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Thermodynamic and kinetic control of ATP synthesis in yeast mitochondria: role of ΔpH

Bertrand Beauvoit, Michel Rigoulet and Bernard Guerin

Institut de Biologie Cellulaire et de Neurochimie du CNRS, 1 rue Camille Saint-Saens, 33077 Bordeaux Cedex, France

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ATP synthesis rate, measured as the variation in external P_i concentration, varied as a linear function of either $\Delta\tilde{\mu}H^+$ or ΔGpin, in such a manner that the ΔGpin/$\Delta\tilde{\mu}H^+$ ratio increased while V_{ATP} increased. We also observed a linear dependence of the flux control coefficient of the P_i carrier on ΔpH. All the results presented can be explained by a relatively large ΔpH drop when V_{ATP} increases.

ATP synthesis; Phosphate carrier; Thermodynamic control; Kinetic control; ΔpH; (Yeast mitochondria)

1. INTRODUCTION

At low external phosphate concentration, phosphate transport in isolated yeast mitochondria controls neither oxygen consumption flux, nor ATP synthesis rate [1], whereas, at high phosphate concentration, this transport does become a controlling step. Indeed, for both fluxes measured at steady state, a transition from zero to high control coefficient (up to 0.5) can be observed for the threshold value of around 2 mM external phosphate. Since the phosphate carrier catalyses an electroneutral co-transport, H^+/P_i^-, we have proposed that a decrease in ΔpH during the increase of oxidative phosphorylation could play a key role in control coefficient evolution.

Here, we studied the distribution of the transported substrates in extra- and intra-matrix spaces as a function of the ATP synthesis rate. In parallel, the flux control coefficient of phosphate transport was obtained from specific inhibitor titration experiments according to [2]. Our results showed that the flux control coefficient is directly linked to the ΔpH. Moreover, we also observed linear relationships between ATP synthesis flux and either the intramatrix phosphate potential (ΔGpin), or $\Delta\tilde{\mu}H^+$. Kinetic control of ATP production by the phosphate carrier is discussed in the framework of the tight dependences of proton translocator components of the ATP-producing system on thermodynamic strengths, i.e. $\Delta\tilde{\mu}H^+$ and phosphate potential.

Correspondence address: B. Guerin, IBCN, 1 rue Camille Saint-Saens, 33077 Bordeaux Cedex, France

2. MATERIALS AND METHODS

2.1. *Preparation of mitochondria*

Cells of diploid wild-strain *Saccharomyces cerevisiae* (yeast foam) were grown aerobically at 28°C in complete medium [1% yeast extract, 0.1% potassium phosphate, 0.12% ammonium sulfate (pH 4.5), supplemented with 2% lactate as carbon source]. Cells were harvested in the logarithmic growth phase. Mitochondria were isolated from protoplasts as in [3]. Protein concentration was measured by the biuret method using bovine serum albumin as standard.

2.2. *Phosphorylation assay*

$^{32}P_i$ incorporation into nucleotides was carried out at 27°C in a 3 ml chamber equipped with a Clark oxygen electrode (Gilson). Mitochondria (0.3–1 mg protein) were suspended in the following basal medium: 0.59 M mannitol, 10 mM Tris-maleate, 0.3% (w/v) bovine serum albumin (pH 6.7) containing 1 mM ADP, 10 μM RbCl, 0.3 μg valinomycin and 1 mM NADH or 0.6% ethanol as substrates. When ethanol was the substrate, 10 mM arsenite was added in order to inhibit substrate-level phosphorylation. ATP synthesis rate was determined as in [4].

Under our experimental conditions, $^{32}P_i$ incorporation into

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nucleotides remaining on the filter was due only to net ATP synthesis, as determined by bioluminescence assay [5] with a luminometer 1250 (LKB-Wallac). On the other hand, it was verified that the presence of an ATP trap (glucose, hexokinase) was not necessary to measure the initial rate of ATP synthesis.

2.3. *Measurements of ΔpH, Δψ and P_i, ADP and ATP distribution*

The matrix space was determined by using [^{3}H]water and inner-membrane-impermeable [^{14}C]mannitol. ΔpH and Δψ were measured by distribution of [^{3}H]acetate and ^{86}Rb (in the presence of valinomycin), respectively [6]. Routinely, after equilibration (2 min), mitochondria were separated from the medium by rapid centrifugation through a silicone oil layer (silicone AR 200 fluid).

Extra- and intra-mitochondrial phosphate concentrations were estimated either by isotopic measurement of the $^{32}P_i$ distribution after separation of P_i and nucleotides (see above), or by P_i assay according to Berenblum and Chain [7] or Summer [8]. Extra- and intra-matrix ADP and ATP concentrations were measured either after isocratic separation by HPLC as in [9], or by bioluminescence assays. For calculation of the internal [ATP]/[ADP]·[P_i] ratio, the matrix ADP and P_i contents were corrected for bound species, as described in [9].

3. RESULTS AND DISCUSSION

Different steady states of ATP synthesis linked to NADH or ethanol oxidation were obtained in the presence of a saturating concentration of ADP and of various concentrations of external P_i. Under these conditions, we measured in parallel experiments (i) the rate of ATP synthesis, (ii) the extra- and intra-mitochondrial concentrations of P_i, (iii) the transmembrane difference of pH, and (iv) the control exerted by the phosphate carrier on ATP synthesis flux. When an irreversible inhibitor is used, the flux control coefficient can be obtained directly from the inhibition curve:

$$C_{iJ} = -\frac{dJ/J}{dI/I_{max}}$$

where I_{max} is the amount of inhibitor required for total inhibition of the enzyme [2]. Control of the P_i transport step was evaluated with mersalyl as described [1].

The relationships between either ΔpH or flux control coefficient of the P_i carrier, and extramatrix P_i concentration, are shown in fig.1A (NADH as substrate) and B (ethanol as substrate). For each substrate tested, a symmetrical change in flux control coefficient of the P_i carrier and ΔpH is observed: the lower the ΔpH, the greater is the

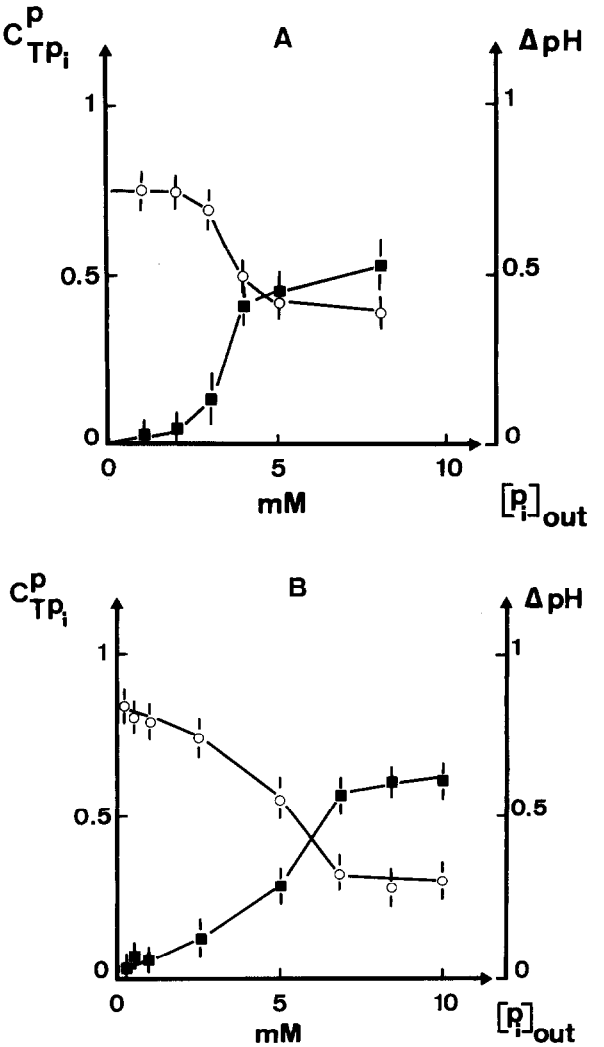

Fig.1. Relationships between either ΔpH or flux control coefficient of phosphate carrier and extramatrix P_i concentration. Mitochondria (1 mg protein) were incubated in the respiratory medium as indicated in section 2 supplemented with either 1 mM NADH (A) or 0.6% ethanol in the presence of 10 mM arsenite (B) and various concentrations of P_i. ΔpH was determined as given in section 2 and the flux control coefficient of P_i carrier calculated as described in the text. (○) ΔpH, (■) flux control coefficient.

flux control coefficient. The dependence of either the flux control coefficient or ΔpH as a function of external P_i is roughly sigmoidal, although the transition is more pronounced with NADH (fig.1A) than ethanol (fig.1B) as substrates. It is worth noting that these transitions can be related to that

occurring between the two phenomenological hyperbolas fitting the dependence of the ATP synthesis rate on external P_i (see [1] for NADH and [4] for ethanol). Moreover, fig.2 indicates a unique linear relationship between control coefficient and ΔpH values irrespective of the respiratory substrate. This fact supports our previous hypothesis [1] that control by the P_i carrier, at high external P_i concentration, exhibits a change in the balance, at a given steady state, between P_i influx and efflux induced by ΔpH change.

Control exerted by the P_i carrier prompted us to check the dependence of the ATP synthesis rate on intramitochondrial phosphate potential (ΔGpin). Fig. 3 shows a unique relationship between V_{ATP} and ΔGpin with NADH or ethanol as respiratory substrates. By extrapolation of the curve to $V_{ATP} = 0$, it is possible to evaluate the maximal ΔGpin of oxidative phosphorylation in yeast mitochondria. By taking into account a standard free energy equal to 28.4 kJ/mol [10], the ΔGpin value is 43.3 kJ/mol, in accordance with [11]. An important point due to the way V_{ATP} varies is that the changes in ΔGpin are essentially due to a variation in the internal phosphate concentration (not shown). Fig.3 also shows the linear relationship between V_{ATP} and $\Delta\tilde{\mu}H^+$. By extrapolation of these curves to $V_{ATP} = 0$, it is possible to evaluate the number n of H^+ used by the ATP synthase to syn-

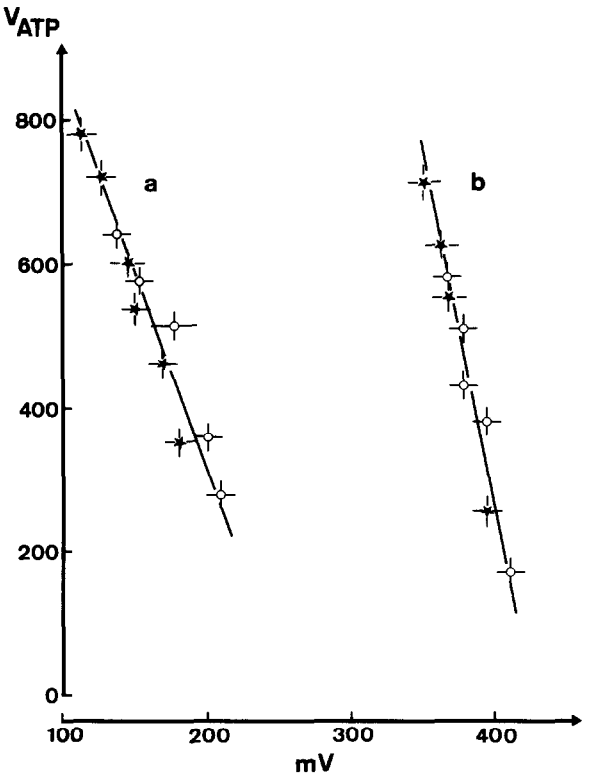

Fig.3. Relationships between ATP synthesis rate and either $\Delta\tilde{\mu}H^+$ (a) or ΔGpin (b). Matrix volume, ΔpH, $\Delta\psi$ and P_i, ADP, ATP concentrations were determined as described in section 2 during ATP synthesis with NADH (★) or ethanol (○) as substrates. V_{ATP} given as nmol ATP·min^{-1}·mg^{-1} protein.

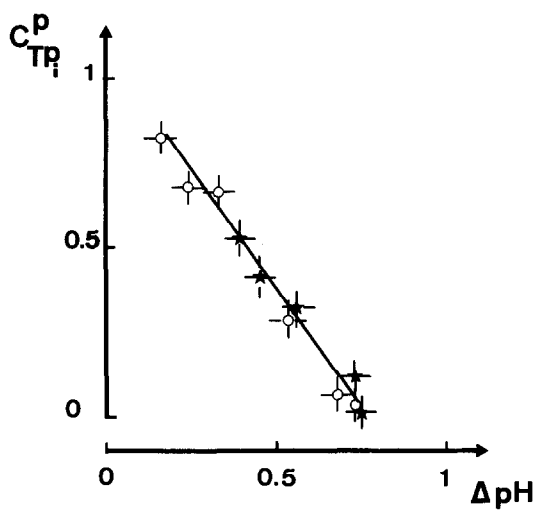

Fig.2. Flux control coefficient of phosphate carrier as a function of ΔpH. Experimental values were from fig.1; the respiratory substrates were NADH (★) or ethanol (○).

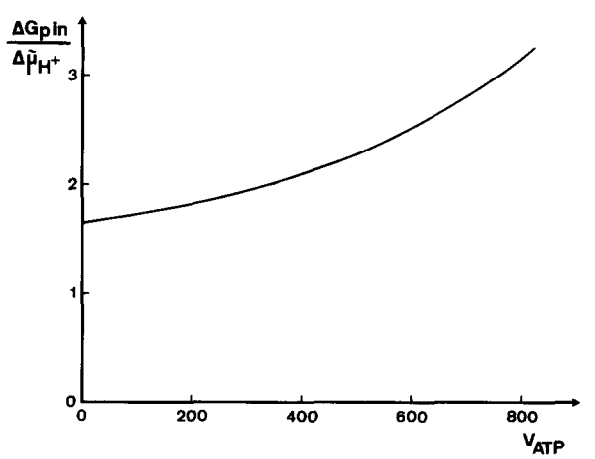

Fig.4. Relationship between ΔGpin/$\Delta\tilde{\mu}H^+$ ratio and V_{ATP}. The curve is a fit from experimental values of fig.3.

thesize one molecule of ATP, assuming a thermodynamic equilibrium at this very point. The value of 1.65 is only an estimation, since this kind of calculation needs some assumptions (equilibrium, substrates not bound, as discussed in [9] for yeast mitochondria) to be taken into account.

One also observes in fig.3 that V_{ATP} is more dependent on variations in ΔGpin than $\Delta\tilde{\mu}H^+$. Consequently, the ATP synthesis rate could strongly depend on the enzymatic steps determining the size of ΔGpin. Indeed, under our experimental conditions, the P_i carrier activity controls the ΔGpin drop according to the variation of the external P_i concentration.

Fig.4 shows the variations in $\Delta G\text{pin}/\Delta\tilde{\mu}H^+$ as function of V_{ATP} deduced from fig.3. In assuming thermodynamic equilibrium for $V_{ATP} = 0$ and no change in the H^+/ATP stoichiometry, the curve would indicate that the higher is the ATP synthesis rate, the further from equilibrium is the system.

Control of ATP synthesis by the P_i carrier appears as a consequence of the relatively large decrease in ΔpH (as compared to $\Delta\tilde{\mu}H^+$) when the external P_i concentration rises. Thus, although the contribution of ΔpH to $\Delta\tilde{\mu}H^+$ is weak, it plays an essential role in the control exerted on ATP synthesis.

REFERENCES

[1] Mazat, J.P., Jean-Bart, E., Rigoulet, M. and Guerin, B. (1986) Biochim. Biophys. Acta 849, 7–15.
[2] Groen, A.K., Wanders, R.J.A., Westerhoff, H.V., Van der Meer, R. and Tager, J.M. (1982) J. Biol. Chem. 257, 2754–2757.
[3] Guerin, B., Labbe, P. and Somlo, M. (1979) Methods Enzymol. 55, 149–159.
[4] Rigoulet, M., Ezzahid, Z. and Guerin, B. (1983) Biochem. Biophys. Res. Commun. 113, 751–756.
[5] Lundin, A., Rickardsson, A. and Thore, A. (1976) Anal. Biochem. 75, 611–620.
[6] Rottenberg, H. (1979) Methods Enzymol. 55, 547–569.
[7] Berenblum, I. and Chain, E. (1938) Biochem. J. 32, 286–294.
[8] Summer, J.B. (1944) Science 100, 413–418.
[9] Rigoulet, M., Velours, J. and Guerin, B. (1985) Eur. J. Biochem. 153, 601–607.
[10] Rosing, J. and Slater, E.C. (1972) Biochim. Biophys. Acta 267, 275–290.
[11] Lauquin, G. and Vignais, P.V. (1973) Biochim. Biophys. Acta 305, 534–556.