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Active proton leak in mitochondria: A new way to regulate substrate oxidation

Arnaud Mourier, Anne Devin, Michel Rigoulet *

Université Bordeaux 2, 1 rue Camille Saint Saëns, 33077 Bordeaux cedex, France
Institute of Biochemistry and Genetics of the Cell (IBGC) du CNRS, 1 rue Camille Saint Saëns, 33077 Bordeaux cedex, France

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ABSTRACT

The main function of mitochondria is energy transduction, from substrate oxidation to the free energy of ATP synthesis, through oxidative phosphorylation. For physiological reasons, the degree of coupling between these two processes must be modulated in order to adapt redox potential and ATP turnover to cellular needs. Such a modulation leads to energy wastage. To this day, two energy wastage mechanisms have been described: the membrane passive proton conductance (proton leak) and the decrease in the coupling efficiency between electrons transfer and proton extrusion at the proton pumps level (redox or proton slipping). In this paper, we describe a new energy wastage mechanism of interest. We show that in isolated yeast mitochondria, the membrane proton conductance is strictly dependent on the external dehydrogenases activity. An increase in their activity leads to an increase in the membrane proton conductance. This proton permeability is independent of the respiratory chain and ATP synthase proton pumps. We propose to name this new mechanism "active proton leak." Such a mechanism could allow a wide modulation of substrate oxidation in response to cellular redox constraints.

1. Introduction

Mitochondria are commonly known as the cell's powerhouse. Their inner membrane contains all the proteins involved in oxidative phosphorylation i.e. the transformation of redox energy into phosphate potential (that is the free energy of ATP synthesis) [1]. Historically, the respiratory chain has been defined as an ensemble of complexes (I, II, III, and IV), some of which (I, III and IV) couple redox reaction to proton extrusion. Such complexes are called coupling sites. Many studies have been realized on mitochondria isolated from the yeast *Saccharomyces cerevisiae*, at both structural and functional levels. The yeast *S. cerevisiae* mitochondria are somewhat peculiar since they do not exhibit a complex I but harbor a number of external and internal dehydrogenases [2] in the inner mitochondrial membrane,which give their electrons to complex III via the quinones pool (see Scheme 1). *In situ*, multiple substrates are available at the same time to the respiratory chain, and functional studies from our laboratory have shown that electrons coming from certain dehydrogenases have the right of way on electrons coming from other dehydrogenases [3,4]. These functional studies were conducted in non-phosphorylating respiratory conditions (state 4).

The mitochondrial respiratory chain couples substrate oxidation to proton extrusion – through proton pumps – that generates a proton motive force (Δp) across the mitochondrial inner membrane [1]. Substrate oxidation leads to electron transfer through the respiratory chain, with the terminal electron acceptor being oxygen. The respiratory flux thus reflects the substrate oxidation flux. Under non-phosphorylating conditions, the proton motive force is not consumed by the phosphorylating systems, i.e., ATP synthase, ATP/ADP and Pi/H^+ carriers, and reaches its highest value [5,6]. At such a steady state, the respiratory flux and, consequently, the proton extrusion compensate the inner membrane proton permeability which itself only depends on the proton motive force for a considered membrane. Thus for a constant number of coupling sites, the non-phosphorylating respiratory flux is expected to be constant whatever the oxidized substrate. While investigating the respiratory chain electron competition processes, we observed that the non-phosphorylating respiratory flux could increase by 40%, depending on the respiratory substrate(s). This is an unusual observation, since, in *S. cerevisiae*, all the considered dehydrogenases give their electrons to the quinone pool and the number of coupling sites consequently is constant. Under these conditions, any change in the respiratory rate would reflect a change in the inner membrane proton permeability, i.e., proton leak [7–13] or a decrease in the proton pump efficiencies, i.e., redox slipping [14–16].

Our data unravel the origin of this energy wastage. We show that the observed increase in respiratory rate is associated with a decrease in proton motive force. The relationship between these two parameters is similar to the one obtained with a protonophore. Furthermore, the decrease in proton motive force is strictly proportional to the activity of the membrane dehydrogenases and independent of the proton pumps. In conclusion, we show that the membrane dehydrogenases are able to generate what we propose to call an "active proton leak."

* Corresponding author. IBGC du CNRS, Bioenergetics laboratory, 1 rue Camille Saint Saëns, 33077 Bordeaux cedex, France. Tel./fax: +33556999040.
E-mail address: michel.rigoulet@ibgc.u-bordeaux2.fr (M. Rigoulet).

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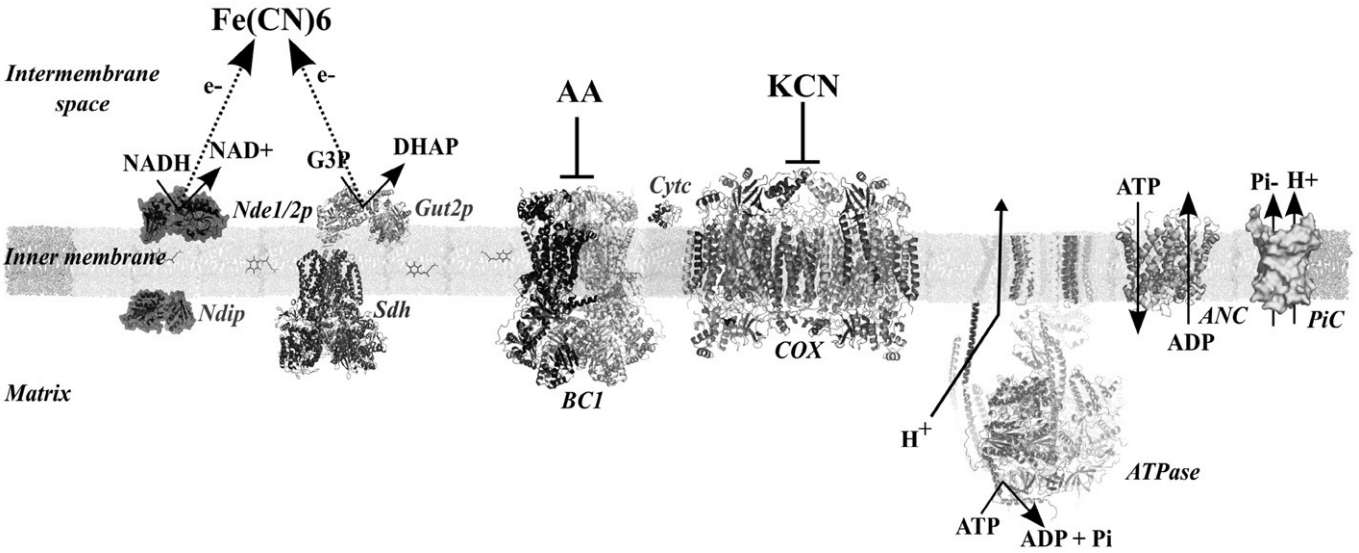

Scheme 1. To determine the effect of the dehydrogenase activities on the proton motive force established by the F1–F0 ATPase, the respiratory chain complexes III and IV were inhibited with antimycin A (AA) and KCN, respectively. In these conditions, ferricyanide ($Fe(CN)_6$) (non-permeant electron acceptor) accepts one electron from the external dehydrogenases: Ndep (external NADH dehydrogenase) and Gut2p (glycerol-3-phosphate dehydrogenase).

2. Material and methods

2.1. Yeast strains and growth conditions

The *S. cerevisiae* strain used in this study was W303-1A (*ade*2-1, *his*3-11, *leu*2-3, 112*trp*1-1a, *ura*3-1, *can*100). Cells were grown aerobically at 28 °C as chemostat cultures in the following medium: yeast extract 1% (w/v), bactopeptone 1% (w/v), KH_2PO_4 0.1% (w/v), $(NH_4)_2SO_4$ 0.12% (w/v), adenine 0.01% (w/v) and glucose 0.5%(w/v) as the carbon source; pH 5.5. The cells were harvested after glucose depletion, i.e., during respiratory growth on ethanol.

2.2. Mitochondria preparation

Yeast mitochondria were prepared as described previously [17] and suspended in the mitochondrial buffer: 0.65 M mannitol, 0.36 mM EGTA, 10 mM Tris-maleate, 5 mM Tris-phosphate, pH 6.8.

2.3. Respiration assay

Oxygen consumption was measured at 28 °C in a 2-ml thermostatically controlled chamber equipped with a Clark oxygen electrode (Gilson) connected to a recording device that provides a display of the oxygen consumption rate. Mitochondria (0.5 mg ml^{-1}) were incubated in the mitochondrial buffer (described above). Unless specified, substrates were used at the following concentrations: NADH (10 mM), glycerol-3-phosphate (10 mM), ethanol (100 mM) and succinate (10 mM).

2.4. Measurement of glycerol-3-phosphate consumption flux (JDHAP) and of NADH consumption flux ($JNAD^+$)

Dihydroxyacetonephosphate (DHAP) was measured fluorimetrically in 7% $HClO_4$/25 mM EDTA extracts neutralized with KOH 2 M, MOPS 0.3 M as described previously in Bergmeyer [18]. NAD^+ was measured fluorometrically in neutralized 7% $HClO_4$/25 mM EDTA extracts as described previously [18].

2.5. Enzymatic activities

NADH-ferricyanideoxydo-reductase and glycerol-3-phosphate-ferricyanide oxydo-reductase activities were assayed in mitochondria in the presence of respiratory chain inhibitors (KCN (2 mM) and antimycin A (0.5 μg ml^{-1})) and of various concentrations of glycerol-3-phosphate with or without 10 mM NADH. Mitochondria were incubated in mitochondrial buffer. The reaction was started by the addition of ferricyanide ($Fe(CN)_6$). Ferricyanide reduction was followed at 420 nm ($\varepsilon = 0.86$ mM^{-1} cm^{-1}).

2.6. Measurement of Δp by labeled probes distribution

Matrix space was determined by using [^{3}H]water and inner membrane impermeable [^{14}C]mannitol. Whichever the experimental condition, matricial volume was not significantly changed. $\Delta\Psi$ and ΔpH were determined by the distribution of [^{3}H]$TPMP^+$ and [^{3}H] acetate, respectively. [^{3}H]$TPMP^+$ is a lipophilic cation and its binding coefficient was determined in our laboratory as being equal to 0.38 [19]. Routinely, after equilibration (3 min), mitochondria were separated from the medium by rapid centrifugation (30 s), through a silicon oil layer and then treated as described previously [20].

2.7. Measurement of ATP hydrolysis flux (JADP)

ADP was quantified fluorimetrically at 340 nm through NADH consumption, via the coupled reaction of pyruvate kinase and lactate dehydrogenase. After a 3-min incubation at 28 °C in mitochondrial buffer supplemented with 5 mM ATP, 0.5 μg/ml antimycin A and 2 mM KCN, samples were removed every minute for 4 min, precipitated in 7% $HClO_4$/25 mM EDTA and then neutralized with KOH 2 M, MOPS 0.3 M. These extracts were analyzed in the following buffer: 75 mM KH_2PO_4, 15 mM $MgSO_4$, 1 mM phospho-enol-pyruvate, lactate dehydrogenase 2 U/ml, 50 μM NADH, pH 7.5). The reaction was started by addition of pyruvate kinase 2 U/ml.

2.8. Measurement of ATP synthesis flux (JATP)

Isolated mitochondria (0.5 mg ml^{-1}) were suspended in the mitochondrial buffer (see above). After addition of the respiratory substrates and 1 mM ADP, both oxygen consumption and ATP synthesis rates were measured. Samples were removed every 20 s and precipitated in 7% $HCLO_4$/25 mM EDTA and then neutralized with KOH 2 M, MOPS 0.3 M. The ATP content in these samples was determined with the ATPlite 1step from PerkinElmer®. In a parallel experiment, oligomycin (1.5 μg/mg protein) was added to the

mitochondrial suspension to determine the non-oxidative ATP synthesis rate.

2.9. Mitochondrial active swelling measurement

Isolated mitochondria were suspended (0.3 mg ml^{-1}) in the following buffer: 0.5 M mannitol, 35 mM potassium phosphate, 10 mM Tris-maleate, 0.36 mM EGTA, pH 6.8, and placed in a thermostatically controlled (28 °C) fluorimeter chamber. After ethanol addition, KCN (2 mM), antimycin A (0.5 μg/ml) and ATP (4 mM) were added. Glycerol-3-phosphate (20 mM) with or without NADH (10 mM) was added in the presence of either 0.6 mM $Fe(CN)_6$ or increasing concentrations of CCCP. Dehydrogenase activities were assessed through $Fe(CN)_6$ reduction flux at ex: 420 nm/em: 420 nm. Mitochondrial swelling was induced by valinomycin addition (0.15 μg/mg). The mitochondrial active swelling was determined at ex: 500/em: 500 (turbidity assessment). The active swelling was fully reversed by the addition of 0.7 μM CCCP.

3. Results and discussion

Most studies conducted in isolated mitochondria are done in the presence of a sole substrate. However, *in situ*, multiple substrates are available to mitochondria. We have previously investigated mitochondrial substrate oxidation rates in the presence of multiple substrates. In a previous paper [3], we have shown that electrons coming from the external NADH dehydrogenases have the right of way on electrons coming from either the internal NADH dehydrogenase or the glycerol-3-phosphate dehydrogenase. In contrast, electrons coming from the succinate dehydrogenase are shared together with the ones coming from the external NADH dehydrogenase. We intended to further study this process, using other substrate associations on isolated yeast mitochondria (see Fig. 1). We assessed both respiratory flux (black bars) and, when duable, substrate consumption fluxes (white/hatched bars). By comparing these fluxes in each experimental condition, we observed that electrons provided by internal dehydrogenases (NADH or succino-dehydrogenase) have the right of way on electrons coming from glycerol-3-phosphate dehydrogenase. A striking observation was also done during the course of these experiments: the respiratory flux assessed under non-phosphorylating conditions could increase by 40% depending on the substrate/substrate association, i.e., from 150 to almost 250 natO/min/mg proteins. As explained above (see Introduction), in *S. cerevisiae*, there is no complex I and for the substrates used in this study, the number of coupling sites (which are complex III and complex IV) is constant. Thus, the non-phosphorylating respiratory flux is expected to be constant whichever the respiratory substrate. However, Fig. 1 shows the ability of isolated mitochondria to substantially vary their non-phosphorylating respiratory rate.

As explained above, the fact that the respiratory rate increases could indicate either an increase in the mitochondrial inner membrane proton permeability, due to a change in the membrane proton conductance or a decrease in the efficiency of the proton pumps, i.e., more electron transfer is needed to achieve that the same amount of protons be pumped out: redox slipping. The only way to sort this out is to assess the proton motive force (Δp) that is known to decrease in the former [7–13] and be stationary in the latter [14,15,21]. When the values of the respiratory rate were plotted against the values of the proton motive force, a linear relationship was obtained (Fig. 2). Thus, when one compares distinct substrates or substrate combination, an increase in respiratory rate is associated to a decrease in Δp. Moreover, these increases in respiratory rate do not depend on the phosphorylating systems (ATP/ADP carrier or ATPsynthase) as assessed with specific inhibitors of these activities (data not shown). The question raised here is whether this decrease in Δp is strictly due to an increase in the inner membrane proton permeability. Some lipophilic weak acids, called uncouplers, are known to increase, in a dose-dependent manner, the inner membrane proton permeability [22]. We titrated the respiratory flux as well as the proton motive force at the lowest value of respiratory flux (i.e., with ethanol as substrate) with an uncoupler. Fig. 2 clearly shows that the relationship obtained in that case is identical to the one with various respiratory substrates. This shows that the increase in respiratory rate we observed under non-phosphorylating conditions and with various respiratory substrates is most likely due to an increase in the mitochondrial inner membrane proton permeability.

Since it is well known that the substrates used here do not have any uncoupling effect by themselves (see also Table 1) the question arose as of the origin of this increase in proton permeability. One hypothesis would be that it originates in the enzymes functioning. There are two kinds of enzymes at the respiratory chain level: the dehydrogenases and the proton pumps (complexes III and IV). In order to be able to distinguish between these two kinds of enzymes and their possible involvement in this process, the following

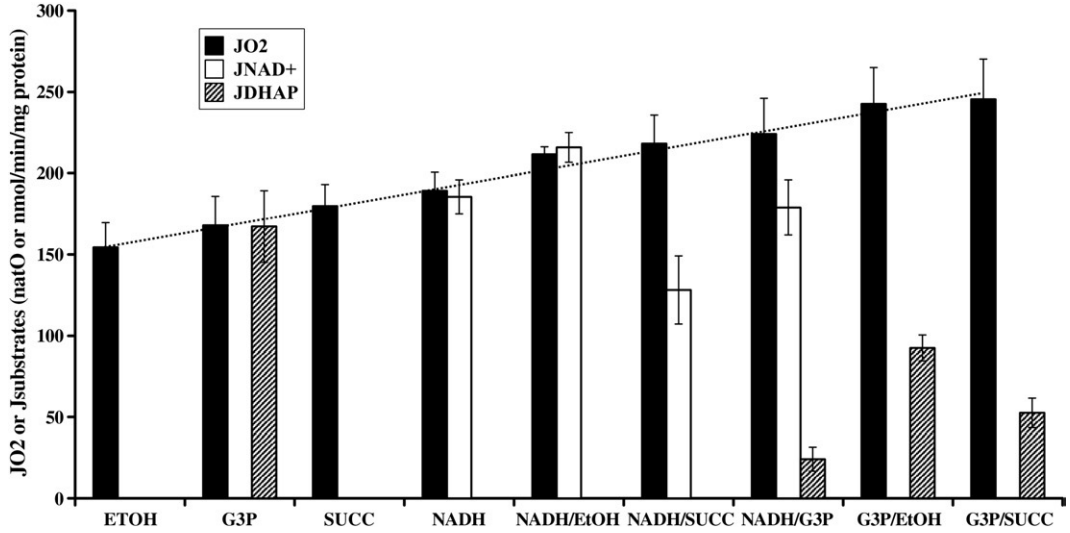

Fig. 1. Respiratory and substrate consumption fluxes in isolated mitochondria. Mitochondria (0.5 mg ml^{-1}) were incubated in mitochondrial buffer (see Material and methods). Substrates were used at the following concentrations: ethanol (100 mM), glycerol-3-phosphate (10 mM), NADH (10 mM), succinate (10 mM). JO_2 (full bars) was expressed in natomes O min^{-1} mg^{-1}, $JNAD^+$ (empty bars) and JDHAP (hatched bars) were expressed in nmol min^{-1} mg^{-1}. Results are means ± S.D. of at least three independent experiments carried out on three different mitochondrial preparations.

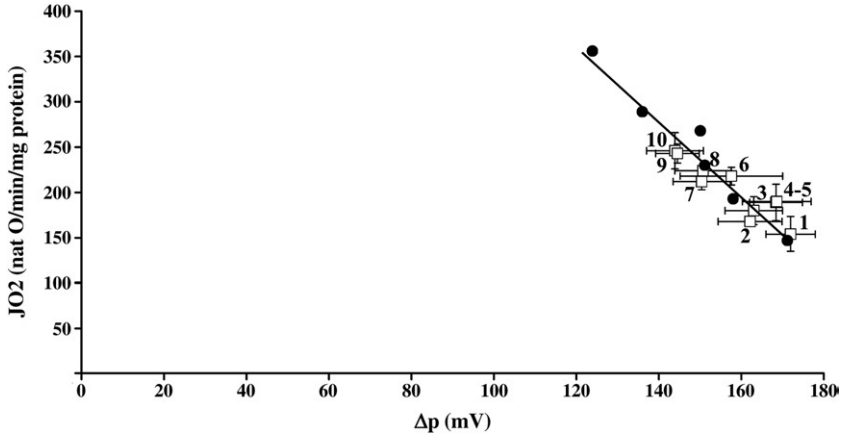

Fig. 2. Relationship between non-phosphorylating respiratory rate and the proton motive force. Mitochondria (3 mg ml^{-1}) were incubated in mitochondrial buffer as described in Material and methods. Respiratory rates were modulated using various respiratory substrates. Ethanol (1), glycerol-3-phosphate (2), NADH + ethanol (3), NADH (4), succinate (5), NADH + succinate (6), succinate + ethanol (7), NADH + glycerol-3-phosphate (8), ethanol + glycerol-3-phosphate (9), succinate + glycerol-3-phosphate (10). Also represented are conditions with ethanol uncoupled by increasing amounts of CCCP (100–360 nM) (●). Matricial volume, ΔpH and $\Delta\Psi$ were measured in the same conditions in parallel experiments as described in Material and methods. Results are means ± S.D. of at least three independent experiments carried out on three different mitochondrial preparations.

experiment was designed (see Scheme 1): the respiratory chain proton pumps were inhibited (complexes III and IV) and the proton motive force was generated by another proton pump, the mitochondrial ATPase. Indeed it is well known that when the respiratory chain is not functional, mitochondria are able to sustain an important proton motive force through ATP hydrolysis [6,15,23]. Then we assessed the relationship between the ATP hydrolysis rate and the Δp when the dehydrogenases oxidize their substrate at various oxidation rates and thus at various enzyme activities. In order for the dehydrogenases to oxidize their substrate when the respiratory chain proton pumps are inhibited, i.e., electrons cannot be transferred to oxygen, an artificial non-permeant electron acceptor ($Fe(CN)_6$) was used [24]. It should be stressed that external dehydrogenase substrate or electron acceptor ($Fe(CN)_6$) additions by themselves do not affect either the Δp or the ATP hydrolysis rate (Table 1). Furthermore, NADH dehydrogenase and G3P dehydrogenase products (NAD^+ and DHAP, respectively) did not affect the respiratory flux (data not shown). The Km of the glycerol-3-phosphate dehydrogenase for its substrate is 75 mM (in the presence of ($Fe(CN)_6$) and ATP; data not shown). We assessed the activity of this enzyme as well as the Δp, in the experimental conditions described above, when the concentrations of glycerol-3-phosphate used were below the Km in the presence or absence of NADH. In the experimental conditions described here, the enzyme substrate oxidation rate corresponds to half the rate of Fe $(CN)_6$ reduction. Indeed, $Fe(CN)_6$ accepts only one electron when the dehydrogenases transfer two. Fig. 3A clearly shows that we were able to induce important variations in the activity of the dehydrogenases, i.e., from 70 to 525 nmol/min/mg proteins and that the Δp decreased accordingly. Indeed, there is a linear relationship between the decrease in Δp and the increase in the dehydrogenase activities. As expected the decrease in Δp induced an increase in the ATP hydrolysis rate (Fig. 3B). Furthermore, this relationship is identical to the one obtained in the presence of increasing amounts of protonophore and in the absence of any dehydrogenase activity (Fig. 3B). Altogether, these results show that the functioning of these dehydrogenases induces an increase in membrane conductance that is proportional to their activity.

These results raised the question of the nature of this permeability induced by the dehydrogenase activity. Since the increase in flux and decrease in proton motive force could be mimicked by a protonophore, we hypothesized that the permeability induced was a proton permeability. Active swelling has been widely used in order to decipher various mechanisms of mitochondrial inner membrane carriers and ionophores [25–28]. When mitochondria are incubated in a saline solution, active swelling (dependent on proton motive force) is induced when both components of the salt are permeant and accumulated. Such a process is possible only if the salt entry is an electroneutral phenomenon. The phosphate carrier is an electroneutral proton/phosphate symport. Consequently, the active swelling in potassium phosphate is dependent on both the proton motive force and an electrophoretic entry of potassium that is catalysed by valinomycin. Thus, in the presence of valinomycin, potassium phosphate active swelling is sensitive to the mitochondrial inner membrane proton permeability as shown by its reversibility in the presence of CCCP (Fig. 4A). The dehydrogenase activities did not induce a potassium leak since there was no swelling before valinomycin addition (data not shown). The proton motive force was established by the ATPase and the swelling was induced by valinomycin addition. In these conditions, the amplitude of the swelling reversion depends on the protonophore concentration. Fig. 4B shows that valinomycin addition induced an important swelling that was partly reversed by the dehydrogenase activities, the amplitude of the reversion being dependent on the electron flux through the dehydrogenase(s) (see Fig. 3). This clearly indicates that the permeability induced by the dehydrogenase activities is a proton permeability.

The results presented above show that under non-phosphorylating conditions, a proton permeability is induced by the mitochondrial inner membrane dehydrogenase activity. It is well known that an increase in proton leak decreases the oxidative phosphorylation yield, i.e., ATP/O ratio [29–31]. There was indeed a decrease in ATP/O ratio – for a constant respiratory rate – in the presence of increasing amounts of protonophore (Fig. 5). Furthermore, in the presence of NADH, when the G3P dehydrogenase activity was increased there was a concomitant increase in respiratory rate that was associated with a decrease in ATP/O ratio (Fig. 5), confirming the increase in proton permeability

Table 1
Effect of ferricyanide and respiratory substrates on the basal ATP hydrolysis flux.

	$J_{ATP\ hydrolysis}$ (nmol/min/mg)	ΔP (mV)
ATP	39 ± 3	163 ± 9
ATP + Fe(CN)6	41 ± 3	161 ± 16
G3P	40 ± 3	163 ± 13
NADH	42 ± 7	161 ± 11

After inhibition of non-phosphorylating respiration (with ethanol) by antimycin A 0.5 μg/ml and KCN 2 mM, JADP and the proton motive force were measured with or without $Fe(CN)_6$ 25 mM, G3P 10 mM or NADH 10 mM.

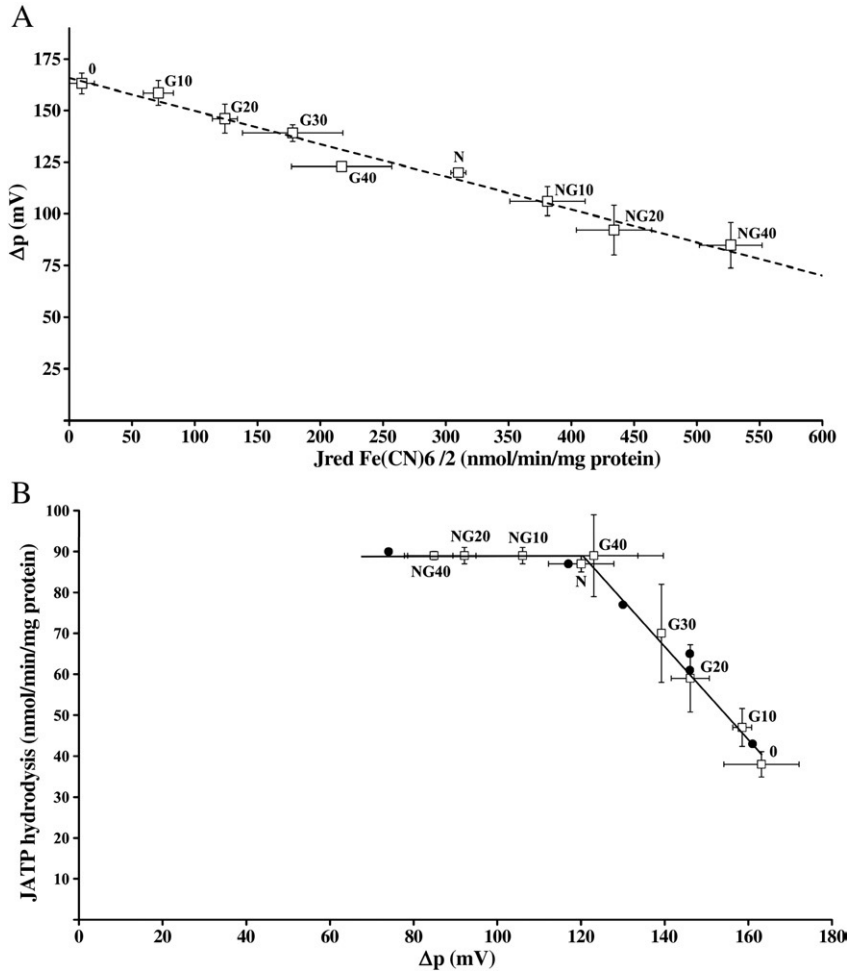

Fig. 3. Relationship between either dehydrogenase activity or ATP hydrolysis flux and proton motive force. (A) Relationship between proton motive force and NADH or G3P dehydrogenases activities. Mitochondria were incubated in mitochondrial buffer. After addition of ethanol, KCN (2 mM) and antimycin A (0.5 μg ml^{-1}), ATP (4 mM) were added. Then, either NADH (N) alone or increasing concentrations of glycerol-3-phosphate (10–40 mM, NG10–NG40) with or without NADH (10 mM) were added in the presence of 25 mM $Fe(CN)_6$. Dehydrogenase activities were assessed through $Fe(CN)_6$ reduction flux. Since $Fe(CN)_6$ accepts only one electron, its reduction rate was divided by two in order to assess dehydrogenase activities, since they transfer two electrons. Results are means ± S.D. of at least three independent experiments carried out on three different mitochondrial preparations. (B) Relationship between ATP hydrolysis rate and proton motive force. Experimental conditions were identical to the ones used in panel A (□). Increasing concentrations of CCCP from 25 to 200 nM were used (●). Results are means ± S.D. of at least three independent experiments carried out on three different mitochondrial preparations.

in these conditions. In contrast to what can be assessed with a classical protonophore, the dehydrogenase activation uncouples the mitochondrial inner membrane in conditions where the increase in respiratory rate allows the maintenance of the ATP synthesis flux. Consequently, the respiratory rate increases without any significant change in ATP synthesis flux.

4. Conclusion

Previously published [3] and present results show that when mitochondria are incubated with multiple substrates, the non-phosphorylating respiratory rate is increased. It should be stressed that the presence of multiple substrates is the most physiological situation. The data reported here clearly show that this increase is due to an increase in proton permeability that originates in the mitochondrial inner membrane external dehydrogenase activities (i.e., external NADH dehydrogenase and glycerol-3-phosphate dehydrogenase). This is a new and previously unknown mechanism that we propose to name "active proton leak." Indeed we have shown in this paper that this process is totally independent of the activity of the proton pumps and directly related to the level of activity of the dehydrogenases. This is a very original mechanism where enzymes that are not involved in energy-dependent proton movements across the inner membrane are able to generate a proton leak.

The molecular mechanisms that generate passive proton leak are unknown even though numerous factors are known to influence its extent. These factors are the mitochondrial inner membrane surface [32], its protein content [33] and the fatty acids membrane composition [34–36]. Since all our experiments were carried out in mitochondria isolated from the same strain, none of these factors could be responsible for the increase in respiratory rate. Furthermore, there are some proton carriers that are able to increase the mitochondrial inner membrane permeability. These carriers belong to the UnCoupling Protein (UCP) family and are highly regulated [37]. Moreover, under certain conditions, the respiratory chain proton pump efficiency can be modified, i.e., redox slipping, which induces a modification of the relation between the respiratory rate and the proton motive force [21,38]. However, there are no UCP carriers in yeast and it has been shown that deletion of any carrier of the mitochondrial inner membrane does not modify the proton permeability [39]. Furthermore, the relationship that we obtained between the respiratory rate and the proton motive force either with the respiratory chain or the ATPase cannot be explained by a change in the proton pump efficiency.

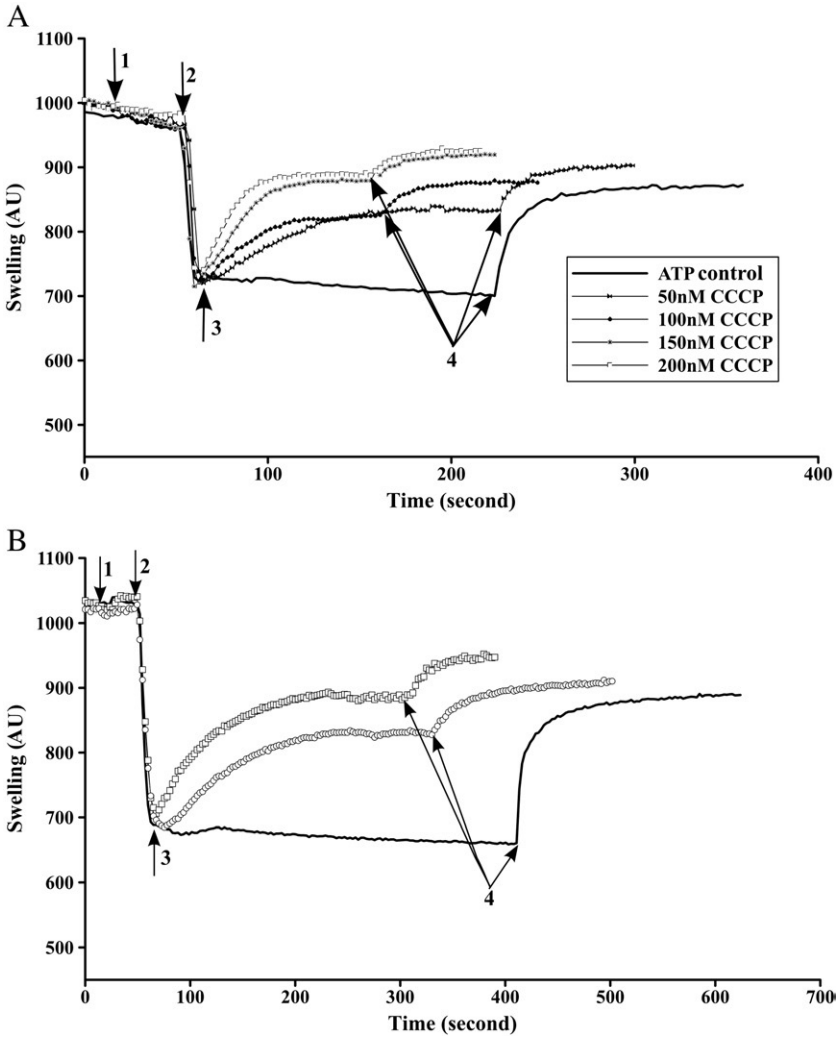

Fig. 4. Active mitochondrial swelling. (A) Active swelling in K-Pi when increasing concentrations of CCCP are added: Isolated mitochondria (0.3 mg ml^{-1}) were incubated in the following buffer (0.5 M mannitol, 35 mM potassium phosphate, 10 mM Tris-maleate, 0.36 mM EGTA, pH 6.8). After sequential addition of ethanol, KCN (2 mM), antimycin A (0.5 μg/ml), ATP (4 mM) was added (arrow 1). Mitochondrial swelling was induced by Valinomycin addition (0.15 μg/mg) (arrow 2). Then, increasing concentrations of CCCP were added (arrow 3). Active swelling was completely reversed by adding 0.7 μM CCCP (arrow 4). (B) Active swelling in K-Pi when external dehydrogenases are functioning: isolated mitochondria (0.3 mg ml^{-1}) were incubated in the following buffer (0.5 M mannitol, 35 mM potassium-phosphate, 10 mM Tris-maleate, 0.36 mM EGTA, pH 6.8). After sequential addition of ethanol, KCN (2 mM), antimycin A (0.5 μg/ml), ATP (4 mM) was added (arrow 1). Mitochondrial swelling was induced by Valinomycin addition (0.15 μg/mg) (arrow 2). Glycerol-3-phosphate (20 mM) with (○) or without (□) NADH (10 mM) were added in the presence of 0.6 mM $Fe(CN)_6$(arrow 3). Active swelling was completely reversed by adding 0.7 μM CCCP (arrow 4).

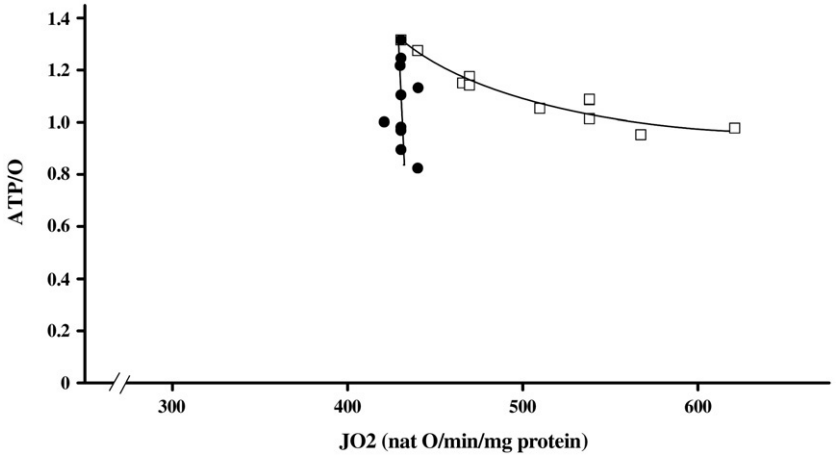

Fig. 5. Relationship between the oxidative phosphorylation yield and the respiratory rate: mitochondria (0.5 mg ml^{-1}) were incubated in mitochondrial buffer (see Material and methods). The respiratory substrates were added in the presence of ADP (1 mM) and ATP/O was assessed with increasing concentrations of glycerol-3-phosphate (5–20 mM) in the presence of NADH (10 mM) (□). ATP/O was also assessed with increasing concentrations of CCCP (50–250 nM) in the presence of NADH (10 mM) (●). Results are means ± S.D. of at least three independent experiments carried out on three different mitochondrial preparations.

It is noteworthy that a number of mitochondrial processes allow the modulation of the proton leak. Two categories can be considered (i) passive proton leak and (ii) catalysed proton leak. Passive proton leak, for a defined mitochondria or membrane, is only dependent on the proton motive force and consequently cannot be widely modulated. By contrast, the catalysed proton leak, namely "active proton leak" and UCP activities, can be finely tuned. For example the UCP's activities are highly regulated by GDP and fatty acids [37]. The peculiarity of the "active proton leak" is that the increase in proton permeability is strictly dependent on the activity of external dehydrogenases. Furthermore, the weak uncoupling of oxidative phosphorylation allows an important increase in respiratory rate when the cytosolic redox state increases without any major alteration in ATP synthesis flux (data not shown but see Fig. 5).

The fact that there are so many processes and different regulation levels for the proton leak leads one to consider that this process is central to cell survival. Indeed mitochondria play a central role in pathways such as substrate oxidation, ATP synthesis and ROS production. Even though these pathways are obviously linked through mitochondrial activity, depending on the cellular needs it might be necessary to modulate the coupling between them with a limitation in ROS production. This might be the major function of the "active proton leak."

Acknowledgments

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