18 Efficiency of *Acanthamoeba castellanii* uncoupling protein in energy-dissipating processes

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Introduction

Uncoupling proteins (UCP) are widespread in the whole eukaryotic world. They were known from long time in animal and have been discovered more recently in plants [1], protozoan [2], and fungi [3].

UCPs are located in the inner mitochondrial membrane and their activity dissipates the proton electrochemical gradient built up by respiration and decreases ATP synthesis. The action of UCPs is to mediate free fatty acid (FFA)-cycling H^+ re-uptake driven by transmembrane electrical potential ($\Delta\Psi$, negative inside) and pH difference acidic outside [4]. UCPs are activated by FFA and are allosterically inhibited by purine nucleotides. For instance, addition of linoleic acid (LA) results in mitochondrial uncoupling revealed by an increase in state 4 respiration and a decrease in $\Delta\Psi$. On the other hand, addition of GTP which inhibits UCP and BSA which removes FFA cancels LA-induced respiration and restores $\Delta\Psi$.

In this work we showed that UCP of *Acanthamoeba castellanii* (AcUCP) can be activated by FFA and can efficiently divert energy from oxidative phosphorylation through a protonophoretic process and that alternative oxidase (AOX) and AcUCP have additive effect on energy conservation.

[1]Abbreviations: UCP, uncoupling protein; FFA, free fatty acids; $\Delta\Psi$, mitochondrial potential; LA, linoleic acid; AcUCP, uncoupling protein of *Acanthamoeba castellanii*; AOX, alternative oxidase; FCCP, carbonyl cyanide *p*-trifluoromethoxyphenylhydrazone

Materials and methods

Amoeba mitochondria were isolated and purified on a self-generating Percoll gradient (31%) as described before [5].

Oxygen uptake was measured polarographically using a Rank Bros. oxygen electrode in 2.7 ml of standard incubation medium (25 °C) containing: 120 mM KCl, 20 mM Tris-HCl pH 7.4, 3 mM KH_2PO_4, and 8 mM $MgCl_2$, with 1.5–1.7 mg of mitochondrial protein. Membrane potential of mitochondria was measured simultaneously with oxygen uptake using a tetraphenylphosphonium-specific electrode according to Kamo *et al.* [6]. All measurements were made in the presence of 10 mM succinate, 1.5 mM benzohydroxamate (BHAM, an inhibitor of alternative oxidase), 170 μM ATP, and 15 μM rotenone.

Results

Concentration dependency of the LA-induced stimulation of state 4 respiration LA-induced BHAM-resistant respiratory rate should represent part of total AcUCP activity. This activity was measured for various LA concentrations (from 0 to 24 μM) in FFA-depleted amoeba mitochondria. LA-induced respiratory rate increased with the increasing concentration of LA and 50% of maximal stimulation by LA ($S_{0.5}$) was reached at 8.1 ± 0.4 μM.

Coupling state of phosphorylating mitochondria In *A. castellanii* mitochondria, both AcUCP and AOX dissipate energy and therefore must lead to the same final effect i.e., decrease in oxidative phosphorylation efficiency. In order to estimate this effect, ADP/O ratio has been measured in four conditions in FFA-fully depleted amoeba mitochondria. In control conditions (+0.5% BSA, +1.5 mM BHAM) the ADP/O ratio measured with succinate + rotenone was 1.40 ± 0.03 (S.D., n = 6). When AcUCP was activated with a low LA concentration (3.7 μM), ADP/O decreased to 0.80 ± 0.05 (S.D., n = 4). When AOX was activated with 0.6 mM GMP, the ADP/O decreased to 0.96 ± 0.04 (S.D., n = 6). When both dissipating systems were activated, the ADP/O dropped to 0.63 ± 0.06 (S.D., n = 4) indicating combined effect of both dissipating pathways on the oxidative phosphorylation efficiency. The ADP/O measurements have been used to determine the contribution of each dissipating pathway in state 3 respiration according to the method used to calculate the AOX contribution in amoeba mitochondria [7]. The ADP/O ratios with 3.7 μM LA (0.80) and without LA (1.40) have been used to estimate the contribution of AcUCP activity (i.e., part of the cytochrome pathway activity that is finally dissipated, $V_{\text{cyt diss}}$) and the contribution of ATP synthase activity (i.e., part of the cytochrome pathway activity that is finally conserved into ATP, $V_{\text{cyt cons}}$) at

a given state 3 respiration rate (+ BHAM). As

$$V_{\text{cyt cons}} = V_3 \times \frac{(\text{ADP/O})_{+\text{LA}}}{(\text{ADP/O})_{-\text{LA}}} \tag{18.1}$$

and

$$V_{\text{cyt diss}} = V_3 - V_{\text{cyt cons}} \tag{18.2}$$

$V_{\text{cyt cons}}$ represents 57% and $V_{\text{cyt diss}}$ 43% of state 3 respiration at 3.7 μM LA. These calculations are valid only if several requirements are fulfilled. Nevertheless, they show how change in the cellular FFA concentration can decrease the efficiency of oxidative phosphorylation through an activation of AcUCP. The same type of calculations can be made for AOX and cytochrome pathway contributions in state 3 respiration (+ BSA). Taking the ADP/O ratio with GMP (0.96), the contribution of the cytochrome pathway represents 69% and the contribution of AOX 31% of state 3 respiration. As LA did not inhibit AOX activity in amoeba mitochondria, the cumulative effect of both dissipating pathways on oxidative phosphorylation can be observed but not distinguished. Taking ADP/O value in the presence of LA and GMP (0.63), Vcyt cons represents 45% and the total dissipating pathway-sustained respiration 55% of state 3 respiration. Thus, 55% of redox energy can be dissipated when both dissipating pathways are activated even for such a low LA concentration (3.7 μM).

Voltage dependence of electron flux in the respiratory chain If LA-induced respiration is only due to a proton recycling by AcUCP, it must correspond to a pure protonophoric effect of LA not distinguishable from the effect of other well known protonophores like carbonyl cyanide *p*-trifluoromethoxyphenylhydrazone (FCCP). Fig. 18.1 reports a relation between couples of $\Delta\Psi$ and respiration rate measurements in the presence of BHAM: (i) in state 4 + olig with increasing concentrations of FCCP (∘), (ii) in state 4 + olig with increasing concentrations of LA (▲), (iii) in state 3 with increasing concentrations of oligomycin (□). The maximal respiration in uncoupled state, in state 3, and in the presence of LA (22–33 μM) were not different. A set of conditions constituted a single force-flow relationship indicating that LA did not interact with the respiratory electron transport chain in amoeba mitochondria and had a pure protonophoric effect. Moreover, respiratory rates measured with saturating LA and FCCP were almost the same indicating that maximal electron flux was reached by activation of AcUCP.

Discussion

The close equality observed for maximal respiratory rate induced by FCCP, LA, and ADP means that the three systems (i.e., ATP synthesis, AcUCP and uncoupling by FCCP) have the same ability to consume H^+ electrochemical gradient and to lead

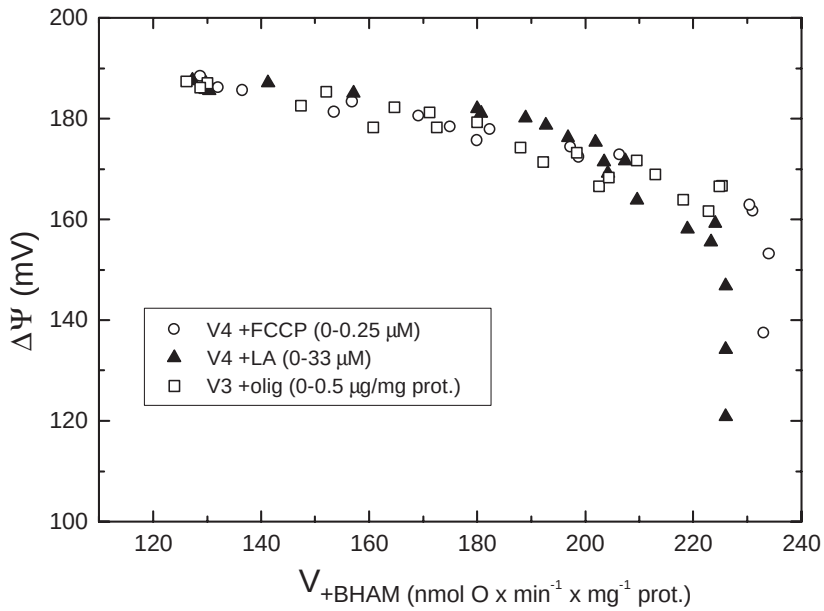

Fig. 18.1 Relation between ΔΨ and mitochondrial respiration rate in the presence of BHAM. All measurements were made in the presence of 10 mM succinate, 5 μM rotenone, 1.5 mM BHAM, and 170 μM ATP. State 4 (V4) was measured in the presence of 2 μg/mg of protein oligomycin. State 3 (V3) was reached by the addition of 2 mM ADP. (○), state 4 with increasing concentration of FCCP (0–0.25 μM); (▲) state 4 with increasing concentration of LA (0–33 μM); (□), state 3 with increasing concentration of oligomycin (0–0.5 μg/mg of protein).

to the maximal electron flux in the cytochrome pathway of the respiratory chain. These observations strongly support the view that in amoeba mitochondria LA did not only induce a mild uncoupling thruogh other carriers non-specialized in the FFA-induced H^+ recycling but also that a UCP-like carrier is actually implied in the LA effect. The close equality of LA-stimulated respiration and state 3 respiration also means that LA cannot induce important acceleration of state 3 respiration (as also shown in tomato mitochondria [8]) but it does not mean that AcUCP activity is not induced by LA addition. Indeed, if H^+ electrochemical gradient built by state 3 respiration in the presence of LA can be shared between oxidative phosphorylation and AcUCP activity, ADP/O must decrease in the presence of LA. Such decrease has been observed with as low LA concentration as 3.7 μM and has been used to determine the contribution of AcUCP activity in state 3 respiration. Contributions of AOX and of AOX plus AcUCP have been determined too. These calculations show that AOX can share electron flux with the cytochrome pathway during phosphorylating respiration and that in our conditions both dissipating pathways can dissipate together more than 50% of redox energy.

Modulation of the force (ΔΨ) either by phosphorylation potential (with oligomycin), by H^+ permeability (with FCCP) or by LA addition leads to the same mod-

ification of the flow (respiratory rate). These observations indicate that LA has only a protonophoric effect and does not act directly on the activity of the respiratory chain. It can be concluded that the presence of LA gives rise to a proton recycling only and that AcUCP activity can efficiently divert energy from oxidative phosphorylation in state 3.

The discovery of AcUCP in *A. castellanii* indicates that UCPs, as specialized proteins for FFA-linked H^+ recycling, emerged very early during phylogenesis and before the major radiation of phenotypic diversity in eukaryotes more than a thousand million years ago, and maybe even earlier after the acquisition of mitochondria in Eucarya. The presence of both UCP and AOX in *A. castellanii* also demonstrates that the appearance of intracellular organellar mitochondrial oxidative phosphorylation coupling was accompanied by the emergence not only of a protein carrier specialized in FFA-mediated H^+ recycling but also of an energy-dissipating oxidase that can modulate the tightness of coupling between respiration and ATP synthesis, thereby maintaining a balance between energy supply and demand in the cell.

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