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# Enzyme distribution and metabolite exchange in the symbiosis between the deep-sea tube worm, *Riftia pachyptila*, and its bacterial endosymbiont

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**Abstract**

*Riftia pachyptila* is a tube worm living around the deep-sea vents along the East-Pacific Rise. Lacking a digestive track it is dependent on an obligate symbiosis with sulfide-oxidizing bacteria which are located in the bacteriocytes constituting the tissue of the worm called trophosome. The enzymological and biochemical aspects of this symbiosis were studied with respect to the anabolic and catabolic pathways involved in pyrimidine and arginine metabolism. The results obtained demonstrate the importance of the metabolic exchanges between the two partners and the complexity of their interactions.

**Keywords**: Symbiosis, *Riftia pachyptila*, deep-sea vents, endosymbiont, pyrimidine enzymes

## 1. Introduction

The biotopes around the deep-sea hydrothermal vents at the volcanic ridges found in the deep-sea were discovered at the end of the seventies (Lonsdale, 1977; Corliss et al., 1979). In complete absence of light, these very diverse populations of organisms rely on the ability of chemolithoautotrophic microorganisms to extract metabolic energy from simple chemical reactions such as the oxidation of sulfide (Felbeck et al., 1981; Gaill, 1993; Fisher, 1996; Minic et al., 2006). They include a large variety of microorganisms (Eubacteria, Archaea, mainly extremophiles) and more than five hundred animal species from twelve different phyla (Desbruyères et al., 2006).

*Riftia pachyptila* is one of the most impressive organisms living in these environments. It is a giant tube worm (up to 1.5 m long) and very common on the East-Pacific Rise where it lives in clusters in the immediate vicinity of the vents. A schematic anatomical organization of this worm is shown in Fig 1. This animal lacks a digestive track (no mouth, no guts) and lives in an obligate symbiosis with a bacterial endosymbiont that extracts metabolic energy from the oxidation of sulfide. This bacterium is strictly localized in so-called bacteriocytes that constitute the densely vascularized part of the worm called trophosome (Felbeck, 1981; Hand, 1987) (Fig. 1). The plume is the only part of the worm that emerges from the tube. It has a well perfused branchial system via which the worm absorbs, from the surrounding waters, the inorganic metabolites that are necessary for itself and for it's bacterial endosymbiont ($CO_2$, $NH_3$, $O_2$, $NO_3^-$, $SH_2$). The energy needed for the fixation of carbon from $CO_2$ by the bacteria originates from an electron transfer chain through which each molecule of sulfide oxidized provides one molecule of ATP. It can then be used to fix one molecule of $CO_2$ into 3-phosphoglycerate by a Calvin-Benson cycle identical to that involved in the photosynthetic process in plants (Felbeck et al., 1981; Robinson et al., 1998) (Fig. 2). This very interesting similarity confirms the theory that photo-synthesis, complex as it is, appeared very early during evolution in the ancestors of cyanobacteriae. The bacteria produce organic molecules (succinate, aspartate etc.) that they use for themselves as well as translocate to the host.

The preparation of the samples, and the various enzymatic tests performed, have been described previously (Minic et al., 2001, 2002, 2003). In this paper, we review the enzymological and biochemical aspects of this very

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intimate symbiosis particularly the pyrimidine and arginine metabolism.

## 2. The Enzymes of the Pyrimidine *de novo* Biosynthetic Pathway

The examination of the activities of the first three enzymes of the de *novo* pyrimidine biosynthetic pathway showed that there are no measurable activities of glutamine-dependent carbamylphosphate synthetase (CPSase-P), aspartate transcarbamylase (ATCase) and dihydroorotase (DHOase) in all the tissues or parts of the worm with the exception of the trophosome. The analysis of the catalytic and regulatory properties of these enzymes (especially ATCase) and of their molecular form (Minic et al., 2001) in both the trophosomal extracts and in the purified bacteria revealed that these enzymes are present only in the bacteria. Thus, the worm does not express the gene for CAD, the multifunctional protein, which, in eukaryotes, catalyses these three reactions. Although it appears not to be expressed, this gene should be present in the worm's DNA,

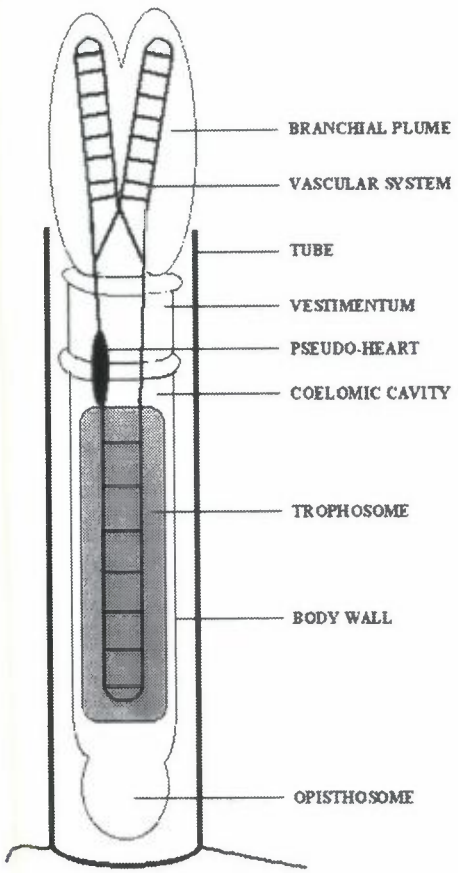


Figure 1. Anatomical organization of *Riftia pachyptila*. The legend identifies the different organs or tissues assayed for the different enzymatic activities. (Adapted from Minic et al., 2001. *J. Biol. Chem.* 276: 23777–23784, with permission.)

since the symbiosis between the worm and the bacteria is re-established at each worm generation (Jones and Gardiner, 1988; Nussbaumer et al., 2006) implying that the larvae must have these activities. Work is in progress to verify the presence of the CAD gene in the genome of the adult worm. Aspartate transcarbamylase is a marker of cells that are actively dividing. In resting cells and mature tissues this activity declines and disappears (Hervé and Xi, 1991). It was thus possible that the absence of these three enzymes of the *de novo* pathway was due to their determination in adult *Riftia* individuals. To test this possibility, a juvenile worm (10 cm long instead of 1.5 m) was studied. In spite of the fact that in such a young worm the symbiosis is already established, its growth could have still required the pyrimidine *de novo* pathway. The results were identical, showing that during its development, *Riftia pachyptila* becomes fully dependent, very early, on its bacterial endosymbiont for the *de novo* biosynthesis of its pyrimidine nucleotides.

The catalytic and regulatory properties of the partially purified bacterial ATCase were analyzed. These properties, in particular its allosteric regulation by the nucleotides CTP, UTP and ATP, are identical to the regulatory properties of the ATCase from *Pseudomonas aeruginosa* (Simon et al., 2000; Vickrey et al., 2002), suggesting that this bacterial endosymbiont could be closely related to the Pseudomonidae. This hypothesis was consistent with the sequence of its 16s rRNA gene (Naganuma et al., 1997). However, this conclusion must now be revised on the basis of the sequence of the entire genome of the bacterium, which, unexpectedly, shows an important heterogeneity in comparison with other genomes and a high degree of polymorphism (Robidart et al., 2006).

The presence of some of the following enzymes of the *de novo* pathway was also investigated in both the bacteria and the different tissues of the worm (Table 1). Dihydroorotate dehydrogenase (DHODase), orotate phosphoribosyltransferase, (OPRTase) and CTP synthetase (CTPSase) are present in both the bacteria and the different tissues of the worm. Apparently, the worm can use the dihydroorotate produced by the bacterial dihydroorotase. For each of these enzymes the specific activities measured in the different parts of the worm and in the bacteria are of the same order of magnitude, with the exception of the CTPSase activity, which is much lower in the trophosome than in the other compartments. However, the most interesting feature is that the DHODase activity is much higher in the bacteria than in the worm. This observation is consistent with our unexpected result that the blood of the worm contains at least three times more orotate than dihydroorotate. Possibly, the bacterial DHODase provides much more orotate to the worm than the host's own enzyme. This unexpected observation is probably linked to the very unfavorable equilibrium constant of the reaction catalyzed by dihydroorotase that is much in favour of the

formation of carbamylaspartate. Under physiological conditions dihydroorotate can be produced efficiently only because dehydroorotate dehydrogenase pulls the equilibrium of the dihydroorotase reaction by using dehydroorotate as soon as it is produced. This process is facilitated if the two enzymes are located in the same compartment, as here in the bacteria.

### 3. The Pyrimidine Salvage Pathway

Since the worm does not posses the entire *de novo* pathway, it is dependent on the bacteria for the complete synthesis of its pyrimidine nucleotides It should also be able to use the salvage pathway that allows it to reconstruct these nucleotides from the products of nucleic acids degradation. Indeed, relatively high levels of cytidine deaminase (CDase), uridine kinase (Ukase) and uracil phosphoribosyltransferase (Uracil-PRTase) were found in all the tissues of the worm (Minic et al., 2001). Surprisingly, the activities of CDase and Ukase were about ten times higher in the body wall than in the other tissues. In contrast, none of these activities can be demonstrated in the bacteria, showing that this microorganism relies exclusively on the *de novo* biosynthetic pathway. This observation indicates that the bacteria is actively growing in the bacteriocytes, a conclusion that is consistent with the significant lysis of bacteria in the peripheral part of the trophosome (see section 5).

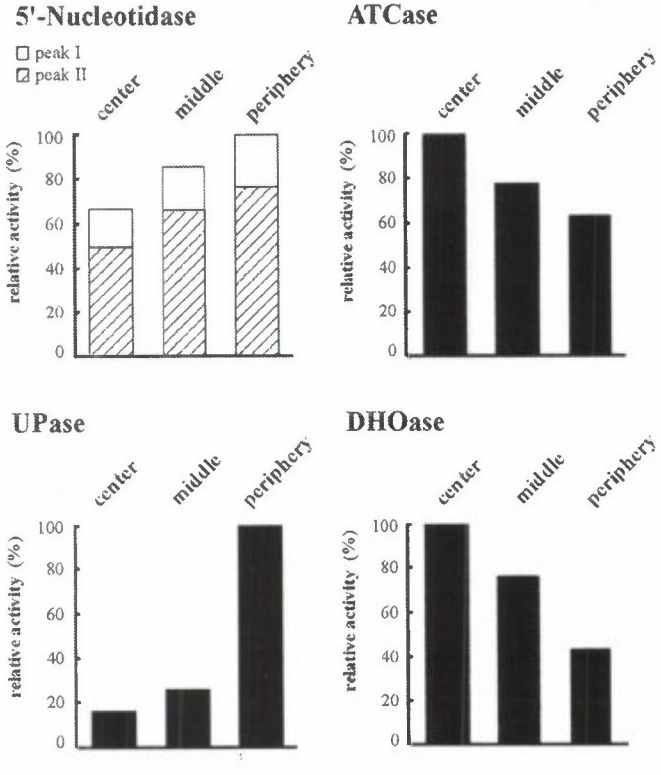


Figure 2. Variations of anabolic and catabolic activities across the trophosome. ATCase: aspartate transcarbamylase; Upase: uridine phosphorylase; DHOase: dihydroorotase. (Adapted from Minic et al., 2002. *J. Biol. Chem.* 277: 127–134, with permission.)

### 4. Enzymes Involved in Nitrogen Assimilation

Nitrate and ammonia are present at relatively high concentrations in the waters around most deep-sea vents (Johnson et al., 1988; Lee and Childress, 1996) and must represent an important source of nitrogen for these organisms (Johnson et al., 1988; Lilley et al., 1993). On the contrary, inorganic nitrogen must contribute poorly to their nitrogen metabolism since its availability appears to be very low in these environments (Johnson et al., 1988; Karl, 1995). Thus, $NH_3$ is provided to the worm either directly from the environment or after reduction of nitrate by nitrate reductase and nitrite reductase (Hentschel and Felbeck, 1993; Lee et al., 1999; Girguis et al., 2000; Minic et al., 2001). $NH_3$ is then incorporated in organic molecules by a series of assimilating enzymes such as glutamine synthetase, glutamate dehydrogenase and carbamyl-phosphate synthetase to produce metabolites such as aminoacids and nucleotides (Minic et al., 2004). The different tissues of the worm and the bacteria were tested for nitrate reductase and glutamine synthetase activities to verify the presence of these pathways in *Riftia*. From our results and others (Hentschel and Felbeck, 1993; Lee et al., 1999; Girguis et al., 2000; De Cian et al., 2000; Minic et al., 2001) it appears that nitrate reductase is present only in the bacteria and that glutamine synthetase is present in both the worm and the bacteria. The activity of the latter enzyme is especially high in the branchial plume (Minic et al., 2001) suggesting that ammonia is trapped in this tissue as soon as it is taken up from the sea water.

### 5. The Catabolic Pyrimidine Pathways

Several published reports indicate that, in spite of their relatively high concentrations, the inorganic nitrogen and carbon sources present in the environment limit the growth of *Riftia* (Childress et al., 1993; Scott et al., 1994; Minic and Hervé, 2003). Internal recycling of N and C containing metabolites must therefore be important for the development of the worm. In view of this prediction, enzymes of pyrimidine catabolism were tested from different parts of the worm and in the bacteria (Table 2). It appears that UMP nucleotidase, CMP nucleotidase, uridine phosphorylase and uracil reductase activities were found in all of the tissues of the worm. These activities were especially high in the trophosome, the bacteria-harboring tissue. Activities of these enzymes were absent in the bacteria except for a small UMP/CMP nucleotidase activity.

Table 1. Activity of the enzymes of the pathway of *de novo* pyrimidine synthesis (after CAD) in the tissues of *Riftia pachyptila* and its bacterial symbiont.

| Tissue | DHODase | OPRTase | CTPSase |
|---|---|---|---|
| Branchial plume | 32 ± 5 | 0.072 ± 0.004 | 0.225 ± 0.026 |
| Vestimentum | 26 ± 6 | 0.037 ± 0.003 | 0.204 ± 0.012 |
| Trophosome | 24 ± 4 | 0.147 ± 0.013 | 0.016 ± 0.010 |
| Body wall | 53 ± 7 | 0.112 ± 0.009 | 0.124 ± 0.048 |
| Opisthosome | 20 ± 6 | 0.180 ± 0.015 | 0.196 ± 0.090 |
| Isolated bacteria | 164 ± 40 | 0.072 ± 0.007 | 0.142 ± 0.017 |

The enzymatic activities are expressed in nmole of product formed per minute per mg of protein. The averages and standard deviations were calculated from three different determinations made on three different worm specimens. DHODase: dihydroorotate dehydrogenase; OPRTase: orotate phosphoribosyltransferase; CTPSase: Cytidine triphosphate synthetase.

Table 2. Activity of the enzymes involved in the catabolism of pyrimidine nucleotides in the tissues of *Riftia pachyptila* and its bacterial symbiont.

| Tissue or body part | UMP nucleotidase | CMP nucleotidase | UPase | UracilRase |
|---|---|---|---|---|
| Branchial plume | 0.14 ± 0.13 | 0.26 ± 0.23 | 0.78 ± 0.15 | 1.63 ± 0.57 |
| Vestimentum | 0.25 ± 0.11 | 0.32 ± 0.05 | 1.58 ± 0.16 | 1.76 ± 0.73 |
| Trophosome | 0.50 ± 0.09 | 0.53 ± 0.10 | 0.34 ± 0.12 | 0.78 ± 0.38 |
| Body wall | 0.05 ± 0.02 | 0.04 ± 0.02 | 7.33 ± 1.73 | 1.38 ± 0.39 |
| Opisthosome | 0.21 ± 0.19 | 0.36 ± 0.24 | 0.20 ± 0.09 | 1.64 ± 0.59 |
| Isolated bacteria | ND | ND | ND | ND |

The enzymatic activities are expressed as nmole of product formed per minute per mg of protein. The averages and standard deviations were calculated from three different determinations made on three different worm individuals. Upase: uridine phosphorylase; UracilRase: uracil reductase. ND: not detectable.

Table 3. Activity of the enzymes of the arginine biosynthetic pathway in the tissues of *Riftia pachyptila* and its bacterial symbiont.

| Tissue or body part | CPSase A | OTCase | ASSase |
|---|---|---|---|
| Branchial plume | 0.230 ± 0.050 (6) | 128 ± 73 (5) | 147 ± 20 (3) |
| Vestimentum | 0.367 ± 0.150 (6) | 251 ± 62 (5) | 315 ± 93 (3) |
| Trophosome | 0.667 ± 0.183 (12) | 68 ± 8 (6) | 320 ± 71 (3) |
| Body wall | 0.050 ± 0.017 (3) | 306 ± 83 (5) | 12 ± 3 |
| Opistosome | 0.150 ± 0.117 (4) | 176 ± 13 (6) | 108 ± 34 (3) |
| Isolated bacteria | 0.190 ± 0.065 (3) | 30 ± 9 (3) | 64 ± 16 (3) |

The enzymatic activities are expressed as nmole of product formed per minute per mg of protein. The averages and standard deviations were calculated from the numbers of determinations indicated in parentheses, made on different worm individuals. CPSase A: carbamylphosphate synthetase specific of the arginine pathway; OTCase: anabolic ornithine transcarbamylase; ASSase: arginosuccinate synthetase.

Thus, the worm, has the ability to use the degradation of nucleic acids as a source of carbon and nitrogen especially in the trophosome. The catabolism of pyrimidine nucleotides can also lead to the production of ribulose-phosphate. This could be transferred, from the worm to the bacteria, to be used in the Calvin-Benson cycle, suggesting that metabolic exchanges between the two organisms are operating in both directions.

## 6. Heterogeneity of the Trophosome

The trophosome represents about 15% of the body weight of the worm (Childress et al., 1984) and the bacterial volume represents between 15 and 35% of the total volume of the trophosome (Powell and Somero, 1986). This simple fact supports the essential role played by the trophosomal tissue in the metabolism of the two symbiotic partners. This

organ is not simply a blood-irrigated bag of bacteriocytes. There is a complex physiology going on in this tissue. The bacteriocytes are organized into lobules surrounding the blood capillaries, and some heterogeneity of the bacteriocytes was observed within these lobules. Some enzymatic activities tested in the present work were also measured in the center, the periphery and the intermediary region of the trophosome. The results obtained are shown in Fig. 2. The activities of the anabolic enzymes (ATCase, DHOase) decrease from the center of the trophosome to the periphery. In contrast, the activities of the catabolic enzymes (5'-nucleotidase, uridine phosphorylase) increase from the center to the periphery. Electron microscopy also shows a regular increase of the number of lysosomes and partially degraded bacteria from the center of the trophosome towards its periphery (F. Gaill, personal communication). Thus, some heterogeneity is also observed in the entire trophosome. Therefore, not only the worm takes advantage of the ability of the bacteria to extract metabolic energy from the oxidation of sulfide, but it also use them directly as a source of nutrition.

## 7. Arginine Metabolism

Because they have a common metabolite, carbamyl-phosphate, the pyrimidine and arginine pathways are related. A single carbamylphosphate synthetase provides this metabolite to the two pathways in prokaryotes while in eukaryotes two specific enzymes have this role. Exchanges between the two pathways, for this metabolite, are possible in some organisms; in others, a strict compartmentalization, prevents such an exchange. The second reaction of the arginine biosynthetic pathway involves ornithine transcarbamylase which catalyses a reaction very similar to that catalyzed by aspartate transcarbamylase in the pyrimidine biosynthetic pathway. For this reason, our investigations were extended to the enzymes of arginine metabolism. Both organisms, in the symbiosis, posses the enzymes for the biosynthesis of this amino acid. Activities of the ammonium-dependent carbamylphosphate synthetase (specific to the arginine pathway), the anabolic ornithine transcarbamylase and arginosuccinate synthetase were found in all the tissues of the worm and in the bacteria (Table 3). However, due to its instability the bacterial carbamylphosphate synthetase can be detected only in fresh preparations of this microorganism (Minic and Hervé, 2003).

The catabolism of arginine, through the arginine deiminase pathway, involves in sequence arginine deiminase, catabolic ornithine transcarbamylase, and carbamate kinase (Cunin et al., 1986). This pathway could therefore be beneficial to the worm since it produces ATP and provides carbon dioxide and ammonia as carbon and nitrogen sources for the worm and its symbiont. However, none of these enzyme activities could be detected in any part of the worm. Arginine can also be catabolized though the action of arginine decarboxylase (ADase) or ornithine decarboxylase (ODase), after its transformation into ornithine by arginase. These decarboxylase reactions produce agmatine and putrescine, which are precursors of polyamines. Unexpectedly, a high activity of these two enzymes (0.3 nmole/min/mg of protein) was found in the isolated bacteria, but none in the tissues of the worm. This result strongly suggests a dependence of the worm on the bacterial symbiont for the production of the polyamines that are necessary for the metabolism of nucleic acids.

## 8. Conclusions

The results, reported above, exemplify the degree of integration in the physiology of *Riftia pachyptila* and its bacterial endosymbiont and the complexity of their metabolic exchanges. One of the surprising observations is the presence only in the bacteria, of the enzymes allowing the synthesis of the precursors of polyamines. The worm therefore appears to be dependent on its symbiont for the synthesis of these molecules for the metabolism of its nucleic acids. A similar observation was made in the case of the human and animal filarial worm parasites *Dirofilaria immitis, Brugia patei* and *Litomosoides* (Wittich et al., 1987). As in *Riftia pachyptila*, a lack of the three activities of the CAD protein has been discovered in the aerobic protozoan parasites *Giardia lamblia, Trichomonas vaginalis* and *Tritrichomonas foetus* (Linmark and Jarroll, 1982; Jarroll et al., 1983; Wang and Cheng, 1984). These observations suggest similarities in the biochemical and metabolic adaptation involved in symbiosis and parasitism. Polyamine transport systems have been described in other organism (Kandpal and Tekwani, 1997; Cabella et al., 2001; Satriano et al., 2001). Because of the possible involvement of the *Riftia* symbionts in the synthesis of host polyamines, the existence of these systems in *Riftia pachyptila* should be further investigated.

The absence of a particular enzymatic activity does not strictly indicate that the corresponding enzyme is absent. However, in the present case, there is absolutely no reason to hypothesize that all the enzymes whose activity is absent in the worm or in the bacteria would be all completely inhibited.

There is no doubt that the very intimate symbiosis between *Riftia pachyptila* and the bacteria must be regarded as a mechanism of adaptation to the very unusual and extreme conditions in which these organisms are living. This system deserves detailed study, particularly in view of the probability that life emerged on earth in these very special environments (Di Giulio, 2005).

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