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CALORESPIROMETRY AND SPECTROPHOTOMETRY OF THE CILIATED GILL OF THE MARINE MUSSEL *Mytilus edulis* (L.) *

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SUMMARY

The energy metabolism of the ciliated gill of the marine mussel *Mytilus edulis* (L.) was studied under different conditions of energy supply and demand, using calorespirometry and spectrophotometry. When ciliary beat frequency was increased approximately two-fold at 30-155 Torr p_{O_2} (4-20.7 kPa), metabolic rate nearly doubled. Metabolism remained aerobic and dissipative, with very little anaerobic capacity. Mitochondrial cytochrome reduction showed the same sensitivity to p_{O_2} as metabolic rate, whether gills had basally active or stimulated cilia. These methods will be useful to determine the effects of natural stresses and aquatic pollutants on the control of energy flux in working ciliated tissues.

INTRODUCTION

To investigate the control of energy metabolism in the working tissues of marine invertebrates, we ask: how is the balance between the supply of ATP and the demand for ATP controlled, and how might this balance be altered by environmental or toxicological stresses? In this pilot study, the energy metabolism of the ciliated gill of the marine mussel *Mytilus edulis* was examined using calorespirometry and spectrophotometry. Electron flux through the mitochondria of an aerobic tissue, measured as oxygen flux, provides a general index of the level of metabolic activity of that tissue. Simultaneous measurements of heat flux and oxygen flux (calorespirometry) indicate whether the metabolism of a tissue is aerobic and fully dissipative or anaerobic (refs. 1,2). Metabolic status is also determined spectrophotometrically as the oxidation/reduction state of mitochondrial cytochromes, intracellular indicators of energy demand (ref. 3,4).

Ciliated gills of bivalve mollusks are situated at an interface between animal and environment. As such, gills are exposed to a variety of ambient environmental conditions, including low p_{O_2}, low ionic strength seawater, and perhaps aquatic pollutants. Gills are important working tissues of bivalve mollusks. Ciliated cells maintain a flow of seawater through the mantle cavity in order to deliver oxygen for respiration and food particles for

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nutrition (ref. 5). Ciliary activity, which is driven by dynein ATPases (ref. 6), requires a continual supply of ATP at a rate sufficient to match the rate of ATP hydrolysis. In the experiments presented here, the energy demand of the gill of *Mytilus edulis* was manipulated by stimulating ciliary beat frequency with the neurotransmitter serotonin. The energy supply of the gill was limited by lowering the ambient partial pressure of oxygen. Physiological status of the gill under these different conditions of energy supply and demand was monitored as metabolic rate, measured as heat flux and oxygen flux using calorespirometry, and as mitochondrial state, measured as the oxidation/reduction state of cytochromes using spectrophotometry. These two techniques in combination indicate flux through the energy-producing pathways as well as the metabolic state of those pathways, both of which are necessary to understand control mechanisms involved in energy metabolism.

MATERIALS AND METHODS

Mytilus edulis

Individuals from three populations of *Mytilus edulis* were used in this study. Animals for calorespirometry were collected from Portland, ME, and Lincolnville Beach, ME, during August 1989. Animals for spectrophotometry were collected from City Island, NY, during July-September 1989. Animals were maintained in aerated millipore-filtered seawater for no longer than two weeks before use.

Calorespirometry of *Mytilus edulis* gills

Metabolic rate of gills was measured with the Thermal Activity Monitor (Thermometric, Sweden) and the Twin-Flow respirometer (Cyclobios, Austria) (refs. 1,7). Inflow and outflow capillaries of the perfusion system were of stainless steel to avoid diffusion of oxygen into the flow lines. A piece of gill tissue was placed into the well-stirred perfusion chamber of the calorimeter. Sensors detected a temperature differential which was converted into a rate of heat dissipation. Polarographic oxygen sensors of the respirometer detected the p_{O_2} of the inflowing and outflowing perfusion fluid, giving a measure of the rate of oxygen consumption. Heat and oxygen flux were recorded virtually simultaneously, taking into account instrument response times and lag times due to perfusion rate (ref. 1). Ambient temperature was controlled at 20°C. Perfusion rate was 7.5 µl/s. Ciliary beat frequency was stimulated with the neurotransmitter serotonin, injected into the perfusion chamber through the injection port to give an initial concentration of 10 µM. Serotonin at 10 µM causes ciliary activity to increase by 2-3 fold (beat frequency increases from about 10 Hz for basally active cilia to about 25 Hz for stimulated cilia, ref. 8). In experiments with gills on a microscope,we observed an approximate 2-fold increase in particle velocity, suggesting that ciliary beat frequency had doubled at this concentration of serotonin.

To support the gill tissue during metabolic rate measurements, a gill holder was

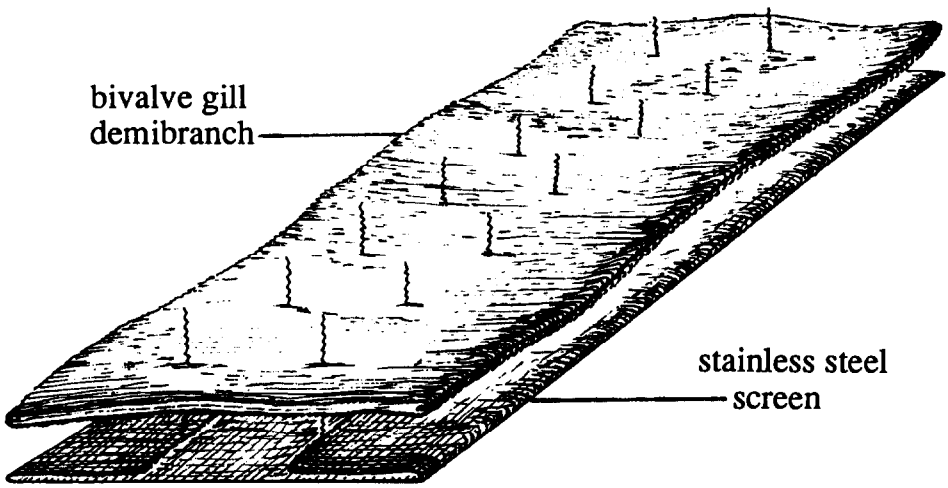

Figure 1. Drawing of stainless steel gill holder with *Mytilus edulis* gill tissue. In this configuration, gill ciliary activity remains unobstructed. Dimensions are 1 X 2 cm.

constructed from 200 mesh stainless steel screen with 50 μm diameter threads (Small Parts, FL) (Fig. 1). Individual threads of the screen were bent at right angles to the base of the holder to form upright posts. A piece of gill, 1 X 2 cm square and about 100 mg wet weight, was eased down onto the posts which slid between individual filaments, suspending the gill above the base of the holder. An important feature of this holder is that ciliary activity remained unaffected; particles continued to move along the surface of the gill into and along the food groove. Consequently, diffusion distances for oxygen remained largely unchanged. The entire apparatus was placed into the perfusion chamber of the calorimeter.

In a calorespirometer experiment, gills were perfused with aerated millipore-filtered seawater containing the antibiotic rifampicin (5 ppm). At steady state, a single 10 μl pulse of deoxygenated serotonin was injected into the perfusion chamber. Washout time for serotonin was approximately 30 minutes, after which the metabolic rate returned to prestimulus levels. Gills were then perfused with seawater at a different p_{O2} and the steps with serotonin were repeated. Gills were exposed to 155, 77.5, and 31 Torr (20.7, 10.3, and 4.1 kPa) p_{O2} of the inflow water, and to anoxia. After each experiment, a baseline was recorded at each p_{O2} used during the experiment, with the empty gill holder in the perfusion chamber.

Spectrophotometry of *Mytilus edulis* gills

Gills suspended on a gill holder in a cuvette containing seawater were placed into the sample chamber of a Cary 14 spectrophotometer equipped with a scattered transmission

accessory and digital data acquisition and analysis software (Aviv and Associates, Lakewood, NJ) (ref. 9). Gills were equilibrated for 20 minutes at each p_{O2}, and an optical absorption spectrum (380-650 nm) was recorded. Serotonin (10 μM) was added to the cuvette for experiments with stimulated cilia. Difference spectra of hypoxic or anoxic gills minus aerated gills were generated for the calculation of fractional reduction of mitochondrial cytochromes. Fractional reduction of cytochrome oxidase and cytochrome c was calculated from the difference in optical density between 445 and 470 nm and between 420 and 407 nm, respectively.

RESULTS AND DISCUSSION

Calorespirometry of *Mytilus edulis* gills

Traces of heat flux and oxygen flux of *Mytilus edulis* gills under three conditions of p_{O2}, measured with the calorimeter and respirometer, are shown in Figure 2. In aerated seawater, heat flux (upper trace: air) of gills with basally active cilia nearly doubled as ciliary beat frequency was increased with the addition of 10 μM serotonin to the perfusion chamber. As serotonin was washed out of the chamber, heat flux returned to its pre-stimulus levels. This same response was seen in seawater equilibrated with 77.5 Torr p_{O2} (data not shown). In seawater equilibrated with 31 Torr p_{O2} (upper trace: 20% air), heat flux of gills with basally active cilia was lower than in aerated seawater, but increased with the addition of serotonin.

Oxygen flux (lower trace: air) of gills with basally active cilia under aerated conditions was in close agreement with values reported by others for *Mytilus edulis* gills (refs. 8,10-12). In all conditions of p_{O2}, changes in oxygen flux were similar to changes in heat flux (see Fig. 2 upper and lower traces: air, 20% air).

When gills undergo aerobic dissipative metabolism in aerated seawater, the empirically measured ratio of dissipated heat (kJ) to respired oxygen (moles), called the calorimetric/ respirometric (CR) ratio, should equal the theoretical oxycaloric equivalent, -450 kJ/mol O2, or at least be within the observed range of -430 to -480 kJ/mol O2 (ref. 13). At 77.5 and 155 Torr p_{O2}, the CR ratios of gills with basally active cilia were within this range (Table 1). The CR ratios at 31 Torr p_{O2} lie outside this range, but are not significantly different from the control ratio under aerated conditions. The CR ratios of gills with stimulated cilia, calculated from integration of the traces, were also within the range of the theoretical oxycaloric equivalent (Table 1). For comparison, induction of metabolic peak activity in fish embryos by addition of high concentrations of antibiotics increased heat flux disproportionately more than oxygen flux (ref. 14). This is reflected by a high CR ratio indicative of supplementary anaerobic processes to total metabolism.

In anoxic or microxic seawater, oxygen flux was virtually zero and heat flux remained at less than 1% of the basal rate under aerated conditions, showing only a slight effect of dilution of

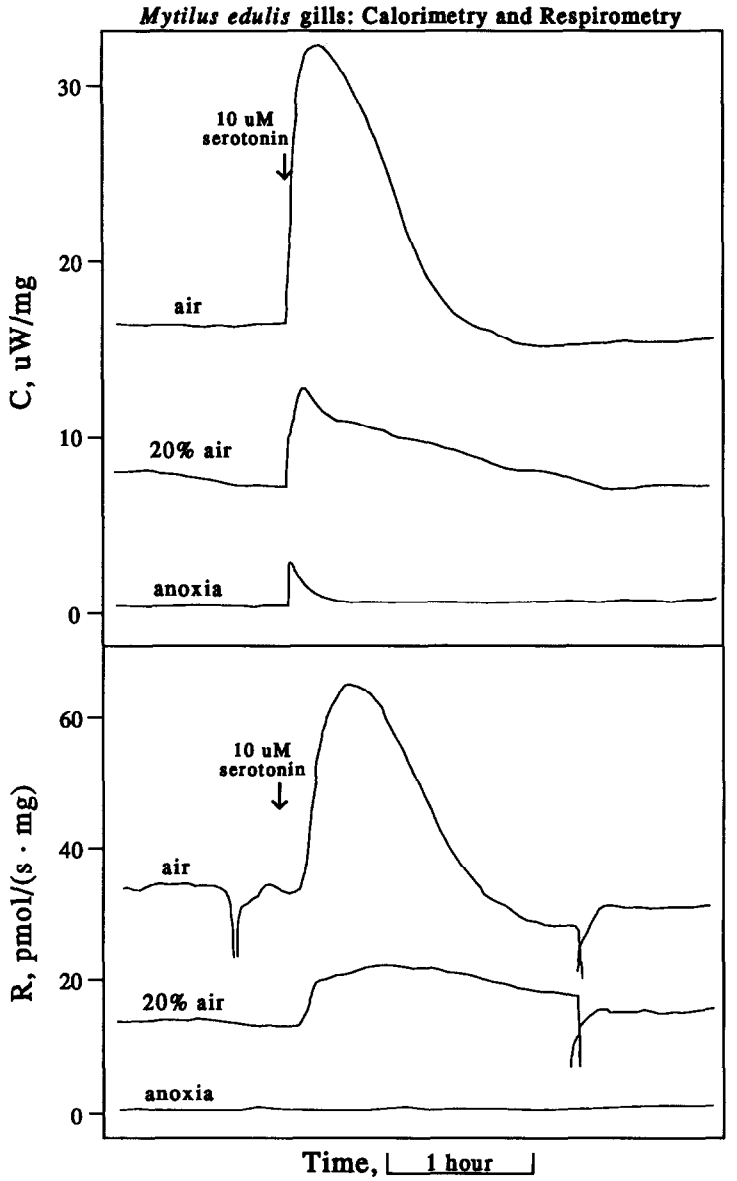

Figure 2. Experimental traces of heat flux, C [mW/g dry mass], and oxygen flux, R [nmol O2/s.g dry mass] of *Mytilus edulis* gills under different conditions of p_{O2} (air, 20% air and anoxia), measured with calorespirometry. Serotonin was injected into the perfusion chamber at arrow. See text for explanation.

TABLE 1
Calorimetric/respirometric (CR) ratio, kJ/mol O2, of *Mytilus edulis* gills with basally active and stimulated cilia.

P_{O2}		CR ratio, kJ/mol O2	
Torr	kPa	Basally active cilia	Stimulated cilia
31	4.1	555 ± 181 (5)[a]	415 ± 75 (3)
77.5	10.3	477 ± 156 (5)	44 0± 2 (3)
155	20.7	468 ± 74 (13)	454 ± 47 (7)

[a] Values given as mean±standard deviation (number of repetitions).

serotonin (Fig. 2, upper and lower traces: anoxia). After 4-5 hr exposure to anoxic seawater, metabolism recovered to aerobic levels within 30 minutes after reintroduction of aerated seawater, with little overshoot of oxygen flux (n=2; data not shown).

Bayne and Thurberg (ref. 12) report that oxygen consumption rate of the excised *Mytilus edulis* gill is not correlated to filtration rate of the same previously intact animal. However, our experiments are in accord with Clemmesen and Jørgensen (ref. 8) who show that oxygen consumption rate of *Mytilus edulis* gills is linearly related to ciliary beat frequency (r^2=0.99), each measurement determined concurrently in fragments from the same gill. Using calorespirometry, we have determined that the metabolism of *Mytilus edulis* gills with basally active and stimulated cilia is mainly aerobic and dissipative at 30 Torr p_{O2} and above. In addition, *Mytilus edulis* gills have little anaerobic capacity. Anaerobic endproducts extracted from gills of *Mytilus edulis* after exposure of whole animals to anoxic conditions (ref. 15) are most likely produced in other tissues and translocated to the gill through the hemolymph.

Spectrophotometry of *Mytilus edulis* gills

Ciliated cells of bivalve gills are densely packed with mitochondria which supply the ATP necessary for dynein ATPase activity and thus for ciliary beating. The geometric design of ciliated bivalve gills makes them ideal for studying mitochondrial activity in vivo using spectrophotometry because the tissue is thin, the cilia provide continual perfusion, and the optical signal from mitochondria is relatively large. Background interference is canceled in difference spectra, revealing the optical spectra of mitochondrial cytochromes.

Figure 3 shows a collection of difference spectra of gills with basally active cilia in hypoxic or anoxic seawater minus the same gills in aerated seawater. The upper spectrum (labeled N2), which is the difference of gills in seawater equilibrated with N2 minus gills in aerated seawater, shows a characteristic reduced minus oxidized mitochondrial cytochrome difference spectrum. Cytochrome *aa*3 is seen by the absorbance peaks at 605 and 445 nm,

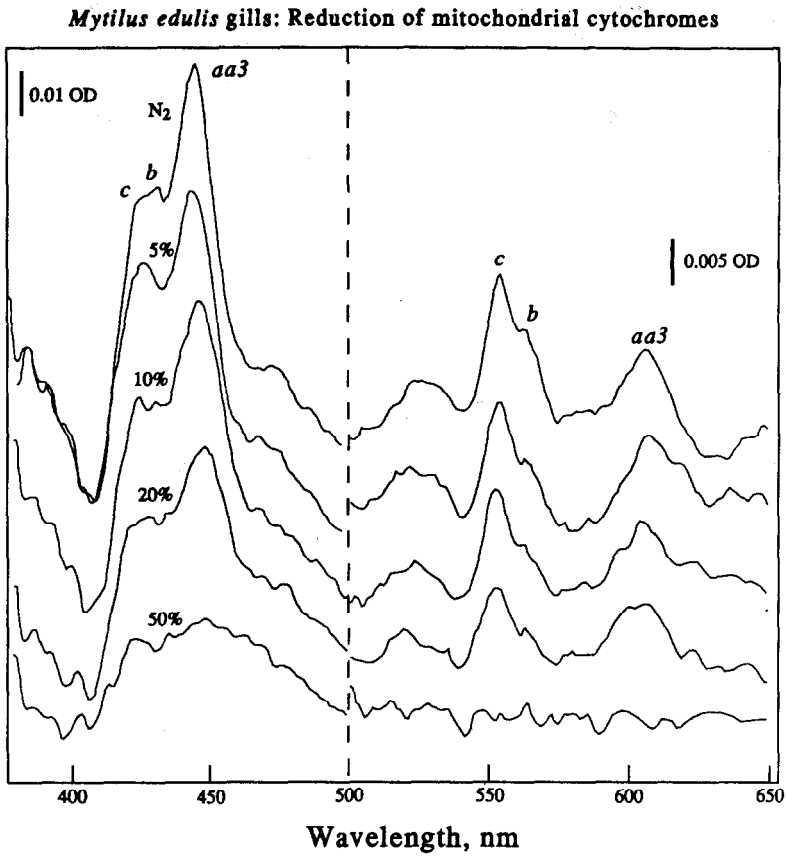

Figure 3. Optical density (OD) difference spectra of *Mytilus edulis* gills in hypoxic or anoxic seawater minus the same gills in aerated seawater. The OD scale of the 500-650 nm region has been halved to increase resolution of the spectra. See text for explanation.

cytochrome *c* by the peaks at 550, 520, and 420 nm, and cytochrome *b* by the peaks at 560, 535, and 430 nm. The other difference spectra are of gills in seawater equilibrated with different p_{O2} (labeled by the % air in each gas mixture) minus gills in aerated seawater, and show partial reduction of the cytochromes. Gills with stimulated cilia show this same response of cytochrome reduction to p_{O2} (data not shown). Thus, the aerobic *Mytilus edulis* gill with basally active or stimulated cilia begins to show cytochrome reduction at 31 Torr p_{O2} (20% air), the same p_{O2} at which basal and stimulated metabolic rate is decreased.

In a number of studies of the effects of natural stresses and aquatic pollutants on the

function of bivalve gills, metabolic rate of the gills either increased or decreased, depending on the particular set of conditions or the compound used (for example, see refs. 10,11). This suggests that the balance between energy supply and demand in the gills may be affected under these conditions. With the methods presented here, we hope to determine more directly how these stresses alter the energy metabolism of ciliated cells of bivalve gills, and to further understand the basic control of energy flux in working ciliated tissues.

ACKNOWLEDGEMENTS

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