Thermal sensitivity of mitochondrial function in the Antarctic Notothenioid Lepidonotothen nudifrons

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Abstract The thermal sensitivity of mitochondrial function was investigated in the stenothermal Antarctic fish Lepidonotothen nudifrons. State 3 respiration increases with increasing temperature between 0 °C and 18 °C with a $Q_{10}$ of 2.43–2.63. State 4 respiration in the presence of oligomycin, an inhibitor of mitochondrial ATP synthase, quantifies the leakage of protons through the inner mitochondrial membrane, which causes oxygen consumption without concomitant ATP production. This parameter shows an unusually high $Q_{10}$ of $4.21 \pm 0.42$ (0–18 °C), which indicates that proton leakage does not depend merely on ion diffusion but is an enzyme-catalysed process. The differential thermal sensitivity of oxidative phosphorylation (=state 3) and proton leakage (=state 4 in the presence of oligomycin) leads to progressive uncoupling of the mitochondria and decreased efficiency of oxidative phosphorylation under in vivo conditions if the body temperature of L. nudifrons increases.

Key words Antarctic fish · Temperature · Mitochondrial respiration · Proton leakage · Isocitrate dehydrogenase

Introduction

The Antarctic marine fauna is constantly exposed to extremely low temperatures only ranging between $-1.8$ °C and 1 °C. Organisms inhabiting the extreme Antarctic environment must have developed special physiological adaptations to overcome the adverse effects of low temperatures on metabolism.

Despite uncompensated low resting metabolic rates (Clarke 1991), polar animals show a compensatory increase in tissue oxidative capacity (van Dijk et al. 1998), which is reflected in higher mitochondrial densities compared to temperate zone animals (Archer and Johnston 1991; Johnston et al. 1998). Accordingly, some oxidative enzymes show 1.5 to 5-fold increased maximal activities in Antarctic fish tissues (Crockett and Sidell 1990). Cold adaptation may provoke a rise in oxidative capacity by enhancing the mitochondrial density or also by increasing the capacity of the individual mitochondrion. Johnston and co-workers (Johnston et al. 1994; Johnston et al. 1998) carried out an inter-species comparison of mitochondria from fishes of different latitudinal origin. They found that maximal respiration rates of mitochondria from Antarctic species at $-1$ °C were close to those expected from extrapolation of the rates obtained in temperate species, suggesting only modest or no compensatory increase in respiratory capacity.

The physiological adaptation to the Antarctic environment seems to go along with reduced tolerance towards higher temperatures. All Antarctic organisms show strong stenothermality. Upper lethal temperatures as low as 4.5 °C have been observed in the Antarctic brachiopod Liothyrella uva (Peck 1989). Different species of Trematomus die at temperatures between 5–6 °C (Somero and de Vries 1967), while the Antarctic eelpout Pachycara brachycephalum survives temperatures of up to 10 °C (van Dijk et al. 1999).

In a variety of marine species, exposure to critically high temperatures causes the formation of anaerobic...
mitochondrial endproducts which may characterise the lethal limits (Sommer et al. 1997). In Antarctic invertebrates, temperature-induced accumulation of anaerobic end-products has been observed at temperatures only slightly above their habitat temperature: at 2 °C in the clam Limopsis marionensis (Pörtner et al. 1999a) and between 3 °C and 6 °C in Laternula elliptica (H.O. Pörtner, I. Hardewig, L. Peck, in preparation). Only one study so far has determined the critical temperature in an Antarctic vertebrate. The Antarctic eelpout Pachy- cara brachycephalum only accumulates succinate at a temperature of 10 °C (van Dijk et al. 1999) which may indicate that Antarctic vertebrates are more tolerant towards higher temperatures than invertebrates.

The onset of anaerobic energy production suggests that oxidative, thus mitochondrial, energy provision is restrained at high temperatures. Thermal limitations of oxidative metabolism may be due to reduced oxygen provision through the ventilatory and circulatory system, or to the impairment of mitochondrial function. The present study was part of a larger investigation to analyse the effect of temperature increase on mitochondrial respiration of stenothermal Antarctic organisms. Parallel studies were carried out on the clam Laternula elliptica (Pörtner et al. 1999b) and the Notothenioid Lepidonotothen nudifrons (this study) in order to compare the thermal sensitivity of vertebrate and invertebrate mitochondria and to reveal a possible correlation between thermal sensitivity of the mitochondria and the critical temperature of the whole organism.

The benthic, moderately active Notothenioid L. nudifrons is endemic to the Southern ocean where water temperatures remain at about 0 °C all year round. While this investigation was in progress, Weinstein and Somero (1998) published a study regarding temperature effects on mitochondria of the Antarctic Notothenioid Trematodus bernacchii. They found that mitochondrial respiration is impaired at temperature above 18 °C, which is beyond the lethal temperature limit of this species. Our data will be discussed with respect to these results.

**Materials and methods**

**Animals**

*L. nudifrons* were caught by scuba diving at Adelaide Island, Antarctica at a depth of 15-30 m in December 1997. Fish with a mean weight of 24.3 ± 8.4 g were kept in aquaria with continuously running sea water at 0 °C for at least 1 week prior to experimentation. Experiments were conducted at Rothera Base (Antarctica).

**Determination of ventilation rate and lethal temperature**

Fish were incubated in a thermostatically controlled aquarium at 0 °C. The water temperature was increased by 1 °C day^-1_. Ventilation rates were determined at the end of the acclimation period by counting the movements of the opercula of each individual fish over several minutes.

Isolation of mitochondria

The fish were killed by decapitation after slight anaesthetisation with MS 222 (0.3 g l^-1). Immediately after this, approximately 1 g liver tissue was removed, chopped finely with scissors in an ice-cold petri dish, and extracted in 30 ml isolation buffer (50 mM Hepes pH 7.1 at 20 °C, 85 mM KCl, 80 mM sucrose, 5 mM EDTA, 5 mM EGTA, 1% BSA, 1 μg ml^-1 aprotinin) with a Potter-Elvehjem homogeniser. The tissue was dispersed by three passes of a loose-fitting pestle. After centrifugation (12 min at 300 g) the pellet was re-homogenised in 30 ml isolation buffer and centrifuged again. The combined supernatants were spun at 9500 g for 8 min. The mitochondrial pellet was re-suspended in 1–1.5 ml assay medium (50 mM Hepes pH 7.1 at 20 °C, 85 mM KCl, 80 mM sucrose, 5 mM KH2PO4, 1 μg ml^-1 aprotinin) with 1% BSA yielding a concentration of 15–20 mg mitochondrial protein ml^-1. The homogenate was kept at 0 °C during the isolation procedure.

**Mitochondrial respiration**

Oxygen consumption was measured at different temperatures using a Clarke-type oxygen electrode in a thermostatically controlled respiration chamber of 1 ml volume. A 100-μl sample of the mitochondrial suspension (1.5–2 mg mitochondrial protein) was added to 0.9 ml assay medium containing 5 μM ApreA, an inhibitor of myokinase and 3.3 mM succinate or 3.2 mM pyruvate together with 0.5 mM malate as substrates. ΔPH/AT of the assay medium was ~0.013 units °C^-1 which is in accordance with α-stat pH regulation. State 3 respiration was recorded after 3 min after the addition of 0.3 mM ADP. During state 3 respiration in the presence of succinate, 8 μM rotenone was added to inhibit complex I. After all ADP had been phosphorylated state 4 respiration was determined. Finally, oligomycin, an inhibitor of mitochondrial ATP synthase, was added to a concentration of 2 μg ml^-1 (1–1.3 μg mg^-1 mitochondrial protein) and oxygen consumption (state 4) was recorded for another 5 min. After this time respiration rates tended to rise, probably caused by a progressive increase in membrane potential due to inhibition of the ATP synthase (see below).

To determine the effective P/O ratios of oxidative phosphorylation, ATP production was measured during state 3 respiration in the presence of succinate without rotenone. Samples of 10 μl assay medium were withdrawn from the respirometer during each run at defined time points. Samples were diluted in 990 μl buffer (0.2 M Tris-acetate pH 7.75, 1 mM EDTA) and heated briefly to 95 °C to inhibit mitochondrial function. ATP concentrations were determined luminometrically with an ATP assay kit (BioOrbit, Finland) following the rationale of Wibom et al. (1990). Effective P/O ratios were calculated as rate of ATP production divided by respiration rate.

Oxygen solubility in the assay medium at different temperatures was adopted from Johnston et al. (1994). Protein concentration of the mitochondrial pellet was determined by the Biuret method, using 5% deoxycholate to solubilise membrane proteins.

**Enzyme assay**

NADP^-dependent isocitrate dehydrogenase activity was determined in the mitochondrial suspensions after Alp et al. (1976) with slight modifications. Mitochondria were lysed by the addition of 5% deoxycholate. The assay contained 70 mM Tris-HCl pH 7.1, 8 mM MgSO4, 2.5 mM NADP^-1, 1 mM MnCl2, 3 mM D/L-iso-citrate and 20 mM citrate. Enzyme activity was measured following the appearance of NADPH at 339 nm in a thermostatically controlled spectrophotometer at 0, 4.5, 9, 15, 21, 25 and 30 °C.

**Statistics**

All values are given as mean ± standard deviation, n = 5 for mitochondrial respiration experiments and n = 4 for enzyme activities. Statistical significance was tested at the P ≤ 0.05 level using