Mitochondria of Antarctic and North Sea Marine Invertebrates – Ecological Functions of Mild Uncoupling in Water Breathers

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Summary

Antarctic marine ectotherms look back on several million years of adaptation to constant extreme cold temperatures. By contrast, animals from temperate zones face high summer and below 0°C temperatures in winter. Here, we present recent data on mitochondrial ROS production in animals from both climatic environments, and a concept of mitochondrial proton leak as part of thermal adaptation in both groups.

Introduction

Cutaneous respiration is significant in many water breathers (1) and becomes more important at lower water temperatures as water oxygen concentration rises in the cold. Its contribution to routine metabolic rate (VO_2) can account for up to 40% in Antarctic zoarcids and ice fishes, respectively (2). Critical warming of the environment causes an acceleration of mitochondrial oxygen demand in ectotherms, and the metabolic stress response to heat will increase surface oxygen flux to the tissues, especially in small aquatic invertebrates, which depend even more on oxygen surface diffusion. Elevated tissue oxygen flux under stress or during and after exercise, causes a necessity for Antarctic ectotherms, to buffer the tissue redox potential and stock up on antioxidants like á-tocopherol, because of an enhanced probability of reactive oxygen species (ROS) formation. ROS-buffering is achieved by the glutathione system, and a significantly more oxidized glutathione redox ratio is found in marine ectotherms (Tab.1), when compared to many

Tab. 1: Comparison of antioxidant tissue concentrations of α-tocopherol and glutathione (2 GSSG + GSH, µmol g^{-1} wet weight), glutathione redox ratio (GSH:GSSG), and levels of protein oxidation (nmol carbonyls mg^{-1} protein). α-tocopherol for fish in µmol g^{-1} wet weight [(a)], for mud clams in pmol mg^{-1} protein[(b)] from ref. (9).

	Fish (eelpout)		*Mud clams*	
	polar	temperate	polar	temperate
α-tocoph. content	300[(a)]	240[(a)]	10[(b)]	2.7[(b)]
glutathione content	7.9	1.2	0.6	0.34
glutath. redox ratio	0.7	5.6	6.8	3.4
protein oxidation	3.7	2.2	1.9	1.1

higher organisms, where the GSH : GSSG ratio usually ranges >10.

Mitochondria have been described as the major cellular ROS producers (3, 4), especially under physiological stress and in pathological stages (5, 6). Generally, higher H_2O_2 production is observed when mitochondrial membrane potential rises under state 4 conditions (3 for rev.). Miwa and coworkers (7) observed that a lowering of the mitochondrial membrane potential by only 10 % reduced H_2O_2 production of insect flight muscle mitochondria complex I (from reverse electron flow) by 70 % under *in vitro* conditions.

Results

In a recent study comparing mitochondrial functions of warm (summer) and cold (winter) adapted marine worms, *Arenicola marina*, from the North Sea (8), we found a lower membrane potential (mp: 147 mV, Fig. 1) in mitochondria isolated during winter. These mitochondria produced only one quarter of the H_2O_2 released by summer animal mitochondria (mp: 161 mV) under the same *in vitro* conditions.

Reduced mitochondrial density and increased tissue antioxidant defences in lugworm cells during summer could counterbalance the higher ROS production per mitochondrion. More importantly, to support rapid temperature fluctuations on intertidal mudflats during summer, lugworm mitochondria isolated in summer responded to experimental warming with an immediate increase of proton leakage, whereby reducing membrane potential and proton motive force, and ameliorating thermally induced acceleration of hazardous ROS formation. In the case of lugworm body wall mitochondria, we used 3 mM succinate as respiratory substrate, rotenone to prevent reverse electron flow to complex I (4), and no antimycin a. Under these conditions, H_2O_2 can form only at complex III and, presumably to a minor extent, complex II. In other words, at least in isolated mitochondria of this marine worm with relatively low aerobic capacity, there seems to be ROS production during forward electron transport, sensitive to mild uncoupling of proton motive force. This

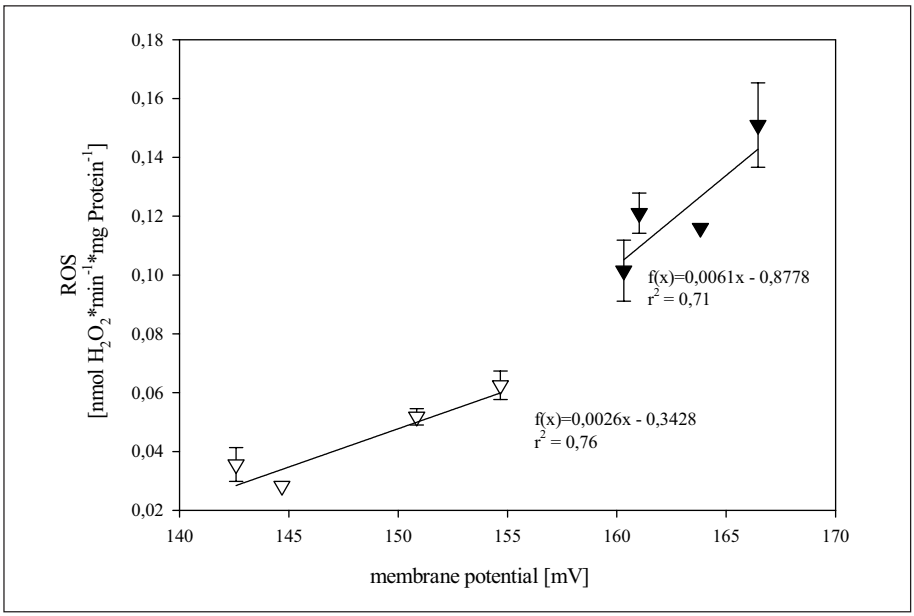

Fig. 1: H_2O_2 formation vs *membrane potential in state 4+ in mitochondrial isolates from* A. marina *winter (white triangles) and summer animals (black triangles). Data are means ± SD from 1-3 isolations per point assayed at 10°C (from ref. 8, ROS assaying by HVA/HRP, catalase sensitive)*

finding contrasts the insect flight muscle study of Miwa et al. 2003. Although we cannot say to which extent complex III or II contribute to overall mitochondrial ROS production, neither *in vitro* nor *in vivo* in lugworm cells (as we did not use NADH linked substrates), this mechanism matches the need for high metabolic flexibility of the oxyconforming and thermoconforming mud dweller *Arenicola marina*.

A controlling function of the proton leak towards forward ROS formation could be an advantage not only under thermal stress, but also during periods of limited oxygen availability. Typically, on intertidal mudflats during summer both critical factors come together. As higher temperatures warm up the mudflat, oxygen is consumed by elevated microbial and heterotrophic demand. Below a species specific critically low oxygen level, aerobic respiration decreases even in hypoxia tolerant infauna species (10). A situation in which oxyconforming marine ectotherms switch on anaerobic metabolism, and which is, moreover, un-experienced by an insect aerobic flight muscle.

Limiting oxygen availability will slow electron transport in the lower part of the respiratory chain, propagating ROS formation from down-

ward electron carriers, like complex III ubiquinone (11, 5). Also, these reduced electron carriers presumably autoxidize, to generate more ROS, during onset of re-oxygenation (see ischemia-reperfusion injury). Theoretically, mild uncoupling might limit excessive ROS formation during re-oxygenation, by oxidizing mitochondrial respiratory complexes, including b-type cytochromes (7).

Presently unknown, while quite possible, reverse electron flow (from succinate-dehydrogenase complex II to NADH-oxidoredutase complex I, (4)) could be fuelled by anaerobic succinate, accumulating during the first 2 h of hypoxia/anoxia exposure of *Arenicola marina* (12). This may cause complex I ROS formation in hypoxic worms, before they induce metabolic reduction, as hypoxic electron transport is slow, but not blocked, and membrane potential presumably kept high, in order to sustain maximal energetic coupling.

It is less likely that Antarctic marine animals face significant fluctuations of environmental oxygen. In contrast, higher oxygen surface diffusion in cold waters in combination with thermally slowed metabolic oxygen consumption in many polar ectotherms can be expected to result in increased cytosolic ROS formation. This is partly warded off by elevated ROS scavenging capacity (see Tab. 1, and ref. 13). Mitochondrial metabolism *in situ* is thought to be idling close to state 4 levels under resting conditions, and this seems especially true for polar marine infauna with generally low activity levels.

Comparing mitochondria of an Antarctic and a North Sea mud clam of similar body size and life style (subsurface filter feeders), we found higher mitochondrial proton leak (58.8±6.5 % of state 3 respiration) at a membrane potential of 122 mV in the Antarctic clam (*Laternula elliptica*), whereas in the North Sea species (*Mya arenaria*) proton leak ranged between 44.4±7.0 % of state 3 respiration at identical membrane potential ((122 mV). Again, elevated proton leakage is linked to lower basal ROS production (state 3 and state 4) in the polar mud clam (14). Recent results from our group confirmed these findings to be consistent also if different age classes from both species are compared (E. Philipp unpubl. data). Thus mild uncoupling could be a basal strategy of Antarctic invertebrates, to control mitochondrial ROS production at least under resting state conditions. However, this strategy seems to function only in species with low and very constant specific metabolism over lifetime, typical for sessile polar fauna. In young North Sea mud clams, metabolic rates were vastly elevated over their elders, and declined dramatically during the first 3.5 yrs. To our surprise, these animals had lowest proton leak rates of all age classes (<45% of state 3), and, moreover, significantly lower *in vitro* ROS production per mol oxygen consumed than aged individuals. So, clearly, in these more active and fast growing young *Mya* specimens, mild uncoupling of proton motive force is not the strategy applied to prevent ROS damage. Actually, in spite of low

ROS production rates *in vitro*, young North Sea mud clams accumulated oxidative damage (protein carbonyls) and age markers (lipofuscin) at higher rates than aged animals.

While we were able to detect net production of ROS in mitochondrial isolates from several types of metabolically sluggish marine bivalves and worms, ROS release from mitochondria that we isolated from more active (scallops) or higher evolved epibenthic animals (fish) was null under all experimental conditions (HVA/HRP assay with complex I and complex II related substrates, respiratory inhibitors rotenone, anitmycin a, SOD addition, high temperature stress). This, although the mitochondria were well coupled and functional.

Discussion

It is intriguing to think that mitochondria of more active marine ectotherms, and of young, fast growing stages, could completely prevent ROS release into the cells by extremely effective scavenging systems, which possibly act on the matrix side. Considering the higher scope for activity in these more active species, tight control of ROS release to the cytosol seems in keeping with a maintenance of cellular homeostatic functions over lifetime. Obviously, more sluggish animals can tolerate the anyway low accumulation of oxidative damage products, while these animals cannot. Damage effects could thus be locked into the mitochondria, and may have an important effect on the rate of cellular aging, only in this cellular compartment. At the same time, the cytosolic compartment stays relatively free of unwanted oxidative by-products, to maintain optimal physiological conditions. A strategy preventing ROS release would, therefore, support cell functioning in young, active animals at the trade-off of (mitochondrial and animal) life expectancy. In deed, we observed a marked decline of specific respiration and of mitochondrial coupling with age in the North Sea mud clams, contrasting only marginal changes in the polar species. Maximal life expectancy in the polar species is 300 % higher than in North Sea clams and, in turn, both mud clams by far outlive swimming scallops. Here the system has been brought to perfection with next to no mitochondrial ROS release to preserve cell functioning at the cost of fast aging in the mitochondria themselves, so that these animals literally “live hard and die young”.

Conclusions

Mild uncoupling of proton motive force might be an important mechanism, to prevent one-electron reduction of oxygen in mitochondria of oxy- and thermoconforming intertidal marine invertebrates. Flexibly inducible leak opening could be part of their “high physiological toler-

ance" program, to withstand natural fluctuations of environmental temperature and oxygen. In polar species with significant contribution of surface oxygen flux to whole animal respiration, a permanent high proton leak might help to control tissue oxygen levels, and limit *in vivo* ROS formation. In combination with elevated cellular ROS scavenging capacity, lower mitochondrial ROS release supports high life expectancies in some polar benthos animals.

Finally, more actively swimming scallops seem to follow another strategy, by locking respiratory ROS damage into their mitochondria. In so doing, they protect their cells from metabolic oxidative stress and support their high activity levels at the cost of enhanced mitochondrial aging.

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