KINETIC CONTROL AND REACTION PRESSURE IN STEADY STATE COUPLED MITOCHONDRIA

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INTRODUCTION

Current studies on mitochondrial respiratory control focus on the role of functional coupling of mitochondrial creatine kinase (Jacobus, 1985; Biermans et al., 1990) and contact sites between inner and outer mitochondrial membrane (Nicolay et al., 1990) in regulating biological energy transformation. Kinetic and thermodynamic models serve as primary tools to investigate the specific mechanisms of such functional coupling (Gnaiger and Jacobus, 1989) and contact sites. We therefore combined kinetic and thermodynamic approaches to investigate fundamental relations of adenylate control in oxidative phosphorylation.

METHODS

Rat liver mitochondria were prepared and biochemical assays carried out as described by Haller (1990). Respiration was studied with the CYCLOBIOS Oxygraph (pp. 13-16) at 37 °C. Mitochondria were preincubated in measuring buffer (see Haller, 1990) containing 20 mmol.dm^{-3} glucose, 1 mmol.dm^{-3} ATP and 2 mmol.dm^{-3} KH_2PO_4, $[P_{i,o}]$. Steady state respiration was initiated by titration of hexokinase (Sigma Type C-301 bakers yeast) into the oxygraph medium. Steady state oxygen flux occurred within a few seconds delay after pulsed addition of the enzyme, and remained linear for several minutes. Samples were withdrawn for biochemical analysis usually upon reaching in the oxygraph 70% air saturation. The time interval was noted from the addition of hexokinase to the sample extraction. Final inorganic phosphate concentrations $[P_i]$ were calculated from the initial P_i concentration $[P_{i,o}]$ and Glu-6-P and ADP formation,

$$[P_i] = [P_{i,o}] - [\text{Glu-6-P}] + [\text{ADP}] \quad (1)$$

RESULTS AND DISCUSSION

A typical hexokinase titration curve is shown in Fig. 1. At saturating hexokinase activities, respiratory fluxes were observed up to 20% above State 3 (addition of saturating ADP concentrations). Binding of the hexokinase to the mitochondria appears at present to be the only explanation of this important phenomenon of "superflux". However, yeast hexokinase does not bind effectively to the porine, the voltage dependent anion selective channel of rat liver mitochondria (Nicolay et al., 1990).

At zero hexokinase addition, a significant oxygen flux may be due to endogenous ATPases. We tested specifically for endogenous (bound) hexokinase activity by incubating liver mitochondria with ATP and glucose. A linear increase was observed of Glu-6-P concentration with time in the oxygraph medium at a rate of 0.3 nmol.min^{-1}.mg^{-1} mitochondrial protein, which is very small in comparison to stimulated respiration.

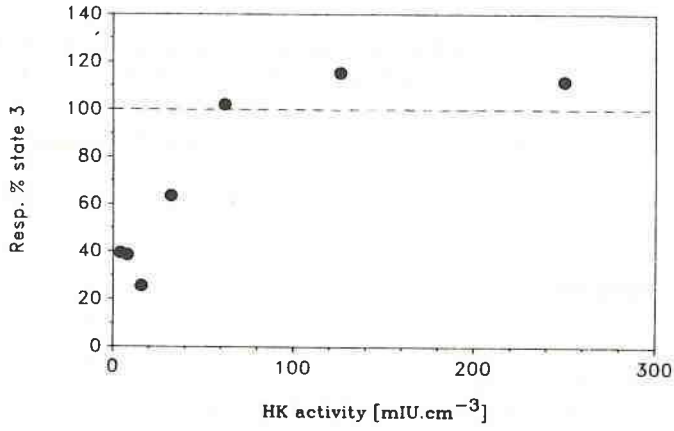

Fig. 1. Titration curve for hexokinase-stimulated respiration. One IU is defined to phosphorylate one μmol glucose.min^{-1} at pH 8.5 and 25 °C. Mitochondrial oxygen flux is expressed in % of ADP State 3 respiratory flux, determined before and after hexokinase stimulated respiration. Note the phenomenon of "superflux" above 100 % State 3.

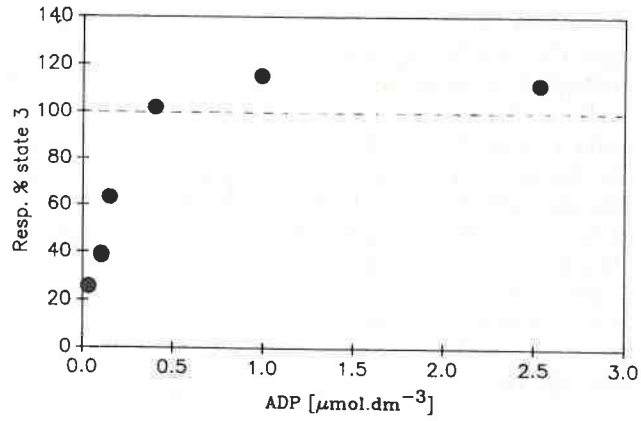

Fig. 2. **Kinetic flux/concentration relation.** Oxygen flux of steady state coupled mitochondria as a function of ADP-concentration.

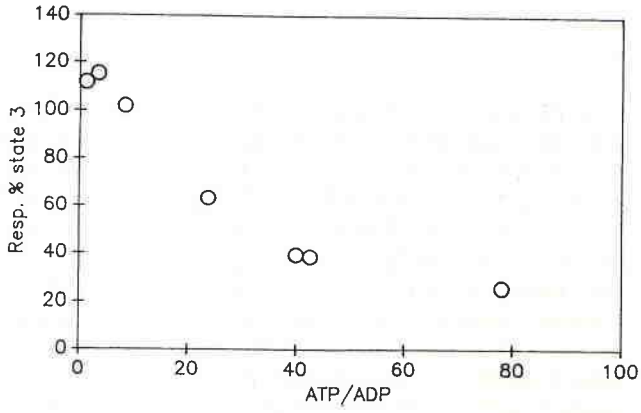

Fig. 3. Oxygen flux of steady state coupled mitochondria as a function of the ATP/ADP ratio.

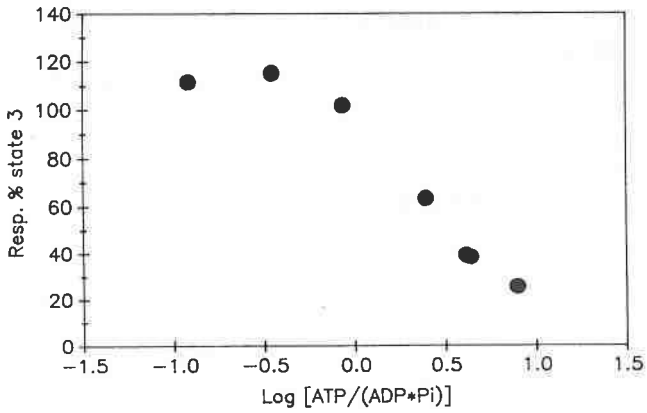

Fig. 4. **Thermodynamic flux/force relation.** Oxygen flux of steady state coupled mitochondria as a function of phosphorylation potential. The plot is apparently linear in the range of phosphorylation potentials corresponding to Gibbs forces, $\Delta_p G$, from 61 to 54 kJ.mol^{-1}.

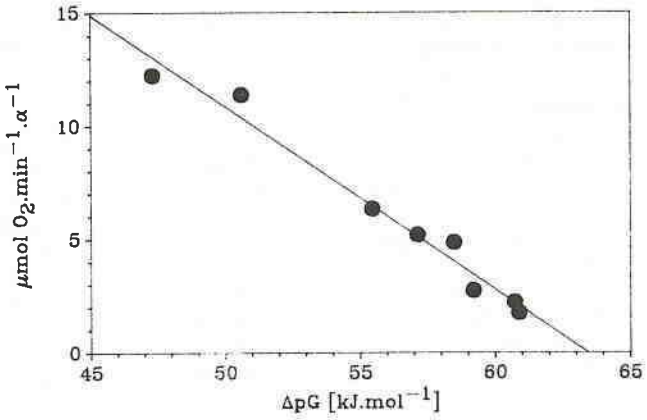

Fig. 5. **Ergodynamic rate/force relation.** The rate of oxygen consumption is the oxygen flux divided by the *free activity*. $\Delta_p G$ is the output force, and the catabolic input force is constant. From the linear rate/force relation, a linear flux/pressure relation can be predicted (cf. Fig. 6).

Figures 2 to 4 show respiratory oxygen flux as a function of measured ADP concentrations, ATP/ADP ratios and phosphorylation potentials. These results are consistent with data reported by Jacobus et al. (1982).

As discussed by Gnaiger and Jacobus (1989), the kinetic (Fig. 2) and thermodynamic (Fig. 4) description of flux can be extended by incorporating the concept of generalized flux/pressure relations (Haller and Gnaiger, 1990). We calculated the *free activity* from the adenylate and phosphate concentrations and pH according to the equations of Gnaiger (1989). The present data are consistent with a linear rate/force relation (Fig. 5) and a linear flux/pressure relation (Fig. 6). Therefore, flux/pressure relations provide a promising approach to study the regulation of mitochondrial energy transformation under physiologically relevant conditions far from equilibrium.

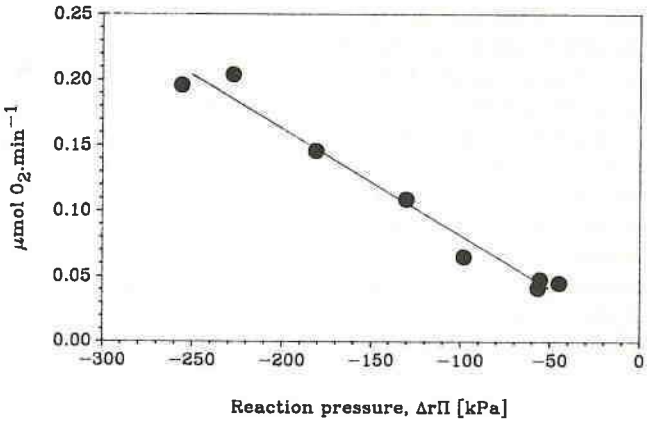

Fig. 6. **Ergodynamic flux/pressure relation.** Oxygen flux of steady state coupled mitochondria as a function of catabolic coupled adenylate reaction pressure.

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