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The burden of independence: Inorganic carbon utilization strategies of the sulphur chemoautotrophic hydrothermal vent isolate *Thiomicrospira crunogena* and the symbionts of hydrothermal vent and cold seep vestimentiferans

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Introduction

Symbiotic and free-living aerobic sulphide oxidizing chemoautotrophs are abundant at sites of diffuse hydrothermal vent flow and at hydrocarbon seeps, and are responsible for a large portion of primary productivity for these communities. As evidenced by the thick white bacterial mats often found encrusting vestimentiferan tubes, free-living and symbiotic sulphide oxidizing chemoautotrophs live within millimetres of one another. However, their chemical microhabitats can be quite different.

The microhabitat of vestimentiferan symbionts is created by the host. Vestimentiferan symbionts are packed into bacteriocyte vacuoles in the trophosome organ, which fills the host body cavity. Inorganic carbon is delivered to the bacteriocytes by blood vessels that extensively perfuse the trophosome, and an abundant coelomic fluid that surrounds this organ (for review, see Childress & Fisher, 1992). Both blood and coelomic fluid are moderately to extremely enriched in inorganic carbon (Table 1). The cytosol of the bacteriocytes has a high activity of carbonic anhydrase, which is believed to facilitate the diffusion of this pool of inorganic carbon to the symbiont-containing vacuoles (Kochevar & Childress, 1996).

In contrast, free-living autotrophs such as *Thiomicrospira crunogena* Jannach et al., 1985 acquire their inorganic carbon directly from the diluted hydrothermal fluid flowing past them. *T. crunogena* is abundant and cosmopolitan; it has been found to be a major component of bacterial mats from the Mid-Atlantic Ridge (Muyzer et al., 1995) and was originally isolated from *Riftia pachyptila* Jones, 1981 tubes at the East Pacific Rise. *T. crunogena* encrusting *R. pachyptila* tubes experience fluctuations in hydrothermal fluid flow over time scales from seconds to days (see references in Childress & Fisher, 1992) which create

Table 1. Kinetic parameters of carbon fixation

Organism	Substrate	Avg. $K_{1/2}$[A] (mM)	Maximum In situ concentrations (mM)[B]
Vestimentiferan symbionts:			
R. pachyptila symbionts	inorganic carbon	2.118	>30[C]
R. piscesae symbionts	inorganic carbon	0.327	3.5 - 11.5
Undescribed seep escarpid	inorganic carbon	0.130	3.7 - 5.8
T. crunogena:	inorganic carbon		>7 (vents)[C]
"low" inorganic carbon cells	inorganic carbon	0.148	0.014 (batch culture)
"high" inorganic carbon cells	inorganic carbon	2.42	46 (batch culture)
Vestimentiferan symbionts:			
R. pachyptila symbionts	carbon dioxide	0.049	>0.35[C]
R. piscesae symbionts	carbon dioxide	0.008	
T. crunogena: "low" inorganic carbon cells	carbon dioxide	0.0015	>1 (vents)[C] 0.00028 (batch culture)

[A]$K_{1/2}$ is the half-saturation constant; (K. Scott, in prep).
[B]Maximum in situ concentrations of inorganic carbon and carbon dioxide for vestimentiferan symbionts are those measured in vestimentiferan blood and coelomic fluid.
[C]pH and inorganic carbon concentrations are from Goffredi et al., 1997.

changes in both inorganic carbon abundance, from 2 to 7 mM, and pH, from 6.2 to 7.8 (Goffredi et al., 1997).

The actual availability in situ of inorganic carbon to vestimentiferan symbionts, as well as to free-living *T. crunogena,* is difficult to assess. Macroscopically, inorganic carbon is abundant. Diffuse flow hydrothermal fluid and vestimentiferan blood are enriched with inorganic carbon compared to the 2 mM found in seawater. Microscopically, however, the concentration of inorganic carbon may be much lower. The pool of inorganic carbon available to both *T. crunogena* and vestimentiferan symbionts may be diffusion limited. Vestimentiferan symbionts are packed in the trophosome. Given their high rates of carbon fixation, it is conceivable that inorganic carbon availability at the symbiont cell surface may be depleted relative to the blood and coelomic fluid. Free-living chemoautotrophs may also face localized depletions in the pool of inorganic carbon at the cell surface, especially when growing in thick mats, which may constrain diffusion of inorganic carbon to the cells in the interior of the mat. *T. crunogena* is capable of growing in the presence of extremely low concentrations of inorganic carbon (<20 µM). Such an ability would allow these cells to survive in low inorganic carbon microhabitats.

Half-saturation constants, as well as the form(s) of extracellular inorganic carbon used by vestimentiferan symbionts and *T. crunogena,* were determined to compare inorganic carbon use by a free-living sulphur chemoautotroph to symbionts inhabiting hosts with extensive morphological and enzymatic adaptations for efficient delivery of inorganic carbon to them.

Methods

Vestimentiferan symbionts have yet proven to be unculturable. As a result, experiments with these microorganisms were conducted at sea. Symbionts were purified from the trophosome by gentle dispersion of the organ and centrifugation through percoll. *R. pachyptila* symbionts prepared in this manner have carbon fixation rates comparable to those reported for the intact symbiosis. For symbionts from all three vestimentiferans, only those experiments with a maximum carbon fixation rate exceeding 6 µmol per gram symbiont protein per hour were considered viable. Symbiont suspensions were incubated at in situ pressures in a vessel plumbed to allow subsampling of these suspensions over the course of the incubation.

As *T. crunogena* is readily culturable, experiments with this microorganism were conducted with cells grown in batch culture at 1 atm. “Low” inorganic carbon cells were grown on a gyrotary shaker. Air was the sole source of inorganic carbon. “High” inorganic carbon cells were aerated with 5% CO_2, 7% O_2 in N_2.

Carbon fixation by symbionts and *T. crunogena* was measured by adding ^{14}C-inorganic carbon to cell suspensions brought to the appropriate pH and inorganic carbon concentration in artificial sea water, buffered with 100 mM HEPES and supplemented with either sulphide (for the vestimentiferan symbionts) or thiosulphate (for *T. crunogena).* Whole cell affinities for extracellular inorganic carbon were estimated using nonlinear regressions modelled after rectangular hyperbolae. Whole cell affinities are expressed as half-saturation constants ($K_{1/2}$), which are the concentrations of extracellular inorganic carbon that result in half-maximal rates of carbon fixation.

Within the pH range at which these cells are found in situ, two forms of inorganic carbon predominate: carbon dioxide and bicarbonate. The form of inorganic carbon used by these cells was determined by varying the concentration of one potential substrate while holding the other constant, and measuring the carbon fixation rate. Cell use of one form or the other may reflect relative abundance at the cell surface in situ. Reliance on carbon dioxide and relatively large half-saturation constants are typically observed in cells adapted to growth with an abundance of available carbon dioxide. Use of both extracellular bicarbonate and carbon dioxide, and small half-saturation constants for both substrates are typical of autotrophic microbes adapted to growth at low inorganic carbon concentrations.

Transmission electron micrographs of these microorganisms were prepared by standard methods and examined for the presence of carboxysomes. Carboxysomes are electron-dark polyhedral organelles packed with Rubisco and a low carbonic anhydrase activity. They are found in many autotrophic bacteria capable of growth at low concentrations of inorganic carbon, and are often absent in autotrophs that require high concentrations of extracellular inorganic carbon to grow. Carboxysomes are believed to play a role in the efficient utilisation of the intracellular bicarbonate that these cells acquire by active transport under low environmental concentrations of inorganic carbon (Kaplan et al., 1994).

Results

Half-saturation constants for vestimentiferan symbiont carbon fixation ranged from 0.130 to 2.118 mM inorganic carbon (Table 1). As these data were heteroscedastic, they were log transformed before analysis of variance. *R. pachyptila* symbiont half-saturation constants for inorganic carbon are significantly larger than those measured for both *Ridgeia piscesae* Jones, 1985 and seep escarpid symbionts, which are not significantly different from each other (Tukey method of multiple comparisons;

family error rate of 0.05). Symbionts purified from both *Riftia pachyptila* and *Ridgeia piscesae* did not use extracellular bicarbonate (K. Scott, in prep.). However, symbionts from both species did use extracellular carbon dioxide, with half-saturation constants ranging from 8 to 49 µM (Table 1).

Thiomicrospira crunogena grown at high concentrations of inorganic carbon fixed carbon with half-saturation constants that were more than an order of magnitude larger than those observed for cells grown with low concentrations of inorganic carbon (Table 1). Half-saturation constants for carbon dioxide were significantly different between low inorganic carbon-grown *T. crunogena* and symbionts from both *Ridgeia piscesae* and *Riftia pachyptila* (Tukey method of multiple comparisons; family error rate of 0.05).

No carboxysomes were observed in symbionts from *R. piscesae* with high or low coelomic fluid inorganic carbon concentrations. Carboxysomes were also not apparent in any electron micrographs examined of *R. pachyptila* or seep escarpid symbionts. However, carboxysome-like inclusions are clearly visible in transmission electron micrographs of *T. crunogena*.

Conclusions

Riftia pachyptila symbionts, which have the lowest affinities for carbon dioxide, have half-saturation constants an order of magnitude smaller than the concentrations in the coelomic fluid and blood of the host (Table 1). Therefore, symbiont carbon fixation rates will be high even if the pool of carbon dioxide at the symbiont cell surface is substantially depleted with respect to the pool measured in the blood. The smaller values of $K_{1/2}$ for carbon dioxide measured in *R. piscesae* symbionts, compared to *R. pachyptila* symbionts, mirror the generally lower internal inorganic carbon concentrations in *R. piscesae* (Table 1). As symbionts from these two hosts are strains of the same species (K. Nelson, pers. com.), this change in $K_{1/2}$ may be either a response to host species, genetic differences between the two strains, or carbon dioxide availability.

Thiomicrospira crunogena grown with low concentrations of inorganic carbon have the smallest half-saturation constants for carbon dioxide measured here. Given the large changes of affinity for inorganic carbon in response to inorganic carbon concentration during growth (Table 1), as well as the presence of carboxysomes in these cells, this high affinity may facilitate the growth of *T. crunogena* in an environment with greater variability in inorganic carbon abundance than that experienced by vestimentiferan symbionts. Although carbon dioxide concentrations can get quite high in the hydrothermal vent fluid bathing the *R. pachyptila* tubes from which *T. crunogena* was originally isolated (Table 1), this pool falls substantially during fluctuations in fluid flow that result in this microhabitat being dominated with alkaline, relatively carbon dioxide-poor bottom water. Under these circumstances, *T. crunogena* would require a versatility of inorganic carbon use that vestimentiferan symbionts, buffered from high-frequency environmental variability by their host, may not.

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