Regulation of uncoupling protein activity in phosphorylating potato tuber mitochondria

Rachel Navet[a], Pierre Douette[a], Florence Puttine-Marique[a], Claudine M. Sluse-Goffart[a], Wieslawa Jarmuszkiewicz[b], Francis E. Sluse[a,*]

[a] *Laboratory of Bioenergetics, Department of Life Sciences, Institute of Chemistry B6c, University of Liège, Sart-Tilman, B-4000 Liège, Belgium*
[b] *Laboratory of Bioenergetics, Institute of Molecular Biology and Biotechnology, Adam Mickiewicz University, Fredry 10, 61-701 Poznan, Poland*

Received 21 June 2005; accepted 5 July 2005

Available online 21 July 2005

Edited by Robert Barouki

Abstract In isolated potato tuber mitochondria, palmitic acid (PA) can induce a H^+ leak inhibited by GTP in the phosphorylating (state 3) respiration but not in the resting (state 4) respiration. The PA-induced H^+ leak is constant when state 3 respiration is decreased by an inhibition of the succinate uptake with *n*-butyl malonate (*n*BM). We show that the efficiency of inhibition by GTP is decreased when state 3 respiration is progressively inhibited by antimycin A (AA) and is restored following subsequent addition of *n*BM. We propose that in phosphorylating potato tuber mitochondria, the redox state of ubiquinone, which can antagonistically be varied with AA and *n*BM, modulates inhibition of the PA-activated UCP-sustained H^+ leak by GTP.

Keywords: Plant mitochondria; Uncoupling protein activity; Inhibition by GTP; Ubiquinone

1. Introduction

Uncoupling proteins (UCPs) reside in the mitochondrial inner membrane and catalyze a H^+ conductance that dissipates the H^+ electrochemical gradient built up by the respiratory chain. As such, UCPs share the protonmotive force with ATP synthase (H^+ partitioning) during phosphorylating (state 3) respiration and partially uncouple electron transport from ATP synthesis [1].

Until the discovery of a plant UCP in potato tubers [2], UCP1 present in brown adipocytes was believed to be a late evolutionary acquisition required for adaptative thermogenesis [3]. UCP homologues are found not only in animal and plant kingdoms but also in fungi and primitive organisms [4]. Since expression of UCP homologues in non-thermogenic tissues and unicellulars (lower size limits) excludes an involvement in thermogenesis [5,6], their physiological role and molecular function are still debated. A consensus role for UCP homologues could be a protection against free radical production by decreasing the reduced state of the mitochondrial respiratory chain as a consequence of energy-dissipation [6–8].

The catalytic activity and regulation of UCP homologues are also poorly understood. On one side, the activation of UCP homologues by free fatty acids (FFA), such as palmitic acid (PA), is currently debated. A direct effect of FFA is well documented but also it seems that superoxide anion could activate UCPs in plants [9] and animals [10] through lipid peroxidation products, such as 4-hydroxy-2-nonenal (HNE pathway) [11,12]. On the other side, the inhibition of H^+ conductance by purine nucleotides (PN) is considered as a diagnostic of UCP activity [13]. However, several UCP homologues are poorly sensitive to PN in isolated mitochondria [3], while they are PN-inhibited in reconstituted systems [14]. So far, only the "superoxide-activated" state of UCP homologues has been claimed to be inhibited by PN during state 4 respiration in isolated mitochondria [8–12].

In the present study, we show that the PA-induced H^+ leak occurs in isolated potato tuber mitochondria depleted of endogenous FFA. The H^+ leak is observed during phosphorylating respiration with succinate as oxidizable substrate (+ rotenone) and remains constant when the respiratory rate is decreased by *n*-butyl malonate (*n*BM) that inhibits a succinate uptake. Moreover, we show that the inhibition by GTP can be cancelled by antimycin A (AA) and subsequently restored following *n*BM addition. A relationship between the efficiency of inhibition by GTP and the redox state of ubiquinone (Q) is proposed.

2. Material and methods

2.1. Mitochondrial isolation

Potato (*Solanum tuberosum*) tubers were purchased from the local supermarket. Mitochondria were isolated from a single batch of potato tubers as described earlier [15]. Mitochondria fully depleted of endogenous FFA were obtained by adding 0.5% FFA-free bovine serum albumin in the isolation media. The depletion was systematically assessed by measuring the effect of bovine serum albumin on the FFA-induced respiration in state 4 [1]. Mitochondrial protein concentration was determined using the biuret method.

2.2. Assay procedures

Oxygen uptake was measured polarographically using a Clark-type electrode (Hansatech) in 1.3 ml of standard incubation medium (sucrose, 125 mM; KCl, 65 mM; $MgCl_2$; 1 mM; KH_2PO_4, 2.5 mM; and

*Corresponding author. Fax: +32 4 366 2878.
E-mail address: f.sluse@ulg.ac.be (F.E. Sluse).

Abbreviations: AA, antimycin A; *n*BM, *n*-butyl malonate; FCCP, carbonyl cyanide *p*-trifluoromethoxyphenyl-hydrazone; FFA, free fatty acids; PA, palmitic acid; PN, purine nucleotides; UCP, uncoupling protein; StUCP, uncoupling protein of *Solanum tuberosum*; Q, ubiquinone; Q_R/Q_T, reduction level of ubiquinone (reduced Q versus total Q); V3, respiratory rate in phosphorylating state 3; V4, respiratory rate in resting state 4; $\Delta\Psi$, mitochondrial membrane potential; $\Delta\Psi 3$, state 3 membrane potential; $\Delta\Psi 4$, state 4 membrane potential

0014-5793/$30.00
doi:10.1016/j.febslet.2005.07.008

HEPES 10 mM, pH 7.2) supplemented with 0.5 mg of mitochondrial proteins at 25 °C. Succinate 10 mM (+ rotenone 5 μM) was used as an oxidizable substrate. To inhibit alternative oxidase present in plant mitochondria, benzohydroxamic acid (2.3 mM) was added. The membrane potential (ΔΨ) was assayed using a tetraphenylphosphonium (TPP^+)-specific electrode according to Kamo et al. [16] as described in [17]. The ADP/O ratio was calculated using the total amount of oxygen consumed during state 3 respiration (V3) induced by a pulse of ADP (230 nmol). Measurements of ΔΨ allowed fine control of the duration of V3. The ADP/O ratio and V3 were used to determine the rate of ADP phosphorylation (ADP/O × V3). The mitochondrial content and redox state of Q (the reduced Q versus the total Q, Q_R/Q_T in %) were determined in steady-state 3 respiration by extraction technique followed by HPLC measurements [18]. Commercially available Q10 was used to calibrate the HPLC peaks of potato tuber mitochondrial Q10. All chemicals were of the highest purity grade.

3. Results

3.1. Effect of PA on respiratory rates and coupling parameters

In order to identify a UCP activity in potato tuber mitochondria, resting (state 4) and phosphorylating (state 3) respiratory rates (V4 and V3, respectively) as well as the ADP/O ratio were measured in the absence or presence of PA with succinate as respiratory substrate (+ rotenone). As shown in Fig. 1 and Table 1, V4 was increased by 7.5 μM PA, while V3 remained unaffected. Moreover, a drop in state 4 membrane potential (ΔΨ4) but not in state 3 membrane potential (ΔΨ3) was observed following PA addition. The respiratory control and ADP/O ratios were lowered by PA, indicating that PA induces the H^+ leak in state 3 as well as in state 4. Similar effect on respiratory rates, ΔΨ and the ADP/O was observed in the presence of 0.20 μM carbonyl cyanide *p*-trifluoromethoxyphenyl-hydrazone (FCCP) (Table 1). The concentration of the uncoupler that gives similar effect on V4 compared to 7.5 μM PA was chosen from the V4-FCCP (or V4-PA) concentration dependency presented in Fig. 2A. As shown in Table 1, addition of GTP (2 mM) in the presence of PA or FCCP did not modify the respiratory rate and ΔΨ values. However, only in the case of the PA-induced uncoupling, the GTP addition resulted in recovery of the ADP/O value to a control value observed in the absence of uncouplers (Fig. 1, Table 1). These results suggest that the PA-induced GTP-inhibited H^+ leak in state 3 could be mediated by uncoupling protein of *Solanum tuberosum* (StUCP) activity that diverts energy from ATP synthesis during phosphorylating respiration in a fatty acid-dependent way. Similar observations were made for the FFA-induced GTP-inhibited H^+ leak in phosphorylating protist and mammalian mitochondria [17,19].

3.2. Effect of PA on voltage dependence of electron flux in respiratory chain of potato tuber mitochondria

State 4 respiration measurements were performed in the presence of 10 μM atractylate in order to inhibit a non-specific FFA-induced uncoupling through the ADP/ATP carrier. State 4 respiration was increased by increasing concentrations of PA, reaching its maximal value at 15 μM PA (Fig. 2A). The PA-induced respiration contributed up to 160 nmol $O \times min^{-1} \times$ mg $prot^{-1}$ to the total V4. A half-maximal stimulation was reached at 5 μM PA. For a comparison, a FCCP concentration-dependent stimulation of V4 is also shown in Fig. 2A.

If PA-induced respiration is only due to a proton recycling by StUCP, it must correspond to a pure protonophoretic effect (i.e., effect related only to a H^+ conductance through the inner mitochondrial membrane) of PA not distinguishable from the effect of other well known protonophores, like FCCP. In order to demonstrate the protonophoretic action of PA, V4 and ΔΨ4

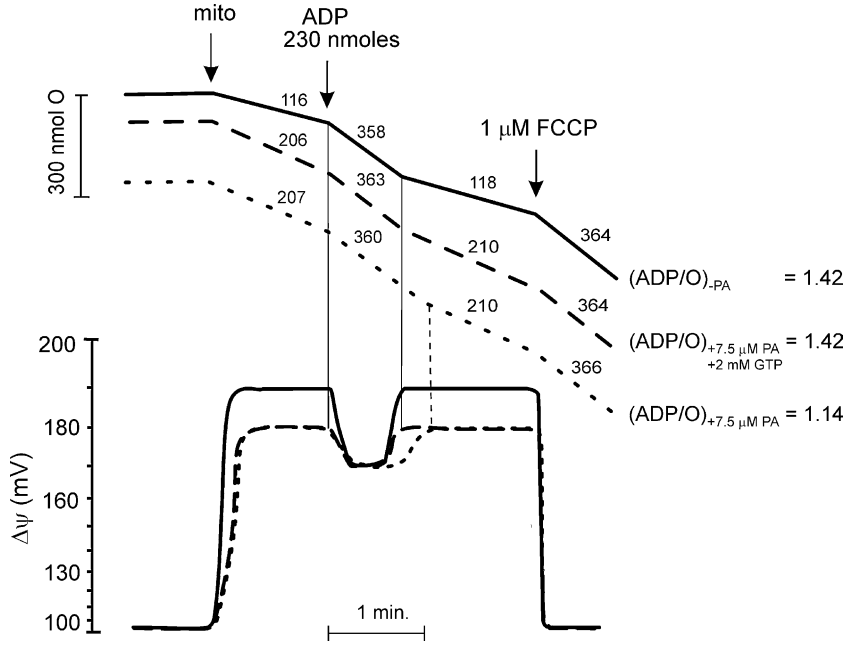

Fig. 1. The effect of PA on coupling parameters of *S. tuberosum* tuber mitochondria. Mitochondria (mito) were incubated as described under Section 2 in the absence (solid line) or presence of 7.5 μM PA (dashed or dotted lines, plus or minus 2 mM GTP, respectively). Examples of oxygen uptake and ΔΨ measurements are shown. Measurements of ΔΨ allowed a fine control of the duration of V3 (vertical lines) and the ADP/O ratio calculations. After the ADP pulse, respiration was uncoupled and ΔΨ was collapsed by 1 μM FCCP. Numbers on the traces refer to oxygen consumption rates in nmol $O \times min^{-1} \times mg^{-1}$ prot.