pool was qualitatively and quantitatively different between species and between tissues. Figure 1 shows the percentages of taurine, hypotaurine and thiotaurine in three species from the East
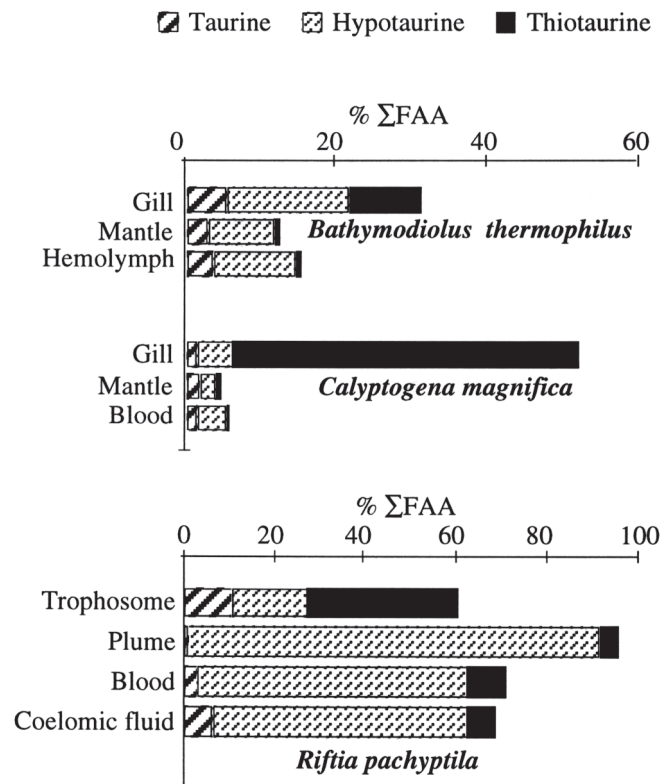


**Figure 1.** Sulphur-amino acid composition of three symbiotic species from the EPR : *Bathymodiolus thermophilus, Calyptogena magnifica* and *Riftia pachyptila*. Mean values are expressed as percentages of the total free amino acids (% SFAA).

Pacific: *Bathymodiolus thermophilus* Kenk & Wilson, 1985 *Calyptogena magnifica* Boss & Turner, 1980 and *Riftia pachyptila*. Larger amounts of these compounds were found in the tissues of the vestimentiferans (average values varied from 61% of the free amino acid pool in the trophosome to 95% in the plume) than in the bivalves (from 13% in the mantle to 32% in the gill of *B. thermophilus* and from 5% in the mantle to 52% in the gill of *C. magnifica*). Taurine and hypotaurine were present in all the tissues, whereas large amounts of thiotaurine were only found in the symbiont-containing tissues: the gill of the bivalves and the trophosome of the tubeworm (from 10% to 45% in the gill of *B. thermophilus* and *C. magnifica* respectively). High levels in thiotaurine were previously seen in several groups of organisms: the vestimentiferan *Riftia pachyptila* (Albéric, 1986; this study), the vesicomyid *Calyptogena phaseoliformis* (Albéric & Boulègue, 1990), several mytilids *Bathymodiolus brevior* Cosel, Métivier & Hashimoto, 1994, *B. elongatus* Cosel, Métivier & Hashimoto, 1994 (see Pranal et al., 1995), *B. thermophilus*, and *B. puteoserpentis* Cosel, Métivier & Hashimoto, 1994 (see Pruski et al., 1997), and two gastropods *Alviniconcha hessleri* Okutani & Ohta, 1988 and *Ifremeria nautilei* Bouchet & Waren, 1991 (see Pranal, 1995). The common

characteristic of these species is that they all harbour sulfur-oxidizing symbionts. Comparison of the 'thiotrophic' mussel *Bathymodiolus thermophilus,* the 'methanotrophic' mussel Seep Mytilid Ia and the 'thio- and methanotrophic' mussel *Bathymodiolus puteoserpentis* (Pruski et al., 1997) shows that thiotaurine is a specific component of sulphide-based symbioses where it occurs only in the symbiont-containing tissues. At present, thiotaurine has been found in 30 sulphur-oxidizing symbiont containing species (Albéric, 1986; Albéric & Boulègue, 1990; Pranal et al., 1995; Pranal, unpublished results; Pruski et al., 1997; Pruski, unpublished results). We therefore propose thiotaurine as a bio-marker of symbioses between invertebrates and sulphur-oxidizing symbionts.

The occurrence of such high amounts of this compound in several groups of organisms raises the question of its origin. Of the different mechanisms proposed in the literature for the synthesis of thiotaurine, the most probable one is the combination of one molecule of $H_2S$ with one molecule of hypotaurine. Although this reaction was not demonstrated in vivo, hypotaurine and $H_2S$ are both present in deep-sea symbiotic species. Furthermore, this reaction can be easily reproduced in vitro by heating a mixture of hypotaurine and sulphide at 100°C for one hour (Albéric & Boulègue, 1990). We have checked the validity of this hypothesis by incubating tissues of hydrothermal organisms in a sulphide enriched medium.

## II. Sulphide stimulation of thiotaurine synthesis

The results obtained for *Calyptogena magnifica* are shown in Figure 2. Incubation of crushed gills in 0.5 mM sulphide sodium rapidly stimulated thiotaurine synthesis: up to 38 µmol of thiotaurine per gram dry weight were produced in 5 minutes. A decrease in the hypotaurine concentration also occurred during the incubation. This decrease was inversely proportional to the rate of synthesis of thiotaurine. After 5 minutes of exposure to sulphide, all the hypotaurine initially present had disappeared and there was no more synthesis of thiotaurine. This result suggests that hypotaurine is the unique precursor of thiotaurine in *Calyptogena magnifica.* Similar results were obtained for several vesicomyids, mytilids and vestimentiferans, although the rates of thiotaurine synthesis differ from species to species (up to 200 µmol g^{-1} D.W. in 5 minutes in *Riftia pachyptila*). These results tend to confirm the synthesis of thiotaurine from hypotaurine and sulphide.

## III. Possible function of thiotaurine in sulphide-based symbioses

In vivo, thiotaurine biosynthesis could be, in association with the other strategies used by these organisms, an efficient way to avoid sulphide toxicity as we saw that up to
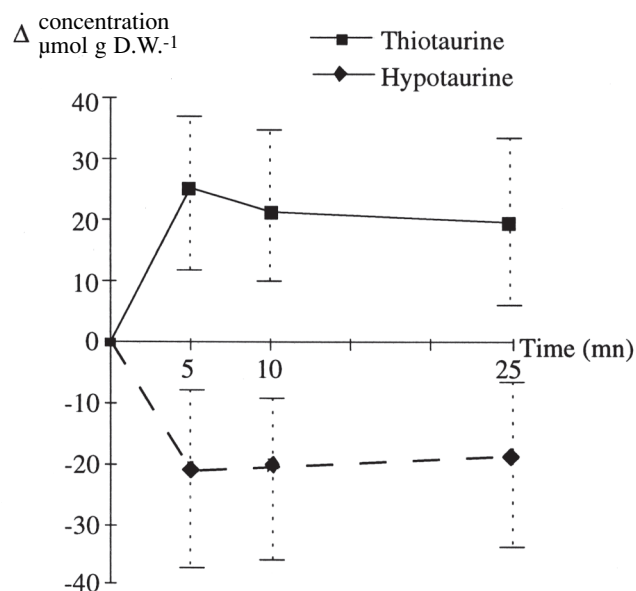


**Figure 2**. Variations of the thiotaurine (solid line) and hypotaurine (broken line) concentrations in crushed gills of *Calyptogena magnifica* exposed to sulphide (0.5 mM). Mean values are expressed in µmol/g D.W., with minimal and maximal values (number of samples = 4).

200 µmol of thiotaurine are synthesized per gram dry weight in 5 minutes by *Riftia*. This rate would correspond to a potential immobilization of 480 µmol of sulphide per gram fresh weight and per hour. In vivo, the hypotaurine level could be a limiting factor for thiotaurine synthesis. To be efficient this reaction would necessitate a fast turn-over to allow hypotaurine replacement, and therefore the consumption of the thiotaurine -SH group. We propose that thiotaurine synthesis inside the bacteriocytes could act as a trap for free sulphide protecting the cells against its toxicity, and serve as a non-toxic reserve of reduced sulphur for the symbionts. The metabolism of thiotaurine has not been studied at this time and further experiments are in progress to see whether or not thiotaurine could be used by the symbionts as a sulphur source.

# References

Albéric P. 1986. Présence de quantités importantes de thiotaurine et d'hypotaurine dans les tissus de *Riftia pachyptila* (Pogonophore, Vestimentifère). *Comptes Rendus de l'Académie des Sciences Paris,* **302**, S. III, **13**: 503-508.

Albéric P. & Boulègue J. 1990. Unusual amino compounds in the tissues of *Calyptogena phaseoliformis* (Japan Trench): Possible link to symbiosis. *Progress in Oceanography,* **24**: 89-101.

Johnson K. S., Childress J. J. & Beelher C. L. 1988. Short-term temperature variability in the Rose Garden hydrothermal vent field: an unstable deep-sea environment. *Deep-Sea Research,* **35**: 1711-1721.

Huxtable R. J. 1992. Physiological actions of taurine. *Physiological Review,* **72**: 101-163.

Martens C. S., Chanton J. P. & Paull C. K. 1991. Biogenic methane fron abyssal brine seeps at the base of Florida Escarpment. *Geology,* **19**: 851-854.

Mopper K. & Lindroth P. 1982. Diel and depth variations in dissolved free amino acids and ammonium in the Baltic Sea determined by shipboard HPLC analysis. *Limnology and Oceanography,* **27**: 336-347.

Nelson D. C. & Fisher C. R. 1995. Chemoautotrophic and methanotrophic endosymbiotic bacteria at deep-sea vents and seeps. In: *Microbiology of deep-sea hydrothermal vents* (D. M. Karl ed), pp. 125-166. CRC Press: Boca Raton.

Pranal V., Fiala-Médioni A. & Colomines J. C. 1995. Amino acid and related compound composition in two symbiotic mytilid species from hydrothermal vents. *Marine Ecology Progress Series,* **119**: 155-166.

Pruski A. M., Fiala-Médioni A. & Colomines J. C. 1997. High amounts of sulphur-amino acids in three symbiotic mytilid bivalves from deep benthic communities. *Comptes Rendus de l'Académie des Sciences Paris,* **320**: 791-796.