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Anaerobiosis in the hydrothermal vent tube-worm *Riftia pachyptila*

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Introduction

The giant tube-worm *Riftia pachyptila* Jones, 1981 grows at hydrothermal vents in the mixing zone of the sulphide-laden anoxic vent effluent and oxygenated ambient seawater. Depending on the vent water flow and the degree of mixing, this environment is extremely unstable and can change quickly. Johnson et al. (1988) showed that *R. pachyptila* and other vent species may experience high sulphide concentrations as well as extreme hypoxia over both short and long periods of time. The sessile tube-worm cannot escape from the frequently hypoxic conditions since it harbours chemoautotrophic, sulphide-oxidizing bacteria in its trophosome cells that require access to the sulphidic vent source as well as to an oxidant. While the symbiotic bacteria could, alternatively, use nitrate or oxygen to oxidize sulphide (Hentschel & Felbeck, 1993), the heterotrophic host *R. pachyptila* has no nitrate reductase and requires oxygen to maintain its normal aerobic metabolism.

We investigated in our study the metabolic capabilities of *R. pachyptila* to tolerate periods of extreme hypoxia as they frequently occur in its habitat. To characterize the anaerobic metabolic changes of the tube-worm, specimens of *R. pachyptila* were exposed to experimental hypoxia for different periods of time.

Material and methods

Experiments were performed on board ship in high-pressure aquaria with variable environmental conditions. At the end of an anaerobic experiment, *R. pachyptila* was sacrificed and samples were taken from the body fluids and the tissues. Neutralized perchloric acid extracts were made to analyse the samples using spectrophotometric assays enzyme activities, glycogen, succinate, malate, L- and D-lactate, HPLC (amino acids) and gas chromatography (short-chain fatty acids).

Results

Our experiments showed clearly a substantial tolerance of *R. pachyptila* to oxygen fluctuations. Worms were shown to survive without oxygen up to 60 hr. However, around this time, the health of *R. pachyptila* appears to deteriorate, even though the tube-worms were still responding to touch stimuli. All specimens experiencing 60 hr of hypoxia were found with retracted plumes, a behaviour which is often observed with dying animals.

When the oxygen availability decreased in the incubation water of *R. pachyptila,* an immediate utilization of the high malate stores in the body fluids (up to 26 mM) and in the tissues (up to 5 µmol g^{-1} f wt) was observed (Fig. 1). The anaerobic degradation of malate appears to occur within minutes, particularly in the body fluids. Animals taken out of their aerobic incubation vessels and stored on ice for about 5 minutes before being sacrificed, showed far lower malate concentration in their body fluids than specimens dissected immediately after incubation. Malate was degraded almost completely after 6 hr of anaerobic exposure. A strong accumulation of succinate was detected in the body fluids (up to 46 mM) as well as in the tissues (up to 23 µmol g^{-1} f wt) of *R. pachyptila*. This accumulation started within the first 6 hr of anaerobic exposure (Fig. 1). Glycogen was found to be the main anaerobic substrate during extended anaerobiosis and was detected mainly in the trophosome tissue (about 100 µmol glycosyl unit g^{-1} f wt, Fig. 2). While no utilisation of glycogen could be found within the first 17 hr of anaerobic exposure, its content decreased significantly by about 60% after 48 hr.

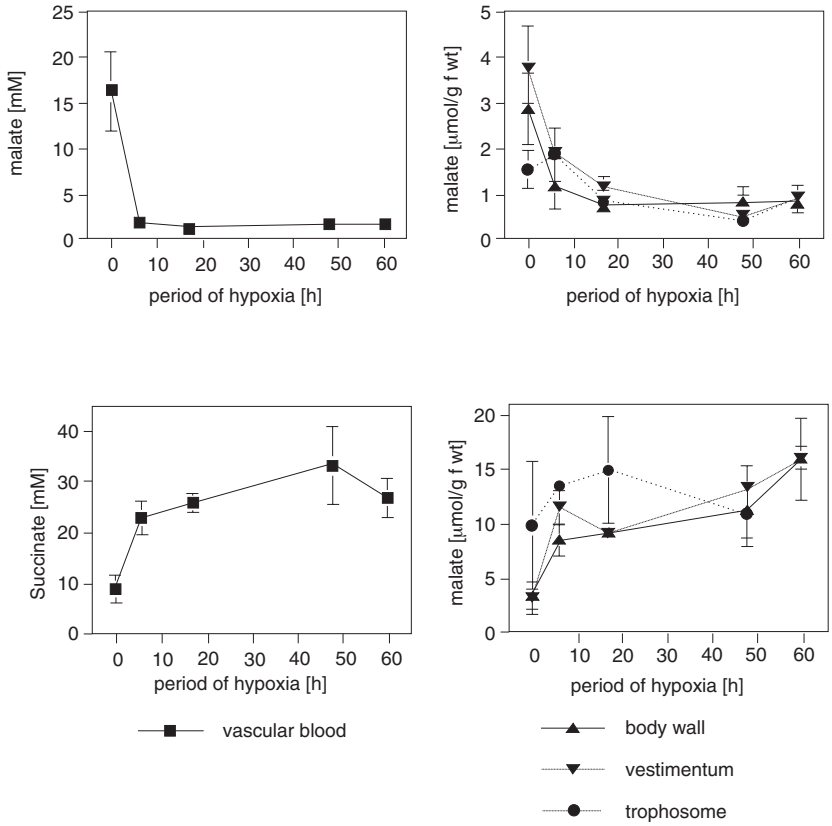

Figure 1. Utilization of malate and production of succinate in the vascular blood and in the tissues of *Riftia pachyptila* during severe hypoxia. Bars represent the mean values ± SD. n = 3 (0 h), 4 (6 h), 5 (17 h), 9 (48 h), 4 (60 h). When no bars are shown, no samples were available. The concentrations in the body fluids, bodywall, and vestimentum changed significantly during the first 6 h ($p<0.05$).

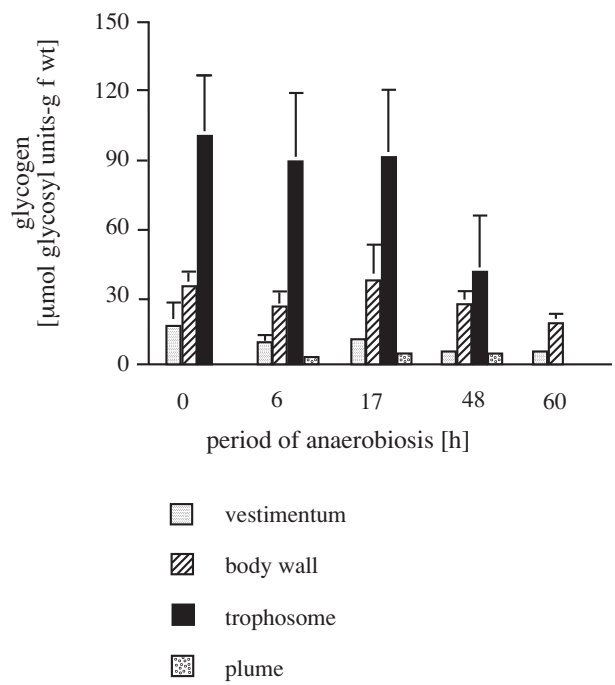

Figure 2. Utilization of glycogen in tissues of *Riftia pachyptila* during severe hypoxia. Bars represent the mean values ± SD. n = 3 (0 h), 4 (6 h), 5 (17 h), 9 (48 h), 4 (60 h). When no bars are shown, no samples were available. The concentration in the trophosome decreased significantly after 48 h ($p<0.05$). Reprinted by permission from *Marine Ecology Progress series,* 1998.

Discussion

The major anaerobic product formed in *R. pachyptila* appears to be succinate. Initially, this accumulation is probably due to a transformation from malate, while after extended hypoxia, glycogen appears to be utilized for the formation of succinate. Succinate concentrations were surprisingly high, even in the body fluids (about 7 mM) and tissues (up to 5 µmol g^{-1} f wt) of worms which were kept under aerobic maintenance conditions. These high levels could be due to the proposed role of these compounds in carrying a portion of the inorganic carbon to the chemoautotrophic symbionts in the trophosome (Felbeck, 1985), in addition to free CO_2 in the blood. Another hypothesis is that the tube-worm primarily uses anaerobic metabolic pathways even when oxygen is available. This could be a response to the high ambient CO_2- concentrations in the blood as well as to the environment of *R. pachyptila*. Carbon dioxide and bicarbonate could enter the tissues of the tube-worm following a diffusion gradient and might promote carboxylation reactions, leading to the production of succinate. Moreover, the formation of organic acids might help to avoid the problem of tissue acidification which is a result of the decreasing pH due to increasing CO_2-levels in the tissues. Succinate is a dicarboxylic acid with a relatively low pK which could take up protons dependent on its concentration (i.e., the number of carboxylic groups available). This metabolic pH-control has also been suggested for the parasitic helminth *Hymenolepis diminuta,* which is frequently exposed to high CO_2-concentrations in its rat host (Podesta et al., 1976). Finally, the excretion of succinate as the metabolic product of the chemoautotrophic symbionts of *R. pachyptila* could also lead to an increase of the succinate level in all body compartments of the tubeworm.

Succinate is to date the only end-product we identified in the anaerobic metabolism of *R. pachyptila*. However, since

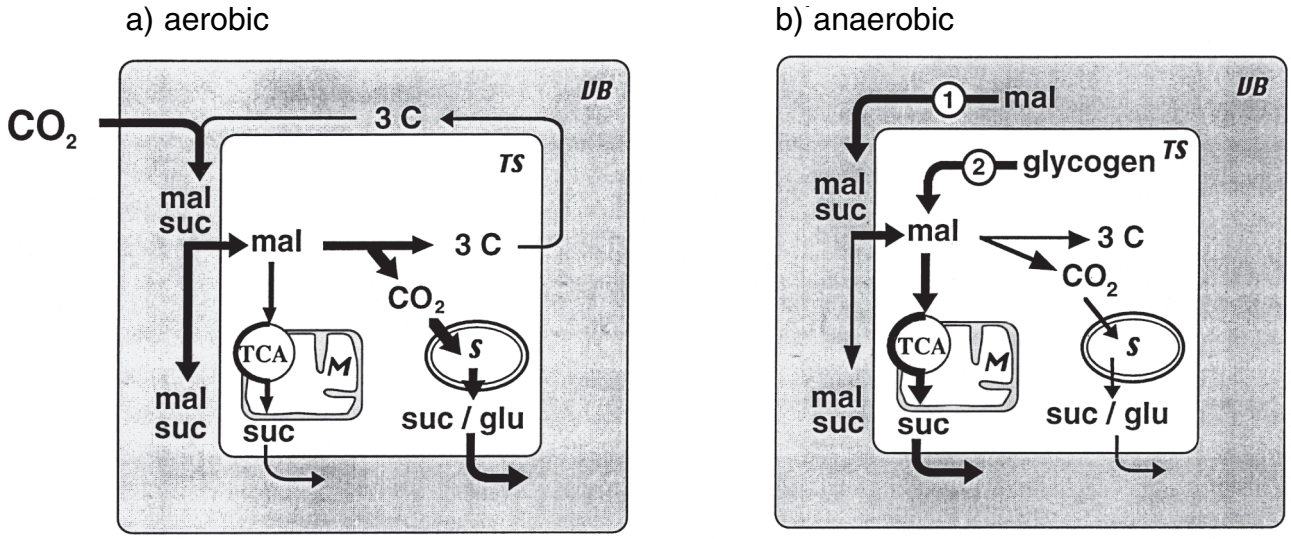

Figure 3. Proposed metabolic scheme of energy-conserving pathways in the hydrothermal vent tube-worm *Riftia pachyptila* during (a) aerobic and (b) anaerobic conditions. a) Under aerobic conditions, malate (mal) formed after CO_2-uptake from the environment is transported via the vascular blood *(VB)* to the chemoautotrophic symbionts *(S)* in the trophosome *(TS)*. Here it is decarboxylated for CO_2 fixation via the Calvin-Benson-Cycle in the symbionts. The organic material formed (succinate and glutamate) is subsequently transferred to the host. Small quantities of malate are proposed to be transformed directly into succinate (suc) via the reversed mitochondrial tri-carboxylic-acid-cycle (TCA cycle). b) Under anaerobic conditions, CO_2-uptake and the autotrophic function of the symbionts appear to be limited. The malate stores in the blood are utilized to generate energy anaerobically (1) while during extended anaerobiosis, glycogen is degraded to produce succinate (2) via the reversed TCA-cycle.

the use of malate and glycogen cannot account for all succinate formed under anaerobiosis in the tube-worm, we expect that further, still unidentified, anaerobic metabolites are produced. While there was no evidence for the activity of opine dehydrogenases forming alanopine, strombine or octopine, we detected moderate activities of lactate dehydrogenase in vestimentum and bodywall (3 and 4 µmol g^{-1} f wt min^{-1}). However, lactate did not accumulate during extended periods of anaerobiosis. Whether volatile, short-chain fatty acids are also involved in anaerobiosis in *R. pachyptila* needs to be evaluated in future research.

Glutamate is the only free amino acid showing a continuous concentration decrease in the trophosome tissue of *R. pachyptila* exposed to extended hypoxia. Since glutamate, in addition to succinate, is a CO_2-fixation product of the tube-worms symbionts (Felbeck & Jarchow, 1998), its concentration decrease could mean a depression of the metabolic activity of the bacteria. A reduction of the food supply to the host would also explain the possibly decreased tolerance of *R. pachyptila* to long-term hypoxia. We have summarized the information obtained to date in a proposed metabolic scheme depicting the differences between aerobic and anaerobic metabolism in *R. pachyptila* (Fig. 3).

References

Arndt C. Schiedek D. & Felbeck H. 1998. Metabolic responses of the hydrothermal vent tube-worm *Riftia pachyptila* to severe hypoxia. *Marine Ecology Progress Serie* (in press).

Felbeck H. 1985. CO_2-fixation in the hydrothermal vent tube-worm *Riftia pachyptila* (Jones). *Physiological Zoology,* **53**: 272-281.

Felbeck H. & Jarchow J. 1998. Carbon release from purified chemoautotrophic bacterial symbionts of the hydrothermal vent tubeworm *Riftia pachyptila. Physiological Zoology,* **71**: 294-302.

Hentschel U. & Felbeck H. 1993. Nitrate respiration in the hydrothermal vent tubeworm *Riftia pachyptila. Nature,* **366**: 338-340.

Johnson M.L., Childress J.J., Hessler R.R., Sakamoto-Arnold C.M. & Beehler C.L. 1988. Chemical and biological interactions in the Rose Garden hydrothermal vent field. *Deep-Sea Research,* **35**: 1723-1744.

Podesta R.B., Mustafa T., Moon T.W., Hulbert W.C. & Mettrick D.F. 1976. Anaerobes in an aerobic environment: Role of CO2 in energy metabolism of *Hymenolepis diminuta.* In: *Biochemistry of parasites and host-parasite relationships* (H. van den Bosche ed), pp. 81-94. Elsevier: Amsterdam.