

# Thermodynamic and kinetic control of ATP synthesis in yeast mitochondria: role of $\Delta pH$

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

ATP synthesis; Phosphate carrier; Thermodynamic control; Kinetic control;  $\Delta pH$ ; (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  $\Delta pH$  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  $\Delta pH$ . Moreover, we also observed linear relationships between ATP synthesis flux

and either the intramatrix phosphate potential ( $\Delta G_{pin}$ ), or  $\Delta\bar{\mu}H^+$ . 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.  $\Delta\bar{\mu}H^+$  and phosphate potential.

## 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  $\mu$ M RbCl, 0.3  $\mu$ g 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 $\Delta pH$ , $\Delta\psi$ and $P_i$ , ADP and ATP distribution

The matrix space was determined by using [ $^3H$ ]water and inner-membrane-impermeable [ $^{14}C$ ]mannitol.  $\Delta pH$  and  $\Delta\psi$  were measured by distribution of [ $^3H$ ]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 Sumner [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  $[ATP]/[ADP] \cdot [P_i]$  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  $\Delta pH$  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  $\Delta pH$  is observed: the lower the  $\Delta pH$ , the greater is the

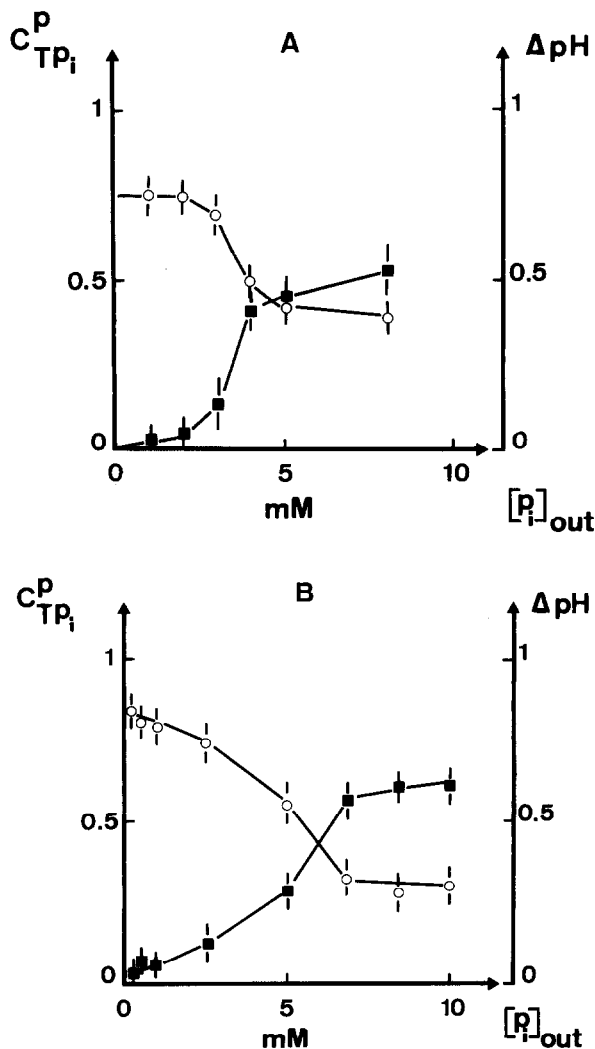


Fig.1. Relationships between either  $\Delta pH$  or flux control coefficient of phosphate carrier and extramatrix  $P_i$  concentration. Mitochondria (1 mg protein) were incubated in the respiratory medium as indicated in section 2 supplemented with either 1 mM NADH (A) or 0.6% ethanol in the presence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

flux control coefficient. The dependence of either the flux control coefficient or  $\Delta pH$  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  $\Delta pH$  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  $\Delta pH$  change.

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

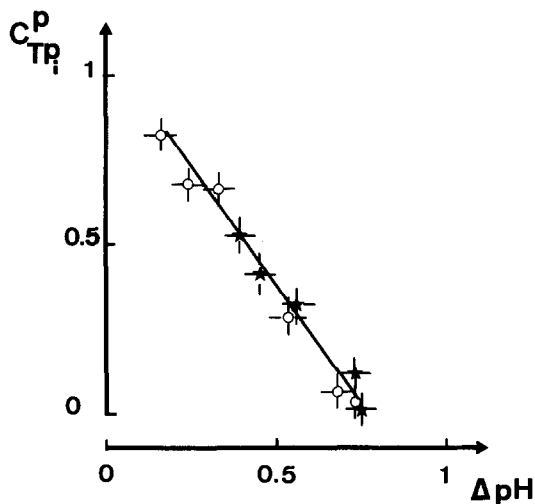


Fig.2. Flux control coefficient of phosphate carrier as a function of  $\Delta pH$ . Experimental values were from fig.1; the respiratory substrates were NADH (★) or ethanol (○).

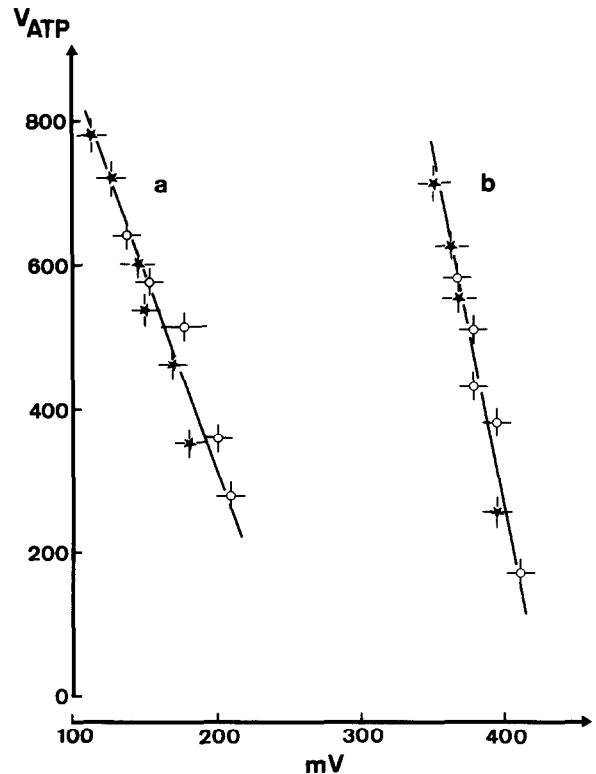


Fig.3. Relationships between ATP synthesis rate and either  $\Delta \mu H^+$  (a) or  $\Delta G_{pin}$  (b). Matrix volume,  $\Delta pH$ ,  $\Delta \psi$  and  $P_i$ , ADP, ATP concentrations were determined as described in section 2 during ATP synthesis with NADH (★) or ethanol (○) as substrates.  $V_{ATP}$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

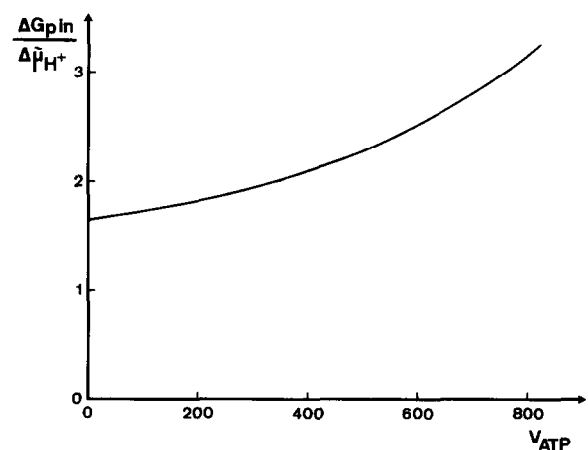


Fig.4. Relationship between  $\Delta G_{pin}/\Delta \mu H^+$  ratio and  $V_{ATP}$ . The curve is a fit from experimental values of fig.3.

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  $V_{\text{ATP}}$  is more dependent on variations in  $\Delta G_{\text{pin}}$  than  $\Delta \mu \text{H}^+$ . Consequently, the ATP synthesis rate could strongly depend on the enzymatic steps determining the size of  $\Delta G_{\text{pin}}$ . Indeed, under our experimental conditions, the  $\text{P}_i$  carrier activity controls the  $\Delta G_{\text{pin}}$  drop according to the variation of the external  $\text{P}_i$  concentration.

Fig.4 shows the variations in  $\Delta G_{\text{pin}}/\Delta \mu \text{H}^+$  as function of  $V_{\text{ATP}}$  deduced from fig.3. In assuming thermodynamic equilibrium for  $V_{\text{ATP}} = 0$  and no change in the  $\text{H}^+/\text{ATP}$  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  $\text{P}_i$  carrier appears as a consequence of the relatively large decrease in  $\Delta \text{pH}$  (as compared to  $\Delta \mu \text{H}^+$ ) when the external  $\text{P}_i$  concentration rises. Thus, although the

contribution of  $\Delta \text{pH}$  to  $\Delta \mu \text{H}^+$  is weak, it plays an essential role in the control exerted on ATP synthesis.

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