Biochimica et Biophysica Acta, 1060 (1991) 293–298

BBABIO 43500

Respiration in non-phosphorylating yeast mitochondria. Roles of non-ohmic proton conductance and intrinsic uncoupling

Rachid Ouhabi, Michel Rigoulet, Jean-Louis Lavie and Bernard Guérin

Institut de Biochimie Cellulaire et Neurochimie du CNRS, Université de Bordeaux II, Bordeaux (France)

(Received 21 March 1991)

Key words: Intrinsic uncoupling; Proton conductance; Mitochondrion; (Yeast)

Respiratory rate, protonmotive force and charge/O ratio were measured under two different kinds of steady state in non-phosphorylating yeast mitochondria: (i) when the electron flux was modulated by a variable limitation in electron supply or (ii) when oxygen consumption was decreased by respiratory chain inhibitor titration. We showed that the relationships between either Δp or charge/O ratio and respiratory rate are different under the two kinds of steady state, indicating different degrees of intrinsic uncoupling in respiratory chain. Moreover, we observed a non-ohmic dependence between H^+-conductance and Δp. We concluded that the high rate of static-head respiration in yeast mitochondria was determined both by the non-ohmic proton conductance of the inner membrane and the saturation of the redox proton pump slipping.

Introduction

In mitochondria isolated from yeast, the static head (state 4) respiration is much higher than that measured with rat liver mitochondria, with the same substrates. The fact that the efficiency of oxidative phosphorylation (i.e., P/O ratio) with succinate or TMPD + ascorbate as respiratory substrates [1–4], and the value of Δp maintained in state 4 [5–7] are similar in these two kinds of mitochondrion, is in opposition with the hypothesis that a high value of proton conductance, alone, is accountable for the static-head respiration of isolated yeast mitochondria. Indeed, an increase in the leakage conductance has been observed to result both in an accelerated respiration and a lowering of Δp [8]. In isolated mammalian mitochondria treated with oligomycin to block ATP synthesis, it is generally observed that the relationship between Δp and respiratory rate is non-proportional [6,9–12]. Indeed, respiratory rate can be significantly decreased by using inhibitors either of electron supply or electron transport with only a weak drop or without significant change in Δp. Two main explanations have been put forward for this behaviour: (i) as the scalar and the vectorial reactions in H^+-pumps are assumed to be tightly coupled, such a relationship is a direct consequence of a large increase in the passive proton conductance of the inner membrane for a weak increase of Δp at high Δp [6,13,14]; indeed, a non-ohmic change in H^+ conductance at high Δp seems to be demonstrated in mammalian mitochondria [12,13,15,16]; (ii) stoichiometry of proton pumping by the respiratory chain (the number of protons translocated vectorially across the respiratory chain per electron transferred) decreases as Δp is raised, a mechanism called 'slip' [10,11,17,18]. Theoretically, it is easy to discriminate between these two processes: the first depends only on the Δp (and the membrane considered), whereas the second could vary in response to many factors: i.e., forces (Δp and $\Delta G_{O/R}$ or ΔG_p) and all the properties of the proton pumps involved. Nevertheless, this long-standing riddle has not yet been resolved, partly because the experimental results are generally contradictory [14,16–18] and also because these two processes may work together (see Ref. 19 for review). More recently, other approaches

Abbreviations: ΔpH, transmembrane difference of pH; $\Delta\Psi$, transmembrane difference of electrical potential; Δp, protonmotive force; $\Delta G_{O/R}$, Gibbs free-energy difference of the oxidation reaction; ΔG_p, free energy of ATP-hydrolysis reaction; J_{out}, charge efflux; J_{in}, charge influx; CCCP, carboxyl cyanide *m*-chlorophenylhydrazone; DCIP, 2,6-dichlorophenol indophenol; PMS, phenazine methosulfate; TMPD, tetramethyl-*p*-phenylenediamine.

Correspondence: M. Rigoulet, IBCN-CNRS, 1, Rue Camille Saint-Saëns, 33077 Bordeaux Cedex, France.

have been proposed to distinguish experimentally between effects of proton leak and redox slip on energy transduction processes [20,21].

Mitochondria isolated from *Saccharomyces cerevisiae* present an ability to oxidize exogenous NADH by an NADH dehydrogenase located towards the outer surface of the inner membrane [22]. By using an NADH-regenerating system, we have observed that, at constant Δp, the ATP/O ratio decreased while electron flux increased [4]. In contrast, when respiratory rate was modulated by antimycin titration, both Δp and ATP/O ratio remained constant. This discrepancy has been interpreted as a difference in the degree of coupling of proton pumps implicated, under these two different kinds of steady state. In this paper, we have compared the Δp, the respiratory rate and the charge/O ratio, measured under two different kinds of steady state in non-phosphorylating yeast mitochondria: (i) when the electron flux through each respiratory unit was modulated by a variable limitation in electron supply; (ii) when the rates of respiration vary in response to a limitation in the number of functional units by using respiratory chain inhibitor. We showed that the relationship between Δp and respiratory rate is different under the two kinds of steady state, reflecting different degrees of intrinsic uncoupling in respiratory chain. When the electron flux through each respiratory unit was modulated, the intrinsic uncoupling of this proton pump was a saturating process which increased when respiratory rate increased. We concluded that the static-head respiration was determined both by the non-ohmic proton conductance of the inner membrane and the saturation of the intrinsic uncoupling of the respiratory chain.

Materials and Methods

Preparation of mitochondria. Cells of diploid wild-type *Saccharomyces cerevisiae* (yeast foam) were grown aerobically at 28°C in a complete medium (pH 4.5) with 2% lactate as carbon source. The cells were harvested in logarithmic growth phase and mitochondria were isolated from protoplasts as described in Ref. 23. Protein concentration was measured by the biuret method using bovine serum albumin as a standard.

Respiration assay. Oxygen consumption was measured at 27°C in a 1.5 ml thermostatically controlled chamber equipped with a Clark oxygen electrode (Gilson) connected to a microcomputer giving an on-line display of rate values. Mitochondria (0.5 mg/ml) were incubated in the following basal medium: 0.65 M mannitol, 0.36 mM EGTA, 3 mM Tris-P_i, 5 μM Tris-acetate, 10 mM Tris-maleate (pH 6.7), 20 μg oligomycin/ml supplemented with 10 μM RbCl and 0.01 μg valinomycin/ml. Substrate supply was an NADH-regenerating system: 4 mM glucose 6-phosphate, 2 mM NAD^+ and various amounts of glucose-6-phosphate dehydrogenase (EC 1.1.1.49) from *Leuconostoc mesenteroides* which is able to work with NAD^+ as cofactor.

Measurement of ΔpH and $\Delta\Psi$ by distribution of labelled probes. Matrix space was determined by using [^{3}H]water and inner membrane impermeable [^{14}C]sucrose, $\Delta\Psi$ and ΔpH by distribution of ^{86}Rb (in the presence of valinomycin) and [^{3}H]acetate, respectively [24]. Routinely, after equilibration (3 min), mitochondria were separated from the basal medium by rapid centrifugation (30 s) through a silicone oil layer (silicone AR 200 fluid). These measurements were made under strictly the same conditions as respiration assays (see above).

Determinations of K^+/O ratio and $\Delta\Psi$ by K^+ electrodes. The technique for measuring electrical charge/O (q^+/O) ratios at steady-state was as described by Murphy and Brand [18]. This method is based on strict equality of the rate of charge efflux from mitochondria, catalyzed by a proton pump, to the charge influx, catalyzed by valinomycin, at a given steady state. Mitochondria (2 mg/ml) were incubated in 8 ml of basal medium (without Rb) supplemented with 2 μg valinomycin/ml at 28°C. Potassium concentration was monitored with a potassium-sensitive electrode (F 2321 K Radiometer). At a given steady-state, obtained either by various amounts of glucose-6-phosphate dehydrogenase (EC 1.1.1.49) or by different amounts of myxothiazol, an inhibitor of b-c_1 complex of respiratory chain (in the presence of saturating concentration of enzyme), the respiratory rate was measured and charge efflux was determined from the initial rate of K^+ efflux following addition of 2 μg myxothiazol/ml. Since the potassium-sensitive electrode gave a logarithmic response to K^+, the initial rate of efflux was determined from linearized replots as described in Ref. 18.

$\Delta\Psi$ (in mV) was calculated from the distribution of K^+ by the Nernst equation:

$$\Delta\Psi = 60 \log([K^+]_{in}/[K^+]_{out})$$

The extramitochondrial K^+ concentration was determined using a K^+-sensitive electrode (see above). The intramatricial K^+ accumulation was estimated using the difference in external K^+ concentrations at given steady states and after complete respiratory chain inhibition. Intramatricial K^+ concentration was calculated using the matrix volume determined in parallel.

ATPase activity measurement. ATP hydrolysis was assayed at 28°C in the following medium: 0.65 M mannitol, 10 mM Tris maleate, 0.36 mM EGTA, 5 mM potassium phosphate, 5 mM $MgCl_2$, 2.5 μg antimycin/ml, 0.5 μg valinomycin/ml and 0.1 mM

carboxyatractylate (pH 6.7). Mitochondria (1 mg protein/ml) were incubated in this medium in the absence or in the presence of 0.1% Triton X-100 (v/v) and the reaction was started by addition of 1 mM ATP. ATP hydrolysis was measured by bioluminescence method as previously described in Ref. 26.

Succinate ferricyanide oxidoreductase. Mitochondria (1 mg protein/ml) were incubated in the basal medium (see above) in the presence of 1 mM KCN, 2 mM ferricyanide, 4 mM succinate and 2.5 μg valinomycin/ml, with or without Triton X-100. Ferricyanide reduction was followed at 436 nm in an Eppendorf spectrophotometer using the absorbance coefficient 0.4 $mM^{-1}\ cm^{-1}$.

Succinate PMS DCIP oxidoreductase. Mitochondria (1 mg protein/ml) were incubated in the basal medium (see above) in the presence of 2.5 μg antimycin/ml, 10 mM succinate, 200 μM DCIP and 2 mM PMS with or without 0.1% Triton X-100. This activity was measured as described in Ref. 27.

Results

The mitochondrial fraction appears to contain less than 5% of broken organelles, as suggested by three sets of experiments (not shown): (i) the ATPase activity insensitive to carboxyatractylate; (ii) the succinate-ferricyanide oxidoreductase activity insensitive to antimycin; (iii) and the succinate-PMS-DCIP oxidoreductase. For each test, the maximum activity corresponding to completely broken mitochondria was obtained in the presence of low concentrations of Triton X-100 (see Materials and Methods).

Relationships between Δp and respiratory rate

Since yeast mitochondria possess an NADH dehydrogenase located towards the outer surface of the inner membrane, it is easy to limit the respiratory rate by NADH supply. For such a purpose, mitochondria were incubated in a medium containing a NADH-regenerating system, i.e., 4 mM glucose 6-phosphate, 2 mM NAD^+ and various amounts of glucose-6-phosphate dehydrogenase inducing different steady-states (conditions 1). As shown in Fig. 1A, the static-head respiratory rate increased as a function of the enzyme concentration to reach a maximal value of 320 natoms 0/min per mg protein for 0.5 unit/ml of enzyme added. This value and the corresponding Δp (see below) are identical to those measured in state 4 when saturating NADH concentration is used as respiratory substrate instead of NADH-regenerating system (not shown). In the presence of 3 μM CCCP, a concentration of this protonophore which completely collapsed Δp, the respiration was not modified for a low amount of glucose-6-phosphate dehydrogenase (up to 0.1 unit/ml) and stimulated above this amount of enzyme (Fig. 1A). This shows that under 0.1 unit/ml glucose-6-phosphate dehydrogenase exerts full control. Maximal uncoupled respiration was obtained for 1.5 unit/ml of enzyme added. Another possibility for modulating the respiratory rate, under coupled (state 4) or uncoupled conditions, was the inhibitor titration. Fig. 1B shows such a titration with myxothiazol (conditions 2). Even when respiratory rate is very low, it is always largely stimulated by Δp dissipation.

ΔpH and $\Delta\Psi$ were measured under the two kinds of steady state corresponding to conditions 1 and 2 in

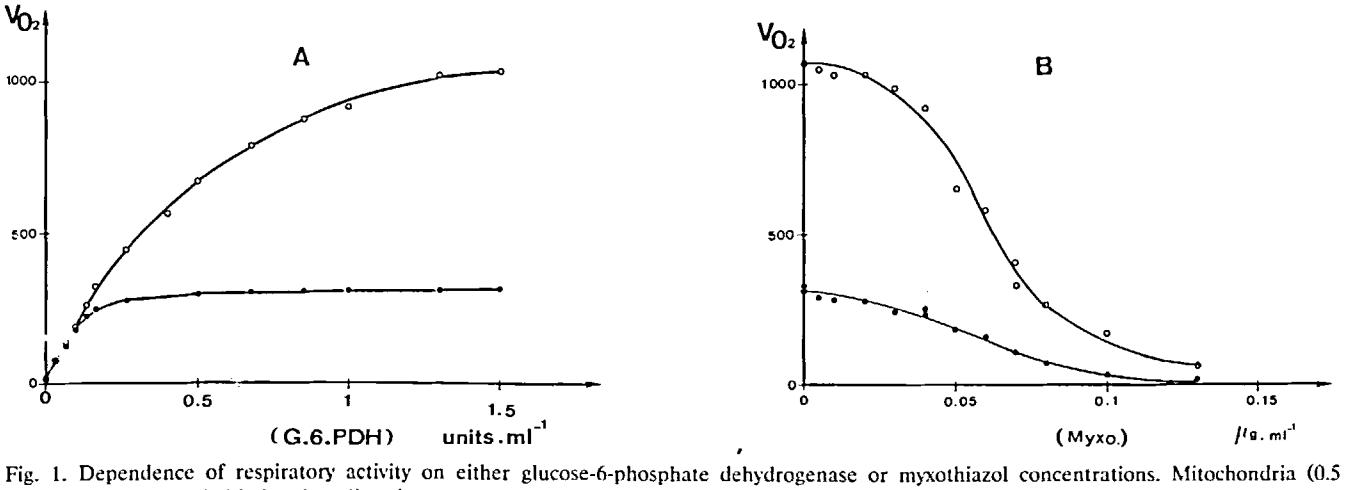

Fig. 1. Dependence of respiratory activity on either glucose-6-phosphate dehydrogenase or myxothiazol concentrations. Mitochondria (0.5 mg/ml) were suspended in basal medium (see Materials and Methods). Respiratory rate was measured either at static-head (•) or after addition of 3 μM CCCP (○). Additions were: in (A) different amounts of glucose-6-phosphate dehydrogenase; in (B) 1.6 unit enzyme/ml with different myxothiazol concentrations. The results for a representative experiment are presented; similar results were obtained with three different mitochondria preparations.

the absence of CCCP. As shown in Fig. 2A, the maximal ΔpH and $\Delta\Psi$ are reached for very low respiratory rate (about 30 natoms O/min per mg protein) when this latter is limited by NADH supply. However, the respiratory rate can be further increased up to a maximal value of 320 natoms O/min per mg protein without any apparent change in Δp (see also Fig. 2C), so that 10% of the maximal respiration seems sufficient to compensate the proton leak. In contrast, under conditions 2 (myxothiazol titration), a slight decrease in respiratory rate corresponded to a decrease in both ΔpH and $\Delta\Psi$ (Fig. 2B). The same result was obtained when antimycin was used instead of myxothiazol (not shown). Consequently, there is more than one relationship between Δp and respiratory rate; at the same respiratory rate, limitation in NADH supply led to a Δp greater than that maintained when respiratory chain itself was partially inhibited (Fig. 2C). In conclusion, the respiratory chain is more efficient under conditions 1, except at very low respiratory rate.

Determination of K^+/O stoichiometry

The electrical charge/O (K^+/O) ratio was determined at different steady states by using the technique described by Murphy and Brand [18]. At a given steady state, it is assumed that the rate of charge efflux (J_{out}) from mitochondria catalyzed by respiratory chain is strictly equal to the rate of charge influx (J_{in}). In the presence of a non-limiting amount of valinomycin, addition of a respiratory chain inhibitor (i.e., myxothiazol), by blocking electron flux and consequently H^+ efflux, leads to a K^+ efflux equal to the H^+ leak. Consequently, the initial rate of K^+ efflux reflects J_{out}. Fig. 3 shows the dependence between either $\Delta\Psi$ (measured by K^+-electrode) or K^+/O ratio and the respiratory rate under the two sets of experimental conditions previously described. $\Delta\Psi$ measured by Rb distribution (Fig. 2A and B) or by K^+-electrode (Fig. 3A and B) were similar except for the low values of respiratory rate (under 60 natoms O/min per mg protein) obtained by myxothiazol titration (compare Figs. 2B and 3B), indicating that addition of non-limiting concentration of valinomycin did not change significantly the membrane properties. Under conditions 1, K^+/O value decreased from 5 to 1 as a function of respiratory rate increase. In contrast, under conditions 2 (myxothiazol titration) K^+/O value was low (approx. 1) and constant when respiratory rate was greater than 40 natoms O/min per mg protein.

It is worth noting that, at low values of respiratory rate (under 20 natoms O/min per mg protein), it is very difficult to determine the right K^+/O ratio, whatever the means used for limiting respiration. Indeed, at low $\Delta\Psi$, the method sensitivity was diminished, resulting obviously in difficulty in interpreting experimental data.

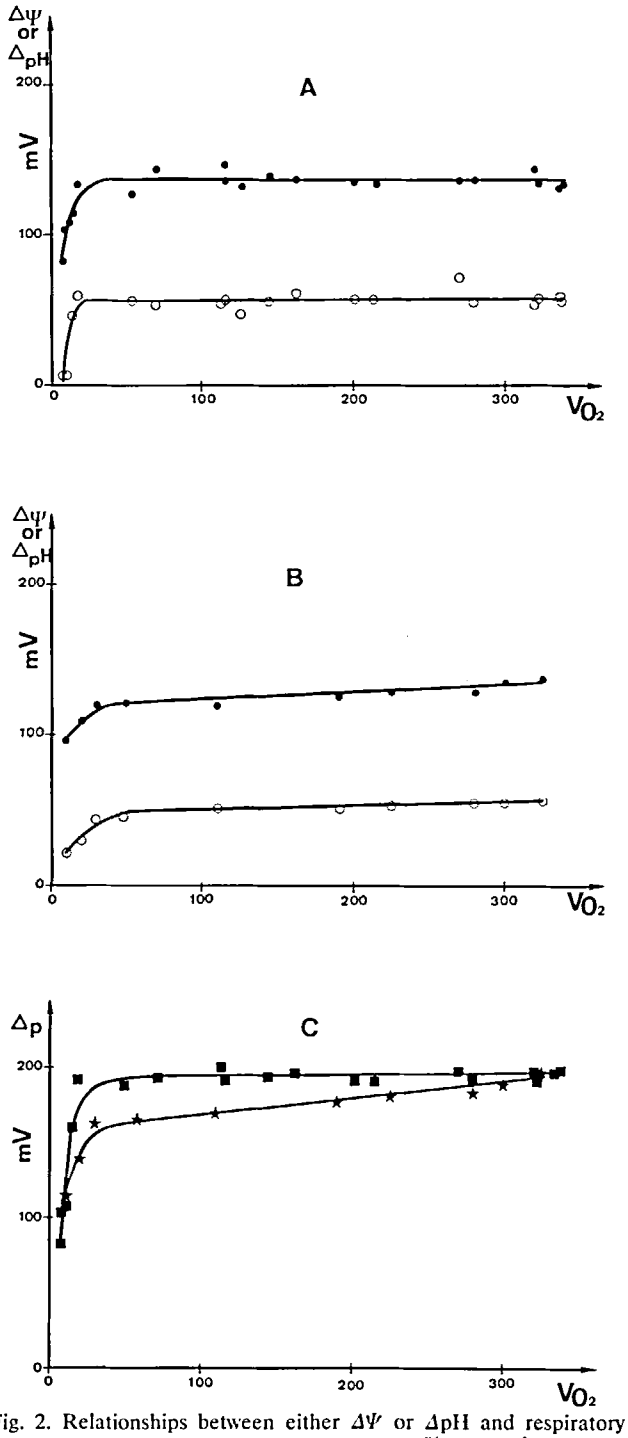

Fig. 2. Relationships between either $\Delta\Psi$ or ΔpH and respiratory rate. $\Delta\Psi$ (●) and ΔpH (○) were determined by ^{86}Rb and [^{3}H]acetate, respectively, as described in Materials and Methods under two kinds of steady state corresponding to either NADH-regenerating system (A) or myxothiazol titration (B). Oxygen consumption and Δp were measured under the same conditions in parallel experiments as detailed in Materials and Methods. Protonmotive force obtained from (A) (■) and (B) (*) against respiratory rate (C). The two kinds of steady state are studied in parallel with the same mitochondria preparation. The points shown are from three different experiments carried out with three different mitochondria preparations. Each point is the means of three or four determinations. Error bars have been omitted since a standard deviation is always smaller than 6 mV for Δp.

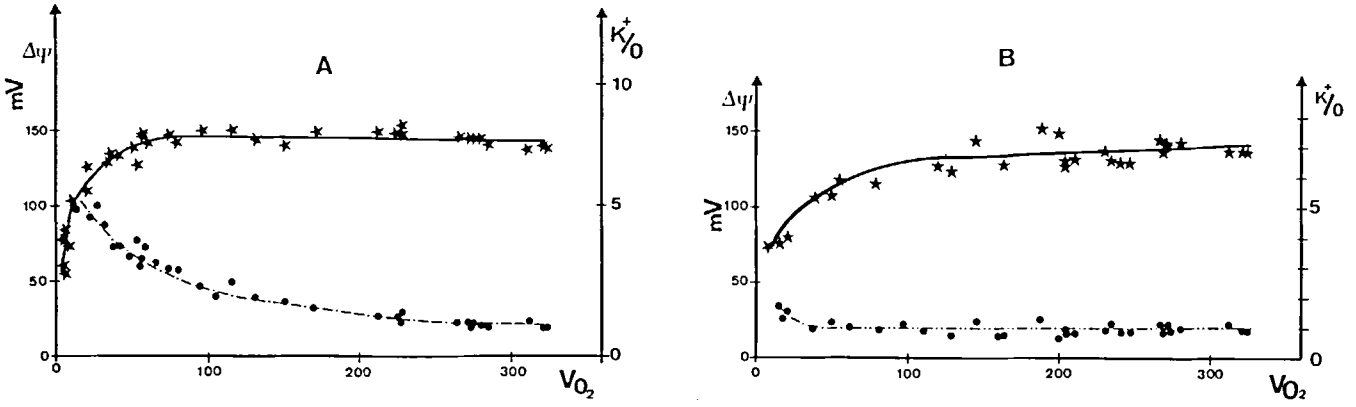

Fig. 3. $\Delta\Psi$ and K^+/O stoichiometry as a function of respiratory rate. K^+/O stoichiometry (•) determination were carried out by simultaneously measuring oxygen and potassium concentrations as described in Materials and Methods. (A) and (B) correspond to different steady states obtained as in Fig. 1A and Fig. 1B, respectively. $\Delta\Psi$ (*) was determined from K^+ concentrations of the medium as described in Materials and Methods. The two kinds of steady state are studied in parallel with the same mitochondria preparation, on the same day. The points shown are from four different experiments carried out with three different mitochondria preparations.

low $\Delta\Psi$, the method sensitivity was diminished, resulting obviously in difficulty in interpreting experimental data.

Taking into account the results presented in Figs. 2 and 3, it is possible to deduce the variation of the H^+ conductance as a function of Δp. This can be realised by dividing J_{K^+} (equal to J_{H^+} leak) by the corresponding Δp value for each respiratory rate induced, either by NADH supply limitation (conditions 1) or by inhibitor titration (conditions 2). Whatever the means used for varying respiratory rate, there was only one relationship between H^+-conductance and Δp (Fig. 4). This result was to be expected if $J_K/\Delta p$ was an actual measurement of H^+ conductance. At low Δp, the H^+ conductance value was 0.2 nmol H^+/min per mg protein per mV; for upper values of Δp, H^+ conductance increased exponentially as a function of Δp, indicating a non-ohmic dependence.

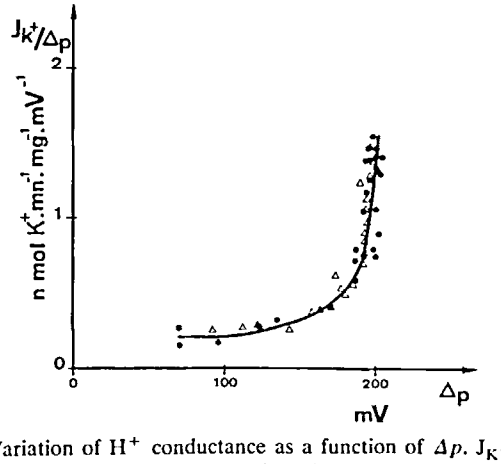

Fig. 4. Variation of H^+ conductance as a function of Δp. J_{K^+} and $\Delta\Psi$ were from Figs. 3A and B. For the calculation of Δp, ΔpH was from Figs. 2A and B. As in previous experiments, two kinds of steady state were compared: different amounts of glucose-6-phosphate dehydrogenase (•) and different myxothiazol concentrations at saturation in NADH supply (△).

Discussion

A non-ohmic dependence between passive proton permeability and Δp has been observed in many biological membranes, including the mitochondrial inner membrane of mammals [6,9–13,16,18]. From Fig. 4, it appears that in yeast mitochondria, the H^+ conductance varies with Δp, as expected if the proton flux is a non-ohmic function of the potential. Moreover, the H^+ conductance of yeast mitochondria was 4-fold higher than that reported in rat liver mitochondria [12,13,15].

The question arises whether such an increase in H^+-conductance can in itself fully account for the non-linear relationship between respiratory rate and Δp in yeast mitochondria, as previously proposed in rat liver mitochondria [6,13,14]. However, another explanation has been suggested in which the stoichiometry decrease in redox proton pump at high Δp is responsible for this behaviour [10,11]. Such an intrinsic uncoupling of a proton pump, also called slip, seems to depend on numerous factors: the value of Δp [16,18,25], the effects of some drugs [29,30], the kinetic regulation [7,31] and the size of the flux through the proton pump considered [4].

In yeast mitochondria, at a given respiratory rate obtained with the same respiratory substrate, Δp is greater when the electron supply is a limiting process than when respiratory chain activity is modulated by inhibitor (Fig. 2C). An explanation could lie in a mitochondrial heterogeneity with different dependences on either the substrate concentration or the inhibitor titration of the uncoupled respiration of the various mitochondrial populations [28]. Such an explanation is unlikely, since the broken mitochondria fraction is less than 5%. Moreover, it is well known that, in the uncoupled state, practically the total control is exerted by the respiratory chain and, in this context, the inhibitor of the respiratory chain will more strongly de-

crease the respiratory rate of mitochondria presenting weak or no coupling. In contrast, when the electron supply is a limiting process, all the kinetic control is exerted by the glucose-6-phosphate dehydrogenase added. Indeed, the heterogeneity of mitochondrial preparation could explain that Δp is more important when the respiratory chain is modulated by inhibitors.

However, the results that we have found are opposite. In assuming an homogeneous mitochondrial population, the facts described in Fig. 2C constitute strong indirect evidence that the stoichiometry of this proton pump is higher under the former conditions except when the respiratory rate fell below 20 natom O/min per mg protein. Direct measurements of the charges translocated as a function of respiratory rate show that the K^+/O ratio is approx. 1 at state 4 respiration. When the rate of respiration varies in response to a limitation in the number of functional units (titration with myxothiazol), the K^+/O ratio remains constant for a respiratory rate above 40 natom O/min per mg protein. Below this value, K^+/O slightly increases. In contrast, when the electron flux through each respiratory chain unit is modulated by a variable limitation in electron supply, the K^+/O ratio increases exponentially from 1 to 5 as the respiratory rate decreases. In conclusion, as previously proposed [4], the decrease in proton pumping efficiency of the respiratory chain seems to depend on the size of electron flow, although at low respiratory rate we cannot exclude an effect of the Δp value. This decrease of proton pump efficiency reflects an increase in pump slippage. Moreover, the increase in slip is a saturating function of the respiratory rate; state 4 respiration which is normally obtained under conditions where the electron supply is not controlling corresponds to a maximal value of the slip.

The experiments reported here show that the energetic status of static-head respiration is controlled by both leak and slip. The exponential increase in H^+ conductance defines the Δp value at this steady state which is buffered at near 200 mV. When electron supply is limited, a low respiratory rate is sufficient for maintaining this force. But, in the absence of such a limitation, respiratory rate in state 4 corresponds to a saturation of slip, a state in which the efficiency of the proton pump is the weakest.

Limitation of respiratory rate by using respiratory chain inhibitor titration is not a suitable method for investigating the relationships between respiratory rate and Δp. Indeed, under this condition the electron flux through each respiratory unit is supposed to remain constant and at maximal slipping value, in contrast to the other method using the NADH-regenerating system. The slip in state 4 appears to be higher in yeast ($K^+/O = 1$) than in rat liver ($K^+/O = 2.3$, see Ref. 18) mitochondria. The fact that the efficiency of P/O in yeast and in mammalian mitochondria is similar (cf. Introduction) can be taken as evidence that the energy dissipation by proton pump slippage decreases more in yeast than in mammalian mitochondria as function of Δp.

References

1 Hinkle, P.C. and Yu, M.L. (1979) J. Biol. Chem., 254, 2450–2455.
2 Beavis, A.D. and Lehninger, A.L. (1986) Eur. J. Biochem., 158, 315–322.
3 Stoner, C.D. (1987) J. Biol. Chem., 262, 10445–10453.
4 Ouhabi, R., Rigoulet, M. and Guérin, B. (1989) FEBS Lett., 254, 199–202.
5 Mitchell, P. and Moyle, J. (1969) Eur. J. Biochem., 7, 471–484.
6 Nicholls, D.G. (1974) Eur. J. Biochem., 50, 305–315.
7 Rigoulet, M., Guérin, B. and Denis, M. (1987) Eur. J. Biochem., 168, 275–279.
8 Nicholls, D.G. (1976) FEBS Lett., 61, 103–107.
9 Nicholls, D.G. (1977) Eur. J. Biochem., 77, 349–356.
10 Azzone, G.F., Pozzan, T., Massani, S. and Bragadin, M. (1978) Biochim. Biophys. Acta 501, 296–306.
11 Pietrobon, D., Azzone, G.F. and Walz, D. (1981) Eur. J. Biochem. 117,389–394.
12 Krishnamoorthy, G. and Hinkle, P.C. (1984) Biochemistry 23, 1640–1645.
13 Brown, G.C. and Brand, M.D. (1986) Biochem. J., 234, 75–81.
14 Brown, G.C. (1989) J. Biol. Chem., 264, 14704–14709.
15 O'Shea, P.S., Petrone, G., Casey, R.P. and Azzi, A. (1984) Biochem. J. 219, 719–726.
16 Zoratti, M., Favaron, M., Pietrobon, D. and Azzone, G.F. (1986) Biochemistry 25, 760–767.
17 Pietrobon, D., Zoratti, M. and Azzone, G.F. (1983) Biochim. Biophys. Acta 723, 317–321.
18 Murphy, M.P. and Brand, M.D. (1987) Nature 329, 170–172.
19 Murphy, M.P. (1989) Biochim. Biophys. Acta 977, 123–141.
20 Groen, B.H., Berden, J.A. and Van Dam, K. (1990) Biochim. Biophys. Acta 1019, 121–127.
21 Luvisetto, S., Conti, E., Buso, M. and Azzone, G.F. (1990) EBEC Rep. 6, 20.
22 Von Jagow, G. and Klingenberg, M. (1970) Eur. J. Biochem. 12, 583–592.
23 Guérin, B., Labbe, P. and Somlo, M. (1979) Methods Enzymol. 55, 149–159.
24 Rottenberg, H. (1979) Methods Enzymol. 55, 547–569.
25 Murphy, M.P. and Brand, M.D. (1988) Eur. J. Biochem. 173, 637–644.
26 Ezzahid, Z., Rigoulet, M. and Guérin B. (1986) J. Gen. Microbiol. 132, 1153–1158.
27 Singer, T.P., Rocca, E. and Kearney, E.B. (1966) in: Flavins and Flavoproteins (Slater, E.C., ed.) pp. 391–419, Elsevier, Amsterdam.
28 Duszynski, J. and Wojtczak, L. (1985) FEBS Lett., 182, 243–248.
29 Luvisetto, S., Pietrobon, D. and Azzone, G.F. (1987) Biochemistry 26, 7332–7338.
30 Luvisetto, S., Pietrobon, D. and Azzone, G.F. (1989) Biochemistry 28, 1100–1108.
31 Rigoulet, M. (1990) Biochim. Biophys. Acta 1018, 185–189.