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Regulation of uncoupling protein activity in phosphorylating potato tuber mitochondria

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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

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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

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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

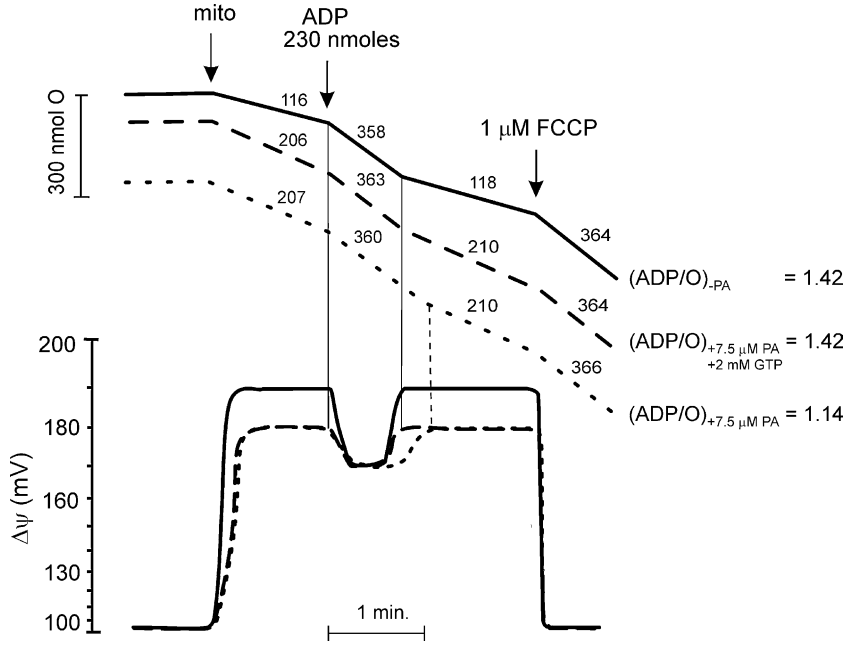

Fig. 1. The effect of PA on coupling parameters of *S. tuberosum* tuber mitochondria. Mitochondria (mito) were incubated as described under Section 2 in the absence (solid line) or presence of 7.5 μM PA (dashed or dotted lines, plus or minus 2 mM GTP, respectively). Examples of oxygen uptake and ΔΨ measurements 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.

Table 1
Influence of various incubation conditions on respiratory rates and ΔΨ during resting and phosphorylating respirations and on the ADP/O ratio

Conditions	V3	ΔΨ3	V4	ΔΨ4	ADP/O
Control	360 ± 10	171 ± 2	115 ± 9	191 ± 3	1.423 ± 0.019
+2 mM GTP	365 ± 10	169 ± 3	116 ± 11	191 ± 4	1.419 ± 0.022
+7.5 μM PA	356 ± 13	170 ± 4	200 ± 10	179 ± 4	1.138 ± 0.026
+7.5 μM PA + 2 mM GTP	362 ± 11	168 ± 3	203 ± 8	180 ± 3	1.420 ± 0.021
+0.20 μM FCCP	366 ± 14	172 ± 4	195 ± 9	182 ± 5	1.132 ± 0.028
+0.20 μM FCCP + 2 mM GTP	360 ± 12	172 ± 4	192 ± 10	183 ± 2	1.130 ± 0.026

Assay conditions were as described in Section 2. Values of V4 after the ADP pulse are shown. Mean values ± S.D. are given for three determinations within one representative mitochondrial preparation. Respiratory rates are in nmol $O \times min^{-1} \times mg^{-1}$ prot, ΔΨ values are in mV.

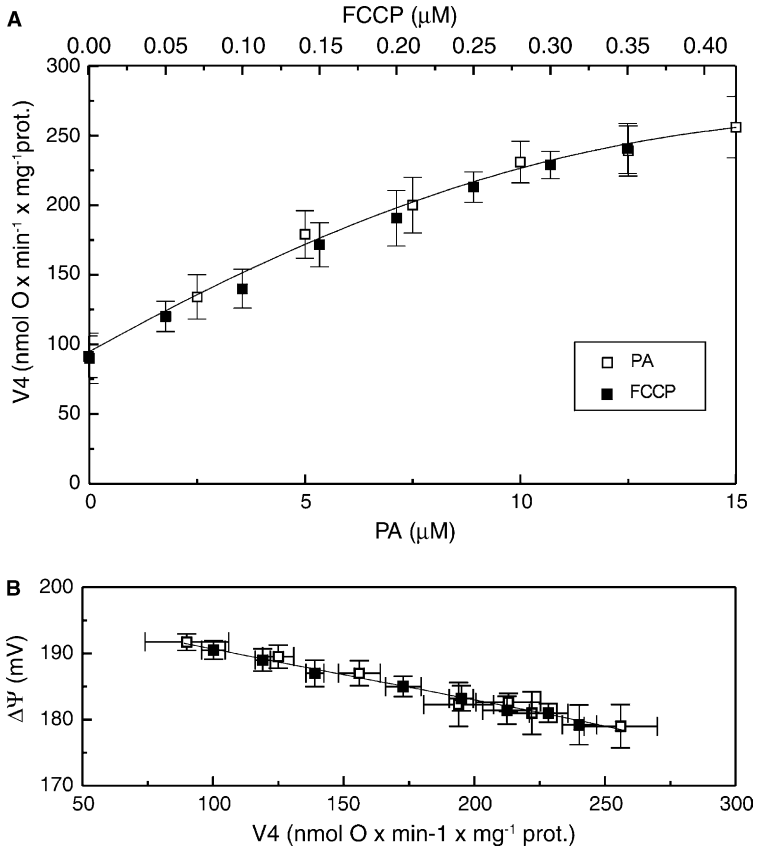

Fig. 2. The effect of PA on state 4 respiratory rates (V4) and membrane potential (ΔΨ). Mitochondria were incubated in the presence of 10 μM atractylate. In A, V4 is plotted versus PA concentration. Mean values ± S.D. are given for three different mitochondrial preparations ($n = 3$). In B, relationship between ΔΨ and V4: (□), state 4 in the presence of increasing concentration of PA (0–15 μM); (■) state 4 in the presence of increasing concentrations of FCCP (0–0.35 μM). The data are the means ± S.D. of four independent experiments within two mitochondrial preparations ($n = 4$).

were determined with increasing concentration of either PA or FCCP. As shown in Fig. 2B, both conditions led to a single force-flux relationship indicating that PA exerts the same effect on the rate of oxygen consumption as the protonophore FCCP. This indicates a pure protonophoretic effect of PA that does not directly interact with the respiratory chain (no slip). The obtained results are similar to those found in protist mitochondria with linoleic acid [4].

3.3. Inhibition of the PA-induced H^+ leak by GTP in state 3 respiration

Amplitude of the FFA-induced H^+ leak can be determined in state 3 by the decrease of ADP/O ratio (thereby phosphorylation rate) when V3 is titrated with various inhibitors [17,19]. In order to describe how the PA-induced mitochondrial uncoupling (that can be attributed to StUCP activity) changes with V3 variations, the rate of Q-reducing (respiratory substrate oxidation) and QH_2-oxidizing (cytochrome pathway activity) pathways was gradually decreased by *n*BM (an inhibitor of succinate uptake) or antimycin A (AA) (an inhibitor of complex III), respectively. Furthermore, in order to study the sensitivity of the PA-induced H^+ leak to GTP, pair measurements of ADP/O ratios and V3 in the presence or absence of 7.5 μM PA, plus or minus 2 mM GTP, were performed for decreasing state 3 respiration (Fig. 3). The titration range of V3 by *n*BM (0–30 mM) or AA (0–8.5 nM) was such that ΔΨ3 was constant (170 ± 2 mV, S.D., $n = 32$) in the absence or presence of PA (plus or minus GTP) (Fig. 3A, all conditions).

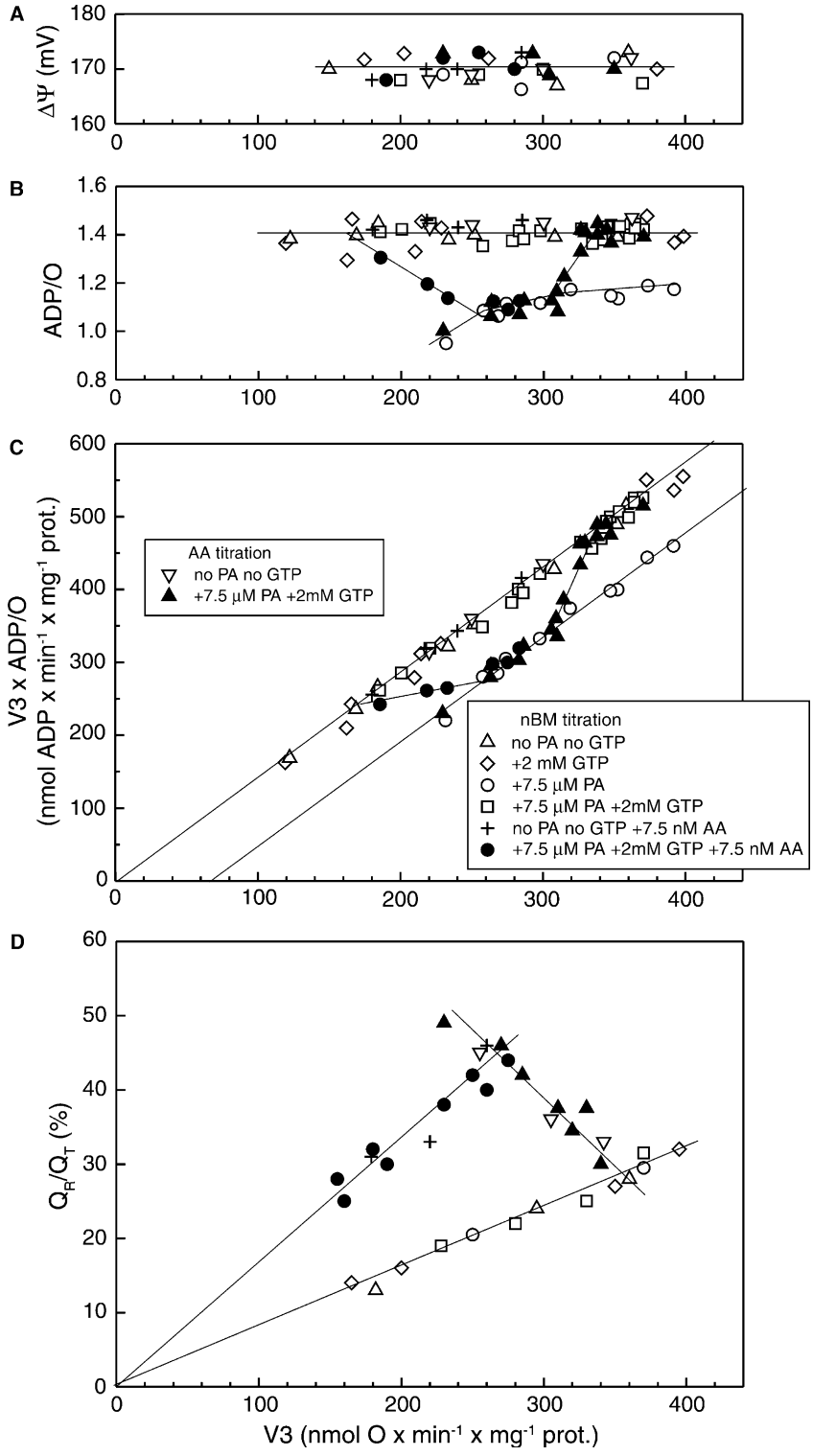

Fig. 3. Inhibitory effect of GTP on the respiration sustained by the PA-induced H^+ leak (StUCP activity). The relationships between ΔΨ (A), ADP/O ratio (B), rate of ADP phosphorylation (C) as well as Q reduction level (D) and the rate of oxygen consumption in state 3 respiration (V3) are presented for nine conditions of V3 titration reported in insets of the part C. V3 is gradually decreased by increasing concentrations of inhibitors, *n*BM (0–30 mM) or AA (0–8.5 nM). Data deal with nine mitochondrial preparations from a single batch of potato tubers. Straight lines are least square regression lines.

As shown in Fig. 3B, a constant ADP/O ratio was observed in all control conditions, i.e., when the Q-reducing pathway was titrated with *n*BM either in the absence of PA (minus or plus GTP, △, ◆), or in the absence of PA (minus GTP, plus 7.5 nM AA, +), or in the presence of both PA and GTP (□) as well as when the QH_2-oxidizing pathway was titrated with AA (∇) in the absence of PA and GTP. The mean ADP/O value was 1.43 ± 0.03 (S.D., $n = 44$). In the presence of PA alone,

the measured ADP/O ratio was lowered to 1.2 and then declined with decreasing V3 (*n*BM titration, ○). Thus, lowering the electron flux amplified the PA-induced decrease in the ADP/O ratio.

Titration of V3 led to a linear relationship between the rate of ADP phosphorylation (V3 × ADP/O) and corresponding V3 (Fig. 3C) in all control conditions (see above) where the ADP/O constancy was found (Fig. 3B). In these conditions, the straight line intersected the abscissa axis close to the origin (the value of intercept was 4.05 ± 3.54, S.D., $n = 44$) as a consequence of the constancy of the ADP/O ratio. This indicates that no basal H^+ leak occurs during state 3 respiration in potato tuber mitochondria. However, addition of PA during *n*BM titration (no GTP, no AA, ○) provoked a shift of the linear relationship between ADP phosphorylation rate and V3 to the right, while the slope of this linear relationship was maintained when compared to control conditions (1.44 ± 0.06, S.D., $n = 10$ and 1.43 ± 0.02, S.D., $n = 44$, respectively). This observation strongly indicates that the intrinsic ADP/O ratio is not affected by stoichiometric modification of both respiratory complexes and ATP synthase in studied mitochondria. In the presence of PA (no GTP, no AA, ○), the respiration sustained by the PA-induced H^+ leak (that is value of shifted to the right intercept with abscissa axis) was 68.3 ± 4.4 (S.D.) nmol $O \times min^{-1} \times mg^{-1}$ prot., $n = 10$.

As shown in Fig. 3D, the Q redox state followed a linear relationship with decreasing V3 titrated with *n*BM (no AA) in the absence or presence of PA (minus or plus GTP) (Fig. 3D, △, ◇, ○, □). This clearly demonstrates that the cytochrome pathway activity is not affected by PA and GTP since *n*BM acts upstream of the cytochrome pathway. The Q reduction level ranged between 32% and 13% in these titrations.

When the Q-reducing pathway activity was decreased (with *n*BM), the ADP/O ratios (Fig. 3B) and the relationship between the ADP phosphorylation rate versus V3 (Fig. 3C) obtained in the presence of PA and GTP (□), when compared to plus PA no GTP conditions (○), reveal the inhibitory effect of GTP on the PA-induced H^+ leak, which thereby can be attributed to the StUCP activity. In order to study if the sensitivity of StUCP activity to GTP depends on the Q redox state, the PA-induced H^+ leak (in the presence of GTP) was followed with the decreasing QH_2-oxidizing pathway activity (i.e., with the increasing Q reduction level). Namely, titration of state 3 respiration was performed with AA in the presence of 7.5 μM PA and 2 mM GTP (Fig. 3, ▲). The progressive decrease of V3 by AA (up to 8.5 nM) led to: (i) a non-linear relationship between the rate of ADP phosphorylation (V3 × ADP/O) and V3 (Fig. 3C, ▲) that moved from the control relationship (the straight line coming from the origin) to the PA-induced linear relationship (Fig. 3C, ○); (ii) a decrease in the ADP/O ratio (Fig. 3B, ▲) that at 7.5 nM AA dropped to the ADP/O value obtained in the presence of PA (no GTP) (Fig. 3B, ○), and (iii) no change in ΔΨ3 (Fig. 3A, ▲). However, The Q reduction level increased from 30% to 50% when V3 was titrated from 360 to 230 nmol $O \times min^{-1} \times mg^{-1}$prot. by AA (Fig. 3D, ▲). Afterwards, another titration at a fixed (7.5 nm) AA concentration with increasing concentration of *n*BM (up to 30 mM) was also made in the presence of 7.5 μM PA and 2 mM GTP (Fig. 3, ●). When state 3 respiration, first inhibited by 7.5 nM AA (till around 280 nmol $O \times min^{-1} mg^{-1}$ prot), was further progressively decreased by *n*BM, reverse changes (when ● titration is compared to ▲ titration, Fig. 3) were observed for the ADP/O, ADP/O × V3 and Q_R/Q_T versus V3 relationships. This set of observations suggest that in phosphorylating potato tuber mitochondria, PA induces a StUCP-mediated H^+ leak and inhibition of this leak by GTP is controlled by the Q redox state.

4. Discussion

In the present study, we demonstrate that in potato tuber mitochondria, PA induces a H^+ leak under phosphorylating conditions and decreases the yield of oxidative phosphorylation through a pure protonophoretic process. Moreover, the contribution of PA in the state 3 respiration was constant and independent of the Q redox state as revealed by the *n*BM titration of V3 in the absence or presence of PA (Fig. 3, △, ○).

Inhibition of the PA-induced H^+ leak by GTP occurs in state 3 and indicates a contribution of StUCP in the phenomenon, excluding non-specific FFA-induced uncoupling through other mitochondrial carriers [20–23]. Moreover, inhibition by GTP can be progressively cancelled by increasing concentration of AA and further restored by subsequent titration with increasing concentration of *n*BM. Regulation of GTP inhibition of StUCP activity by Q redox state is strongly supported by the reverse effects of inhibitors that modify Q_R/Q_T. Indeed, AA inhibits the Q-oxidizing pathway (complex III), while *n*BM inhibits the Q-reducing pathway (succinate uptake). It must be pointed out that in phosphorylating potato tuber mitochondria, inhibition of the PA-induced H^+ leak by GTP is revealed only below 40% of Q_R/Q_T and a transition of GTP inhibitory efficiency (Fig. 3B and C) occurs in a narrow range of the Q redox state (32–39%) that is reversible with AA (from 32% to 39%) and with *n*BM (from 39% to 32%) (Fig. 3D). This observation could explain an inefficiency of GTP to inhibit the PA-induced H^+ leak in state 4 due to a very high reduced state of Q (around 70%). Such conclusion has been also suggested for rat muscle [17] and *Acanthamoeba castellanii* [19] mitochondria, where decreasing of the Q redox state during phosphorylating respiration allows the progressive inhibition of the FFA-induced H^+ leak by GTP, while for non-inhibited state 3, insensitivity to GTP is observed. It is noteworthy that in these mitochondria, the Q redox state reaches around 70% in non-inhibited state 3 respiration that is as high as Q_R/Q_T measured in potato tuber mitochondria in state 4. This could explain the inhibitory inefficiency of GTP on the FFA-induced H^+ leak in mammalian and protist mitochondria respiring in non-inhibited state 3, in contrast with the immediate GTP effect that occurs in potato tuber mitochondria. Moreover, the sensitivity of the FFA-induced H^+ leak to GTP modulated by the Q redox state occurs at Q_R/Q_T below 64% in muscle mitochondria, 55% in protist mitochondria and 39% in potato tuber mitochondria at V3 of 130, 220 and 260 nmol $O \times min^{-1} \times mg^{-1}$ prot, respectively. This likely illustrates differences in a membrane Q content and/or respiratory chain electron flux capacity related to the phylogenetic origin of mitochondria. Anyway, the existence of Q redox state-dependent sensitivity of the FFA-induced H^+ leak to PN in phosphorylating mitochondria of protists, plants and mammals suggests that this regulatory mechanism could take place in the whole eukaryotic world.

The molecular mechanism underlying the regulation by Q redox state has still to be elucidated. It may be hypothesized that reduced Q could decrease the affinity of UCP binding site for PN. Nevertheless, this new regulatory parameter enlightens on the most probable physiological role of UCP homologues, namely the control of energy metabolism balance of the cell. The UCP-sustained H^+ leak can only be inhibited when Q reduction level is low, corresponding to a low availability in oxidizable substrates and a high ATP demand, in order to preserve ATP synthesis efficiency. Contrarily, the high reduced state of Q occurring at high substrate supply or low ATP demand disables for PN inhibition, leading to activation of UCP that consequently decreases the production of harmful oxygen free radicals.

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References

[1] Jarmuszkiewicz, W., Almeida, A.M., Sluse-Goffart, C.M., Sluse, F.E. and Vercesi, A.E. (1998) Linoleic acid-induced activity of plant uncoupling mitochondrial protein in purified tomato fruit mitochondria during resting, phosphorylating, and progressively uncoupled respiration. J. Biol. Chem. 273, 34882–34886.

[2] Vercesi, A.E., Martins, I.S., Silva, M.A.P., Leite, H.M.F., Cuccovia, I.M. and Chaimovich, H. (1995) PUMPing plants. Nature 375, 24.

[3] Klingenberg, M. (1990) Mechanism and evolution of the uncoupling protein of brown adipose tissue. Trends Biochem. Sci. 15, 108–112.

[4] Sluse, F.E. and Jarmuszkiewicz, W. (2002) Uncoupling proteins outside the animal and plant kingdoms: functional and evolutionary aspects. FEBS Lett. 510, 117–120.

[5] Jarmuszkiewicz, W., Sluse-Goffart, C.M., Vercesi, A.E. and Sluse, F.E. (2001) Alternative oxidase and uncoupling protein: thermogenesis versus cell energy balance. Biosci. Rep. 21, 213–222.

[6] Skulachev, V.P. (1996) Role of uncoupled and non-coupled oxidations in maintenance of safety low levels of oxygen and its one-electron reductants Q. Rev. Biophys. 29, 9895–9901.

[7] Popov, V.N. (2003) Possible role of free oxidation processes in regulation of reactive oxygen species production in plant mitochondria. Biochem. Soc. Trans. 31 (6), 13–16.

[8] Brand, M.D., Affourtit, C., Esteves, T.C., Green, K., Lambert, A.J., Miwa, S., Pakay, J.L. and Parker, N. (2004) Mitochondrial superoxide: production, biological effects, and activation of uncoupling proteins. Free Rad. Biol. Med. 37, 755–767.

[9] Considine, M.J., Goodman, M., Echtay, K.S., Laloi, M., Whelan, J., Brand, M.D. and Sweetlove, L.J. (2003) Superoxide stimulates a proton leak in potato mitochondria that is related to the activity of uncoupling protein. J. Biol. Chem. 278, 22298–22302.

[10] Echtay, K.S., Roussel, D., St-Pierre, J., Jekabsons, M.B., Cadenas, S., Stuart, J.A., Harper, J.A., Roebuck, S.J., Morrison, A., Pickering, S., Clapham, J.C. and Brand, M.D. (2002) Superoxide activates mitochondrial uncoupling proteins. Nature 415, 96–99.

[11] Echtay, K.S., Esteves, T.C., Pakay, J.L., Jekabsons, M.B., Lambert, A.J., Portero-Otin, M., Pamplona, R., Vidal-Puig, A.J., Wang, S., Roebuck, S.J. and Brand, M.D. (2003) A signaling role for 4-hydroxy-2-nonenal in regulation of mitochondrial uncoupling. EMBO J. 22, 4103–4110.

[12] Smith, A.M.O., Ratcliffe, R.G. and Sweetlove, L.J. (2004) Activation and function of mitochondrial uncoupling protein in plants. J. Biol. Chem. 279, 51944–51952.

[13] Klingenberg, M. and Echtay, K.S. (2001) Uncoupling proteins: the issues from a biochemist point of view. Biochim. Biophys. Acta 1504, 128–143.

[14] Zackova, M. and Jezek, P. (2002) Reconstitution of novel mitochondrial uncoupling proteins UCP2 and UCP3. Biosci. Rep. 22, 33–46.

[15] Beavis, A.D. and Vercesi, A.E. (1992) Anion uniport in plant mitochondria is mediated by a Mg(2+)-insensitive inner membrane anion channel. J. Biol. Chem. 267, 3079–3087.

[16] Kamo, N., Muratsugu, M., Hongoh, R. and Kobatake, Y. (1979) Membrane potential of mitochondria measured with an electrode sensitive to tetraphenyl phosphonium and relationship between proton electrochemical and phosphorylation potential, in steady state. J. Membr. Biol. 49, 105–121.

[17] Jarmuszkiewicz, W., Navet, R., Alberici, L.C., Douette, P., Sluse-Goffart, C.M., Sluse, F.E. and Vercesi, A.E. (2004) Redox state of endogenous coenzyme Q modulates the inhibition of linoleic acid-induced uncoupling by guanosine triphosphate in isolated skeletal muscle mitochondria. J. Bioenerg. Biomembr. 36, 493–502.

[18] Van den Bergen, C.W., Wagner, A.M., Krab, K. and Moore, A.L. (1994) The relationship between electron flux and the redox poise of the quinone pool in plant mitochondria. Eur. J. Biochem. 226, 1071–1078.

[19] Jarmuszkiewicz, W., Czarna, M., Swida, A., Antos, N., Sluse-Goffart, C.M. and Sluse, F.E. (2005) In phosphorylating *Acanthamoeba castellanii* mitochondria the sensitivity of uncoupling protein activity to GTP depends on the redox state of quinone. J. Bioenerg. Biomembr. 37, 97–107.

[20] Andreyev, A.Y., Bondareva, T.O., Dedukhova, V.I., Mokhova, E.N., Skulachev, V.P., Tsofina, L.M., Volkov, N.I. and Vygodina, T.V. (1989) The ATP/ADP antiporter is involved in the uncoupling effect of fatty acids on mitochondria. Eur. J. Biochem. 182, 585–592.

[21] Samartsev, V.N., Mokhova, E.N. and Skulachev, V.P. (1997) The pH-dependent reciprocal changes in contribution of ADP/ATP antiporter and aspartate/glutamate antiporter to the fatty acid-induced uncoupling. FEBS Lett. 412, 179–182.

[22] Wieckowski, M.R. and Wojtczak, L. (1997) Involvement of the dicarboxylate carrier in the protonophoric action of long-chain fatty acids in mitochondria. Biochem. Biophys. Res. Commun. 232, 414–417.

[23] Zackova, M., Kramer, R. and Jezek, P. (2000) Interaction of mitochondrial phosphate carrier with fatty acids and hydrophobic phosphate analogs. Int. J. Biochem. Cell Biol. 32, 499–508.