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Dependence of yeast mitochondrial unselective channel activity on the respiratory chain

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Abstract

The dependence of yeast mitochondrial unselective channel activity on the respiratory chain was investigated. Modulation of the respiratory chain with different substrates and inhibitors showed that channel activity was dependent on the electron flow rate through the chain and that external NADH only could provide a sufficient rate to activate the channel. These results support the hypothesis that the yeast mitochondrial unselective channel may be involved in the oxidation of cytosolic NADH without coupling to ATP synthesis.

Keywords: Yeast mitochondria; Ionic permeability; ATP; Respiratory chain; NADH-dehydrogenase

1. Introduction

The inner mitochondrial membrane of the yeast *Saccharomyces cerevisiae* supports a large-conductance unselective channel regulated by nucleotides and phosphate (YMUC for yeast mitochondrial unselective channel) ([1] for a recent review). Activation of this channel is the cause of ATP-induced stimulation of the respiration of mitochondria isolated from a laboratory strain, at low phosphate concentration [2]. YMUC activity can also be measured directly by ATP-induced non-energetic swellings of mitochondria suspended in iso-osmotic solutions of different salts [3–5] or in a hypotonic solution of mannitol [6]. The lack of specificity and the large conductance of YMUC led to the proposal that it could be a yeast equivalent of the mammalian permeability transition pore (PTP) ([7,8] for reviews). However, YMUC is neither regulated by Ca^{2+}, nor inhibited by cyclosporin A [6], two major characteristics of PTP.

Under energetic conditions (i.e. in the presence of a $\Delta\Psi$ maintained by the respiratory chain), YMUC characteristics depended both on yeast strains and on respiratory substrates. Prieto et al. [2] and Roucou et al. [5] observed that YMUC of laboratory strains was able to transport protons, whatever the respiratory substrate (glycerol-3-phosphate, ethanol, NADH). On the other hand, Roucou et al. [5] observed that, in an industrial strain, YMUC specificity depended on the respiratory substrate. Upon ethanol-dependent respiration, mitochondria were able to transport K^+ but not Na^+, Li^+ or H^+ [9]. Upon external NADH-dependent respiration, mitochondria were able to transport K^+, Na^+ or Li^+ but

Abbreviations: ADH, alcohol dehydrogenase; BSA, bovine serum albumin; ClCCP, *p*-carbonylcyanide *m*-chlorophenylhydrazone; PTP, permeability transition pore; YMUC, yeast mitochondrial unselective channel

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not H^+. This was confirmed by swelling experiments [5].

This was a crucial point to understand the function of YMUC since its ability to transport cations had consequences on the coupling efficiency between respiration and ATP synthesis [10,11] and on the regulation of phosphate transport [12]. Moreover, evidence for a possible activity of YMUC in whole cells was obtained [13].

In the present paper, we investigated the dependence of the ATP-induced K^+ transport activity of YMUC on the respiratory chain, and we showed that YMUC activity was dependent on the rate of electron flow, and that external NADH dehydrogenase only could provide a rate high enough to induce YMUC activity.

2. Materials and methods

The industrial diploid strain Yeast Foam (PS194) was grown aerobically in a semi-synthetic medium containing 1% yeast extract (Difco), 0.1% KH_2PO_4, 0.12% $(NH_4)_2SO_4$, 2% DL-lactate (pH 5.0 with NaOH) and harvested in mid-exponential growth phase (OD_{550nm} = 4–5). Mitochondria were isolated according to [14]. Proteins were measured with a biuret method.

Mitochondria (0.33 mg/ml) were suspended in a 10 mM Tris/maleate buffer (pH 6.8) added with 0.4 M mannitol, 0.1 M KCl, 2 mM EGTA, in the presence of a low (0.5 mM) or a high (5 mM) phosphate concentration (added as KH_2PO_4). NADH (2 mM), ethanol (40 mM) or glycerol-3-phosphate (4 mM) were used as respiratory substrates. Respiration rates were measured with a Clark-type electrode. Initial rates of ATP synthesis were measured simultaneously, after the addition of 1 mM ADP. At different times, 0.1 ml aliquots were added to 0.1 ml of 7% perchloric acid, 25 mM EDTA. After centrifugation, supernatants were neutralized with 0.065 ml KOH 2 N, MOPS 0.5 M. ATP amounts were measured by bioluminescence with a luciferin/luciferase kit (Bio-Orbit) in a LKB bioluminometer. All products were from Sigma except glycerol-3-phosphate (Boehringer).

Table 1
Effect of ATP on respiration rates of isolated mitochondria

	State 4 (control)	+ATP	Stimulation ratio	
			ATP	ClCCP
Preparation 1				
NADH	444 ± 21	908 ± 26	2.05	2.12
Glycerol-3-phosphate	89 ± 8	98 ± 8	1.10	1.85
Ethanol	159 ± 13	271 ± 18	1.70	2.43
Ethanol+flavone	114 ± 8	118 ± 7	1.04	2.18
Preparation 1+digitonin			Stimulation ratio	
NADH	342 ± 9	619 ± 20	1.81	
Ethanol	76 ± 6	76 ± 10	1.00	
Preparation 2				
NADH	339 ± 14	675 ± 20	1.99	
Glycerol-3-phosphate	151 ± 7	151 ± 9	1.00	
Ethanol	144 ± 8	156 ± 6	1.08	
Ethanol+NAD^+	216 ± 16	380 ± 21	1.76	
Ethanol+NAD^++ADH	232 ± 22	480 ± 19	2.07	

Mitochondria (0.33–0.66 mg/ml) were suspended in a 10 mM Tris/maleate buffer (pH 6.8) containing 0.4 M mannitol, 0.1 M KCl, 2 mM EGTA, 0.3% BSA, 0.5 mM K-phosphate. NADH, glycerol-3-phosphate and ethanol were added at 2 mM, 5 mM and 40 mM, respectively. When added, ATP, ClCCP and flavone were 2 mM, 6 μM and 50 μM, respectively. Digitonin treatment was done according to [18]. Control experiments (not shown), done in the presence of oligomycin to eliminate any possible stimulatory effect of contaminating ADP in ATP, gave similar results. Data (in nat.O/min/mg proteins) are means (± S.D.) of at least four measurements.

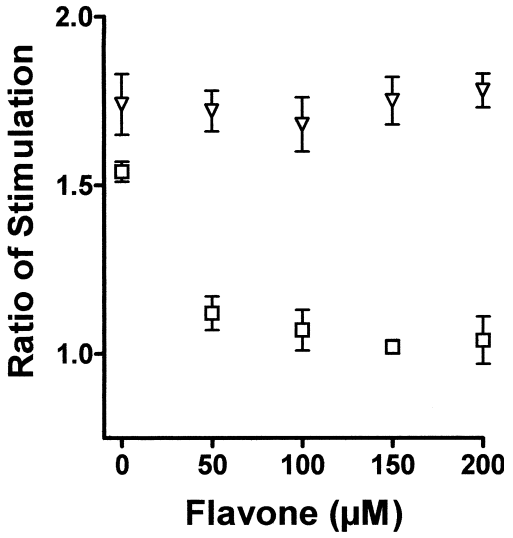

Fig. 1. Effect of flavone on ATP- or ClCCP-induced stimulation. Mitochondria were suspended as indicated in the legend of Table 1, with ethanol as a respiratory substrate, in the absence and the presence of 6 μM ClCCP (triangles) or 2 mM ATP (squares), and the indicated concentrations of flavone. The ratio of ATP- or ClCCP-induced stimulation is indicated. Each point is the mean (±S.D.) of at least four measurements obtained on a single mitochondria preparation, representative of three mitochondria preparations.

3. Results and discussion

3.1. Dependence of YMUC activity on respiratory substrates

We first assayed the dependence of ATP-induced stimulation of respiration rate (in the presence of K^+) on respiratory substrates. Table 1 summarizes data obtained with two different mitochondria preparations, representative of our results. Whatever the preparation, ATP induced a large stimulation of NADH-dependent respiration. Conversely, ATP did not induce any stimulation of glycerol-3-phosphate-dependent respiration. A difference between the two mitochondria preparations was observed with ethanol. With preparation 1, ATP induced stimulation of ethanol-dependent respiration, similar to the stimulation of NADH-dependent respiration. With preparation 2, no significant ATP-induced stimulation of ethanol-dependent respiration was observed.

3.2. Dependence of YMUC on external NADH dehydrogenase

The difference between the two types of mitochondria preparation could be explained by the presence of 'contaminating' cytosolic alcohol dehydrogenase (ADH2) in mitochondria preparation 1, thus allowing the production of external NADH further oxidized by the external NADH dehydrogenase. Ethanol-dependent respiration was measured in the presence of flavone, a non-permeant inhibitor of NADH dehydrogenases (Table 1 and Fig. 1). Flavone inhibited basal ethanol-dependent respiration by 30–40% and fully prevented ATP-stimulated respiration at a concentration that did not affect ClCCP-stimulated respiration. This suggested that part of ethanol-dependent respiration came from the ADH2 → external NADH dehydrogenase route and was responsible for ATP-induced stimulation of respiration.

To confirm this hypothesis, mitochondria preparation 1 was treated with digitonin to solubilize the outer mitochondrial membrane. Under these conditions, NADH-dependent respiration was still stimulated by ATP whereas ethanol-dependent respiration was not, suggesting that the interaction of ADH2

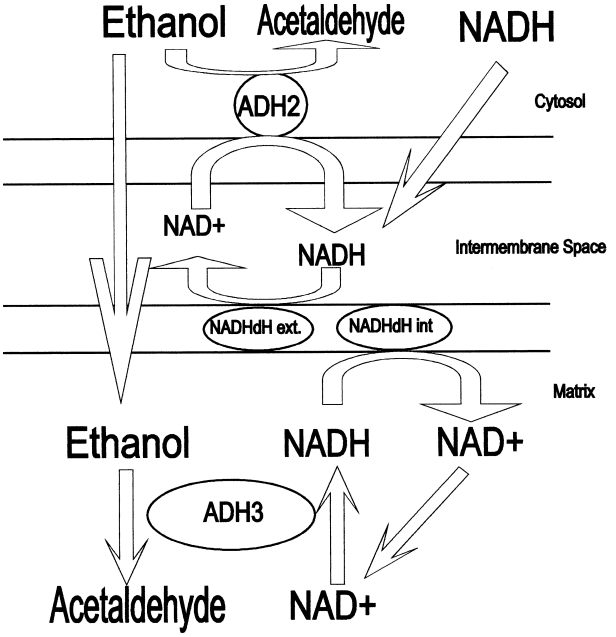

Fig. 2. Dehydrogenases pathways in yeast mitochondria.

with the outer mitochondrial membrane was responsible for the existence of this route.

Conversely, addition of exogenous alcohol dehydrogenase on mitochondria preparation 2 was assayed. The addition of NAD^+ alone increased basal ethanol-dependent respiration, that was further stimulated by ATP. Upon addition of alcohol dehydrogenase, mitochondria preparation 2 retrieved a similar behavior to mitochondria preparation 1.

Results of this first set of experiments are summarized in Fig. 2. The outer mitochondrial membrane of preparation 1 might be intact, allowing attachment of ADH2 to its outer leaflet and trapping of NAD^+ in intermembrane space. The outer membrane of preparation 2 may be altered so that less ADH2 was associated to mitochondria and NAD^+ was released during mitochondria isolation. From data reported above, it can be concluded that external NADH-dependent respiration only (with NADH or external NADH-producing system ethanol/ADH2/NAD^+) allowed stimulation by ATP whereas matrical NADH-dependent respiration (with matrical NADH-producing system ethanol/ADH3/NAD^+) or external glycerol-3-phosphate-dependent respiration did not. This could be explained by (i) different redox potentials, (ii) different electron flow rates through the respiratory chain or (iii) a strict dependence of YMUC on the activity of the external NADH dehydrogenase.

3.3. Dependence of the uncoupling activity of YMUC on electron flow rate

In a second set of experiments, the effect of YMUC activation on energetic coupling between NADH-dependent respiration and ATP synthesis was followed. We reported previously that the ATP/O ratio decreased under conditions where YMUC was able to transport K^+, i.e. at low phosphate concentration (0.5 mM) and in the presence of 100 mM KCl [11]. We then investigated whether a decrease of electron flow (titrated with cyanide)

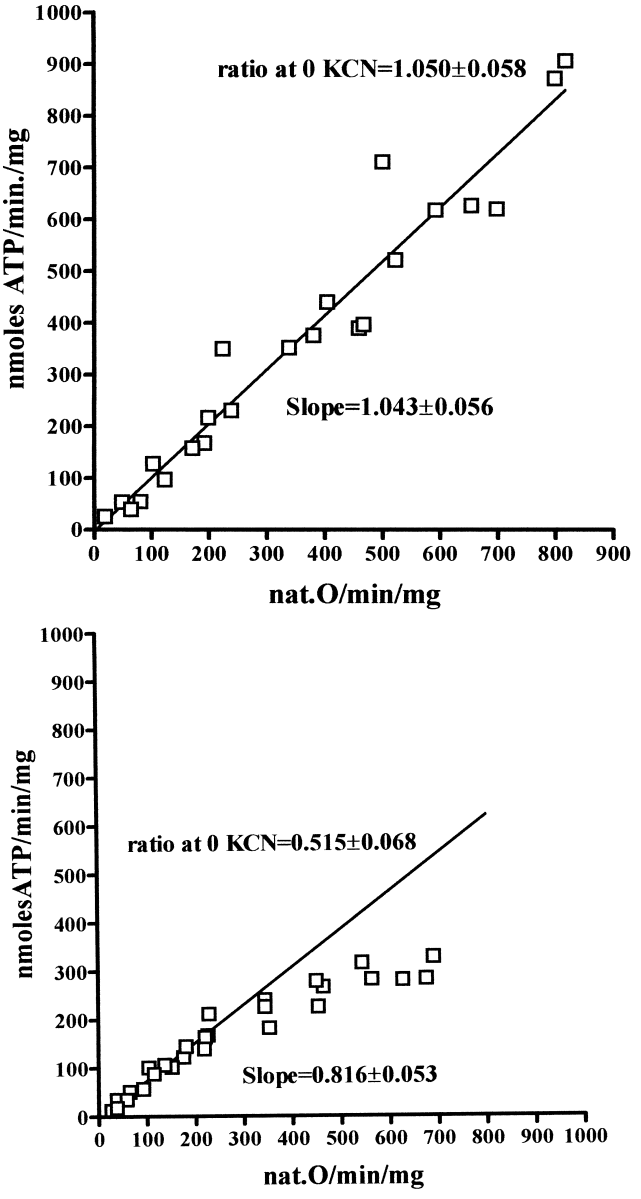

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Fig. 3. Effect of KCN on the coupling efficiency between respiration and ATP synthesis. Mitochondria were suspended as indicated in the legend of Table 1, with 5 mM phosphate (top) or 0.5 mM phosphate (bottom), and 2 mM NADH as the respiratory substrate, in the presence of different KCN concentrations (0–200 μM). The initial rates of ATP synthesis were measured simultaneously (see Section 2). The ATP/O ratio at 0 KCN was calculated as the average of the measurements done at this concentration. The ATP/O slope was calculated by linear regression over the whole curve for 5 mM phosphate (top) and over the points below 300 nat.O/min./mg for 0.5 mM phosphate (bottom). The similarity of the two values shows that the coupling efficiency does not change with respiration rate at 5 mM phosphate, whereas it markedly increases when the respiration rate decreases, at 0.5 mM phosphate. Data from three mitochondria preparations are presented.

could prevent this effect. As a control, the same titration was done under conditions where YMUC is not active, namely at high (5 mM) phosphate concentration. Fig. 3 reports the $J_{ATP} = f(J_{O2})$ curves under both conditions. At high phosphate concentration, the ATP/O value in the absence of cyanide was about 1, comparable to the one obtained in the absence of KCl (see [11]). It can be concluded that, at high phosphate concentration, YMUC was inactive, thus unable to induce any net electrogenic K^+ transport down the transmembrane potential. Conversely, at low phosphate concentration, the ATP/O ratio measured in the absence of KCN was decreased to about 0.5, evidencing a lower coupling efficiency between respiration and ATP synthesis. Since this low value was neither obtained in the absence of KCl, nor in the presence of decavanadate, an inhibitor of YMUC [11], we concluded that YMUC could catalyze a net electrogenic K^+ entry to the matrix. Inhibition of the respiratory chain with KCN increased the ATP/O ratio up to 0.8, a value closer to the one obtained at high phosphate concentration, as demonstrated by the measurement of the slopes at the origin under both conditions. This strongly supports the hypothesis that the uncoupling activity of YMUC decreased with respiration rate. Similar results were obtained by the titration of respiration and ATP synthesis rates with flavone (Fig. 4) instead of KCN, showing that the effect was not dependent on the localization of the inhibition site.

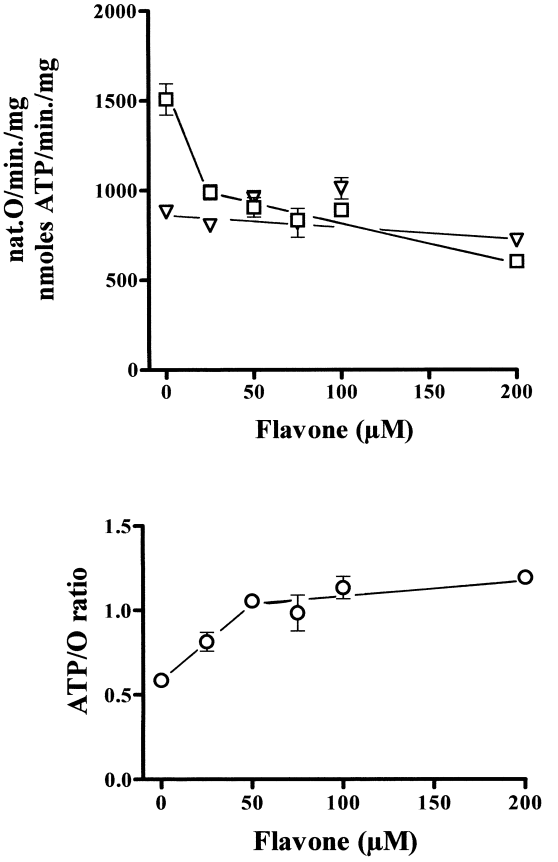

Fig. 4. Effect of flavone on NADH-dependent oxidative phosphorylation. Experimental conditions are similar to those in Table 1 with 0.5 mM phosphate and 2 mM NADH as respiratory substrate. Respiration rates (squares), initial rates of ATP synthesis (triangles) and ATP/O ratios (circles) were measured. Each point is the mean (±S.E.M.) of two determinations on a single mitochondria preparation, representative of three mitochondria preparations.

4. Conclusion

Data reported in this paper demonstrate that ATP-induced K^+ transport activity of YMUC is dependent on the electron flow rate through the respiratory chain. Oxidation of external NADH only is able to provide a sufficient rate to observe the uncoupling effect of YMUC. Neither oxidation of external glycerol-3-phosphate, nor oxidation of matrical NADH could provide an electron flow rate high enough. This was particularly clear when ethanol was used as a respiratory substrate, since it can be reduced both outside and inside the matrix by ADH2 and ADH3, respectively; only mitochondria preparations still containing an intact outer membrane, able to retain both an intermembrane pool of NAD^+ and enough ADH2 associated to the outer membrane, were able to support YMUC activity. It may be noted that, when working on intact or permeabilized spheroplasts, an YMUC activity could be evidenced upon ethanol-dependent respiration [13], suggesting that in whole cells the ADH2/external NADH dehydrogenase pathway was at least as active as the ADH3/internal NADH dehydrogenase pathway.

A recent paper by Fontaine and coworkers [15] describes the dependence of mammalian permeability transition pore (PTP) on the electron flow through complex I. The authors hypothesized that some components of this complex might be involved in the regulation of PTP activity. Although YMUC is not

a strict equivalent of PTP, the two systems have common characteristics [1]. The regulation by the electron flow rate (through complex I in mammalian mitochondria, through the whole respiratory chain in yeast mitochondria) seems to be an additional common point. It is important to note that, from the present paper, the absence of complex I in yeast does not prevent YMUC regulation by the electron flow through the respiratory chain.

This new aspect of YMUC regulation is an additional clue for a role of this system in the modulation of mitochondrial functions, as already suspected from experiments done on whole cells [13]. It is interesting to note that the optimal conditions for the uncoupling effect of YMUC (high ATP, external NADH) correspond to conditions where cells may need to oxidize cytosolic NADH without an obligatory coupling to ATP synthesis. This may be compared to non-phosphorylating alternative respiratory pathways from other organisms. The recent identification of gene(s) encoding the external NADH dehydrogenase(s) [16,17] may be a powerful tool to understand this issue. Further experiments on yeast mitochondria and spheroplasts prepared from wild-type and mutant cells grown under different metabolic conditions will help to confirm this hypothesis.

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