THE KINETICS OF MITOCHONDRIA ISOLATED FROM OLIGOCHAETES: COMPARISON WITH CLASSICAL RAT LIVER MITOCHONDRIA

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INTRODUCTION

Aquatic oligochaetes are well known for their ability to tolerate anoxia. Under these conditions the mitochondria are involved in anoxic electron transport with coupled ATP generation through the fumarate-succinate redox system (Schöttler, 1977; see also Schroff and Zebe, 1980; Schulz et al., 1982). In the present study we investigated mitochondria isolated from these "euryoxic" animals under aerobic conditions, to compare their kinetic characteristics with the classical model of mitochondrial studies obtained from rat liver.

MATERIALS AND METHODS

The experiments with rat liver mitochondria were carried out as described by Haller (1990) except for the experimental temperature. A mixture of tubificids (mainly *Tubifex tubifex*) was obtained from a commercial company and kept under running tap water in the absence of food. 20 g fresh weight of whole worms were homogenized in a Potter-Elvehjem glass homogenizer with a motor driven teflon pestle in 3 volumes of 0.3 $mol.dm^{-3}$ sucrose medium containing 20 $mmol.dm^{-3}$ HEPES, 1 $mmol.dm^{-3}$ EGTA, 0.2% fatty acid free BSA, and Nagarse (from *Bacillus subtilis*; 4 $mg.g^{-1}$ fresh weight; Pande and Blanchaer, 1971) at pH 7.5 and 0 to 4 °C (modification after Schöttler, 1977). After homogenization the homogenate was left on ice for 10 min, then filtered through a series of filters (400, 200 and 100 μm). The filtered homogenate was centrifuged at 1,500 *g* for 10 min, the pellet discarded and the supernatant centrifuged at 13,000 *g* for 10 min. Except for the black layer at the bottom, the pellet was resuspended in buffer without Nagarse and once more centrifuged at 13,000 *g* for 10 min. The resulting mitochondrial pellet was resuspended in ca. 5 cm^3 of isolation buffer, typically yielding a protein concentration of 13 $mg.cm^{-3}$. The isolation procedure was carried out near 0 °C, and the procedure was optimized in the course of preliminary test experiments.

Mitochondrial respiration was measured in a prototype CYCLOBIOS Oxygraph (see pp. 13-16) with a volume of 5 cm^3 at 20 °C. The measuring buffer contained 150 $mmol.dm^{-3}$ KCl, 20 $mmol.dm^{-3}$ HEPES, 1 $mmol.dm^{-3}$ EGTA, 3 $mmol.dm^{-3}$ KH_2PO_4 and 10 $mmol.dm^{-3}$ $MgCl_2$ at pH 7.3 (20 °C). The experiments were started by injection of 200 mm^3 of mitochondrial suspension, with the subsequent addition of Na_2-succinate to yield a final concentration of 2 $mmol.dm^{-3}$ (State 2). State 3 respiration was initiated by the addition of ADP.

Experiments for studying KCN inhibition were initiated by titration of 200 $IU.dm^{-3}$ HK (final concentration) to the mitochondrial suspension containing 20 $mmol.dm^{-3}$ glucose, 1 $mmol.dm^{-3}$ ATP and 2 $mmol.dm^{-3}$ sucrose. First a linear steady state coupled oxygen flux was

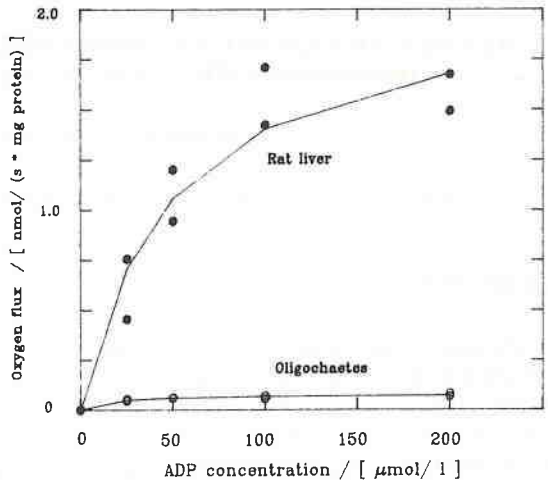

Fig. 1. Dependence of mitochondrial oxygen flux on ADP concentration: Comparison of oligochaete and rat liver mitochondria. Oxygen flux [nmol $O_2.s^{-1}.mg^{-1}$ protein] as shown on the ordinate is the difference of State 3 and State 2 respiration. Duplicate experiments are shown by symbols, and the hyperbolic Levenberg-Marquardt fit is shown by the two lines. The apparent V_{max} of the oligochaete mitochondria was 76 pmol $O_2.s^{-1}.mg^{-1}$ protein, 28-fold lower than for rat liver mitochondria. The apparent K_m for ADP of the oligochaete mitochondria was 14 μmol.dm^{-3}, indicating a 3.6-fold higher affinity in comparison with rat liver mitochondria.

observed for 3 to 4 min. Subsequently, the KCN concentration was increased by step-wise titration of maximally 10 times 50 mm^3 of 4, 6, or 12 mmol.dm^{-3} KCN solution. A linear oxygen flux was then observed for periods of ca. 2 min. The dilution of mitochondrial concentration and the partial extrusion of previously titrated KCN were corrected for. The aqueous KCN solution contained 0.16 mmol.dm^{-3} EDTA, 20 mmol.dm^{-3} KH_2PO_4, 0.1% albumin and 12 mmol.dm^{-3} KCN.

RESULTS AND DISCUSSION

The respiratory control ratios (State 3/State 4 oxygen flux ratio) of the oligochaete mitochondrial preparations varied from 1.5 to 2.5.

Mitochondrial oxygen flux [nmol $O_2.s^{-1}.mg^{-1}$ protein] was approximately 30-fold lower in oligochaete mitochondria compared to rat liver mitochondria, both measured at 20 °C (Fig. 1). This difference can only partially be explained by a larger contamination of the mitochondrial fraction isolated from the oligochaetes in comparison with the more homogenous mitochondrial fraction isolated from rat liver (Rieger et al., 1990).

Cyanide inhibition of respiration was studied using hexokinase steady state coupled mitochondria which enables multiple titrations without the interference of ADP depletion. Liver mitochondria are much more sensitive to cyanide (Fig. 2). The affinity for oxygen of oligochaete mitochondria was higher, and the affinity for cyanide was lower than in rat liver mitochondria. Nevertheless, respiration of the oligochaete mitochondria cannot be considered to be cyanide insensitive as seen by the kinetics of the inhibition experiment (Fig. 2). This agrees with the observation of mitochondria isolated from euryoxic mussel tissues, if the artifact of D-mannitol oxidase based (cyanide insensitive) respiration (Zaba, 1983) is avoided (Burcham et al., 1984).

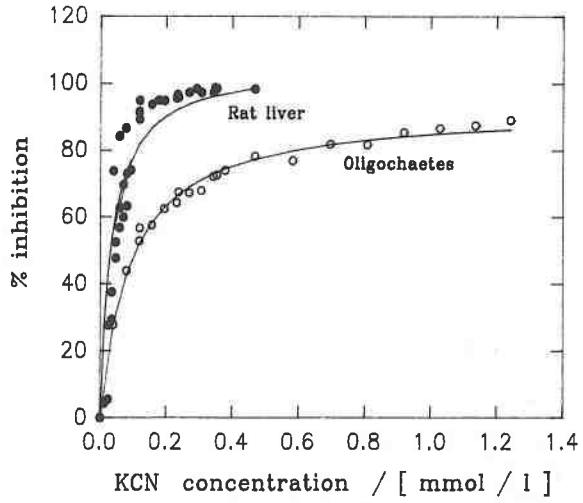

Fig. 2. KCN inhibition of hexokinase induced steady state coupled respiration: Comparison of oligochaete and rat liver mitochondria. The inhibition constant for KCN was 0.09 mmol.dm^{-3} for the oligochaete mitochondria. The hyperbolic fit for rat liver mitochondria did not describe the experiments accurately, but for comparison an inhibition constant of 0.03 mmol.dm^{-3} was calculated, indicating an approximately 3-fold affinity for KCN in rat mitochondria.

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