



H⁺ equivalent elimination by the tube-worm *Riftia pachyptila*

Peter R. GIRGUIS and James J. CHILDRRESS

University of California Santa Barbara

Marine Science Institute

Santa Barbara, CA 93106 USA

Fax: (1) 805 893 4724

e-mail: girguis@lifesci.ucsb.edu

The tube-worm *Riftia pachyptila* Jones, 1981 is one of the most prominent members of the hydrothermal vent community. This organism harbours chemolithoautotrophic bacteria deep within its body in a specialized organ known as the trophosome. The bacteria are capable of sulphide oxidation for energy production and inorganic carbon fixation. As a consequence of its morphology, the tube-worm must provide its symbionts with the compounds required for autotrophy (e.g. inorganic carbon and sulphide). Recent studies have demonstrated that CO₂ is the primary species of inorganic carbon (Ci) which is assimilated by the tube-worm (Goffredi et al., 1997). It is posited that the uptake of CO₂ takes place across the respiratory surfaces via passive diffusion. The influx of CO₂ is expected to shift the Ci equilibrium, resulting in the production of bicarbonate and proton equivalents. Acidification of the tube-worm's body fluids would diminish the gradient favouring the passive influx of CO₂. Thus, we believe that the maintenance of an alkaline internal pH (relative to the environment) is fundamental to Ci uptake.

In addition, sulphide metabolism would also result in a production of proton equivalents (Girguis & Childress, in prep). Depending upon which species is acquired, the uptake of sulphide could produce one proton per sulphide. Furthermore, the oxidation of H₂S or HS⁻ to sulphate (SO₄) will result in the production of one or two protons per sulphide. Due to the substantial rates of carbon fixation and sulphide oxidation, the production of proton equivalents should be considerable. It was our intention to determine the rates of proton elimination as a function of inorganic carbon acquisition and sulphide oxidation, and to assess the dependency of these uptake rates on the maintenance of proton elimination.

Riftia pachyptila were collected during the HOT 96 expedition using the DSV *Nautile* from various sites at 9°N

and 13°N along the East Pacific Rise. Tube-worms ranging from 10 to 20 grams were maintained in pressurized flow through aquaria for no more than 2 days prior to experimentation. Experiments were conducted on board ship using a high pressure, flow-through respirometer system. Three flow-through pressure aquaria, two experimental vessels and one control, were subject to identical conditions. Effluent pH was measured using a double junction pH electrode, and data were plotted on a chart recorder. Seawater Ci, sulphide, and oxygen concentrations were varied singularly. Upon conclusion of the experiment N-ethylmaleimide (N.E.M.), a non-specific ATPase inhibitor, was added to the pressure aquaria at a concentration of 2 mM. The rate of proton elimination was calculated using an algorithm which accounted for the buffering capacity of sea water (Millero, 1979; 1995). Simple regressions were plotted using STATVIEW 4.5 software.

Effluent pH from vessels containing *R. pachyptila* were typically 0.06 to 0.24 pH units lower than control pH (Fig. 1). These ΔpH values corresponded to proton elimination rates from 24 to 121 μeq g⁻¹ hr⁻¹ respectively. Rates of proton elimination significantly correlated with Ci and sulphide (p = .0219 and .0347 respectively). Under hypoxic conditions (e.g. less than 10 μM O₂), proton elimination was severely reduced. The use of N.E.M. resulted in the cessation of proton elimination and a significant decrease in Ci uptake (Fig. 2).

Riftia pachyptila is a prime example of the tight physiological coupling which can occur between host and symbiont. Proton equivalent elimination by the tube-worm can clearly compensate for the production of protons which results from Ci acquisition and symbiont sulphide oxidation. Indeed, an increase in the uptake rates of either Ci or sulphide results in a concomitant increase in proton

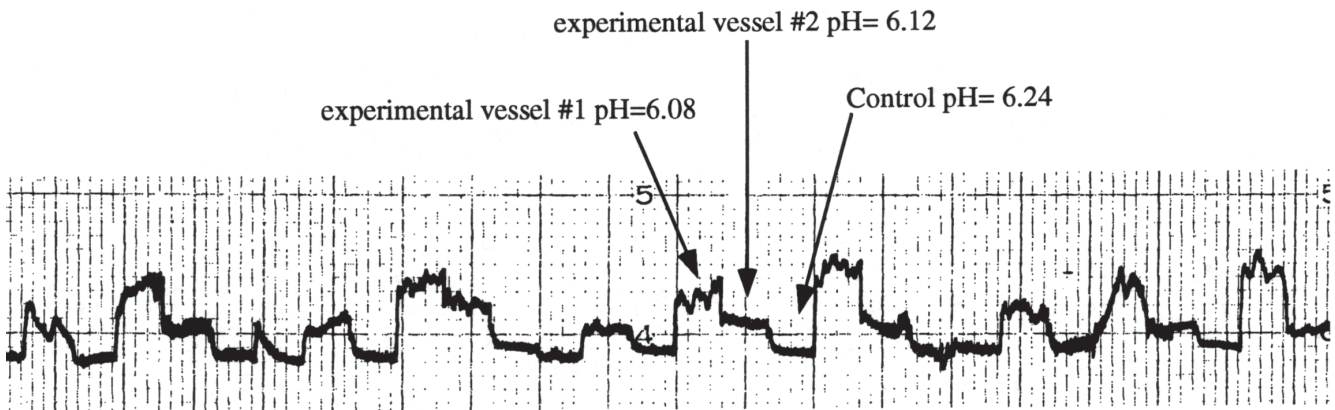


Figure 1. Plot of effluent pH at nominal vent conditions ($\text{CO}_2 = 3500 \mu\text{M}$, $\text{H}_2\text{S} = 200 \mu\text{M}$, $\text{O}_2 = 150 \mu\text{M}$). A stream selection valve was used to divert flow from the three vessels (two experimental and one control) to a pH meter. Effluent pH from the experimental vessels was typically 0.08 to 0.24 pH units lower than the control (as indicated by the arrows).

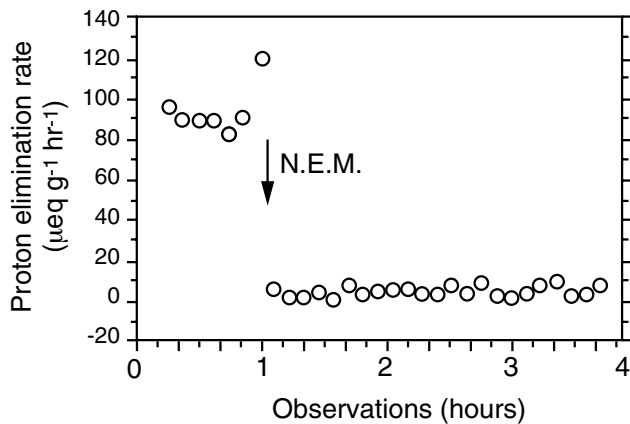


Figure 2. Observational plot of proton equivalent elimination rate vs. time. Arrow indicates the addition of N-Ethylmaleimide (N.E.M), a non-specific ATPase inhibitor, to the vessel.

elimination. The relationship between proton elimination and hypoxia, however, remains unclear. It is possible that hypoxia results in the cessation of host and/or symbiont metabolism, or that the transport of protons is reliant upon the presence of oxygen, e.g. a RedOx proton pump. The

dramatic effect of N.E.M. upon proton elimination and Ci uptake suggests an active mode of proton elimination. It also demonstrates, however, the Ci acquisition continues only when the internal pH is kept alkaline relative to the environment. Under nominal vent conditions, the rates of proton elimination may be the highest ever demonstrated in the animal kingdom, further testimony to the remarkable physiological adaptations of *Riftia pachyptila* to both its environment and symbionts.

References

- Goffredi S.K., Childress J.J., Desaulniers N.T., Lee R.W., Lallier F.H. & Hammond D. 1997. Inorganic carbon acquisition by the hydrothermal vent tube-worm *Riftia pachyptila* depends upon high external CO_2 and on proton equivalent ion transport by the worm. *Journal of Experimental Biology*, **200**: 883-896.
- Millero F.J. 1979. The thermodynamics of the carbonate system in seawater. *Geochimica et Cosmochimica Acta*, **36**: 92-98.
- Millero F.J. 1995. The thermodynamics of the carbon dioxide system in the oceans. *Geochimica et Cosmochimica Acta*, **59**: 661-677.