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Rapid report

The ATP-induced K^+-transport pathway of yeast mitochondria may function as an uncoupling pathway

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Abstract

The effect of the presence of K^+ during oxidative phosphorylation measured on isolated yeast mitochondria was dependent on phosphate concentration. At 0.5 mM phosphate, K^+ did promote an uncoupling of oxidative phosphorylation, which was prevented by decavanadate, a potent inhibitor of the ATP-induced K^+-transport pathway. At 5 mM phosphate, no uncoupling effect of K^+ could be evidenced. These data suggest that the ATP-induced K^+-transport pathway may, under certain conditions, function as an uncoupling pathway of oxidative phosphorylation.

Keywords: Oxidative phosphorylation; Permeability, K^+; Vanadate; Decavanadate; (Yeast mitochondrion)

The recent demonstration of the existence of ATP-induced permeabilities in the inner membrane of yeast mitochondria raised some intriguing questions. Prieto et al. [1,2] first observed a stimulation, by ATP, of the respiration rates of mitochondria isolated from the laboratory strain W303 and proposed that a H^+-conducting pathway was involved. These data could not be reproduced on mitochondria isolated from the baker's yeast Yeast Foam except when K^+ was present [3]. Under 'non-energetic' conditions, osmotic swellings [4,5] and electrophysiological [6] experiments demonstrated that ATP could also open anionic conductances. We recently resolved a part of the apparent discrepancies between all these data by demonstrating the complexity of the regulation of these ATP-induced permeabilities [7].

On the other hand, we investigated the effect of KCl on oxidative phosphorylation on isolated yeast mitochondria at high phosphate concentration [8]: K^+ stimulated both respiration rates and ATP synthesis fluxes without effect on the ATP/O ratio. The stimulation of ATP synthesis could be related to a kinetic stimulation of phosphate/H^+ measured both directly under conditions of phosphate accumulation (state 4) and indirectly by a decrease of the kinetic control by phosphate transport over ATP synthesis. Additionally, we observed that, in the presence of K^+, preventing the stimulation of phosphate transport with low amounts of mersalyl promoted an uncoupling of oxidative phosphorylation (i.e., a decrease of the ATP/O ratio) that pointed out the role of phosphate transport in the kinetic control of ATP synthesis in yeast mitochondria, at high phosphate concentration. This observation confirmed previous results from the laboratory [9,10].

Since the ATP-induced K^+-transport pathway was

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inhibited at high phosphate concentration [3], it was most probably inactive under our conditions for measuring oxidative phosphorylation. In the present paper, we investigated the effect of KCl on oxidative phosphorylation at low phosphate concentration. We used decavanadate, an efficient inhibitor of the ATP-induced permeabilities, to demonstrate that, under these conditions, it could function as an uncoupling pathway of oxidative phosphorylation.

The wild-type bakers's yeast Yeast Foam (PS194) was grown aerobically in a semi-synthetic medium (1% Yeast Extract, 0.1% KH_2PO_4, 0.12% $(NH_4)_2SO_4$, 2% D,L-lactate (pH 5.0 with NaOH) until mid-exponential growth phase. Mitochondria preparation, respiration and ATP synthesis measurements were described in [11] and are detailed in the legend of Table 1.

Decavanadate was prepared by dissolving sodium orthovanadate in water (0.1 M) and adjusting the pH at 6.0 with sulfuric acid. The solution turned to an orange color, typical of polymeric species [12]. It was verified that solutions where the monomeric form was preserved did not have the effects described in this study.

The upper half of Table 1 shows the effect of the presence of K^+ in the respiration buffer on oxidative phosphorylation parameters at low (0.5 mM) and high (5 mM) phosphate concentrations. At high phosphate concentration, as previously reported [8], the presence of K^+ induced a stimulation of respiration rates (state 4 and state 3) and of the initial rates of ATP synthesis. It was shown that, although the respiration rates were stimulated because of a partial drop of $\Delta\Psi$ following K^+-cycling, the ATP/O ratio was maintained following a kinetic stimulation of phosphate transport [8].

At low phosphate concentration, a very different situation emerged. In the absence of K^+, both the state 3-respiration rate and the initial rate of ATP synthesis were lower than at high phosphate concentration, but the ATP/O was nearly the same. This showed that the coupling efficiency was not affected by phosphate concentration, which is in accordance with previous observations in the laboratory [9,10]. On the opposite, in the presence of K^+, the initial rates of ATP synthesis were altered in such a way that the ATP/O ratio was dramatically reduced, showing an affected coupling efficiency.

This effect of phosphate concentration on the coupling efficiency in the presence of K^+, can be clearly visualized in Fig. 1A where the ATP/O ratio was plotted vs. the phosphate concentration. It appears that, below 1 mM phosphate, the ATP/O ratio was significantly altered by the presence of K^+.

This phosphate-dependence of the uncoupling effect of K^+ could be related to the existence, in the inner mitochondrial membrane of the strain Yeast Foam, of an ATP-induced K^+-transport pathway [3]. We demonstrated recently that this pathway was fully inhibited by low vanadate concentrations, under conditions where the polymer decavanadate was the mainly present chemical species [7]. We therefore assayed the effect of decavanadate on oxidative phosphorylation parameters measured as above. The lower half of Table 1 shows that decavanadate did not modify the respiration rates and the ATP synthesis rates measured either in the absence of K^+ or in the presence of K^+ at 5 mM phosphate. On the other hand, in the presence of K^+ and at low phosphate concentration, decavanadate induced both a decrease of state 3-respiration and an increase of ATP synthesis rate in such a way that the ATP/O ratio was now similar to the one measured under the other conditions. The protective effect of decavanadate is also reported in Fig. 1, over all the range of phosphate concentration. This strongly suggested that the opening of the decavanadate-sensitive K^+-transport pathway was responsible for the uncoupling observed at low phosphate concentration.

We investigated whether this uncoupling effect could be related to the mersalyl-induced uncoupling effect observed at high phosphate concentration in the presence of K^+, that was interpreted in terms of prevention of the kinetic stimulation of ATP synthesis by phosphate transport [8]. Fig. 2 shows the effect of mersalyl on the ATP/O ratio, evidencing the previously described uncoupling effect of the inhibition of phosphate transport in the presence of K^+. It appears clearly that decavanadate did not protect oxidative phosphorylation against the uncoupling effect of mersalyl. This demonstrates that the uncoupling effect of low phosphate concentration (Fig. 1) and the uncoupling effect of mersalyl (at high phosphate concentration) (Fig. 2) are unrelated. The kinetic interpretation of the uncoupling effect of mersalyl [8] is therefore still valid.

Table 1
Parameters of oxidative phosphorylation

		Respiration rates (nat. O/min per mg)		RCR	ATP synthesis (nmol ATP/min per mg)	ATP/O ratio
		state 4	state 3			
Controls (without decavanadate)						
Without KCl	P_i = 5 mM	181 ± 1	420 ± 12	2.32 ± 0.07	442 ± 5	1.06 ± 0.05
	P_i = 0.5 mM	173 ± 1	352 ± 1	2.04 ± 0.01	356 ± 26	1.01 ± 0.07
+ KCl 100 mM	P_i = 5 mM	243 ± 4	551 ± 15	2.27 ± 0.03	550 ± 14	1.00 ± 0.00
	P_i = 0.5 mM	260 ± 2	513 ± 12	1.98 ± 0.05	323 ± 26	0.62 ± 0.05
With decavanadate 5 μM (equivalent orthovanadate)						
Without KCl	P_i = 5 mM	175 ± 4	411 ± 4	2.34 ± 0.07	446 ± 14	1.07 ± 0.05
	P_i = 0.5 mM	166 ± 1	312 ± 2	1.88 ± 0.00	320 ± 15	1.02 ± 0.05
+ KCl 100 mM	P_i = 5 mM	232 ± 6	555 ± 7	2.39 ± 0.03	576 ± 28	1.04 ± 0.06
	P_i = 0.5 mM	239 ± 7	432 ± 6	1.81 ± 0.03	448 ± 1	1.03 ± 0.02

Mitochondria (0.67 mg/ml) were suspended at 28°C in a 10 mM Tris/maleate buffer (pH 6.8) containing 2 mM EGTA, 0.3% bovine serum albumin and either 0.6 M mannitol (without KCl) or 0.4 M mannitol and 0.1 M KCl. 40 mM ethanol and 1 mM ADP were added for the measurements of state 4 and state 3. Respiration (measured polarographically) and initial rates of ATP synthesis (incorporation of $[^{32}P]P_i$ into ATP) were measured simultaneously.

As a conclusion, data reported in this paper allow to suggest that the decavanadate-sensitive K^+-transport pathway may act, in vitro, as an uncoupling pathway. Since one of the characteristics of this pathway is to be induced by ATP [3] this may be of critical importance for the regulation of oxidative phosphorylation in yeast. It may be noted that, even under the conditions allowing the opening of the ATP-induced K^+-conducting pathway, the kinetics of ATP synthesis, at least for the first minute within which the measurements were done, remained linear. This observation suggests that the lower coupling efficiency was not a transient phenomenon but that the transmembrane potential was stabilized at a new steady state, lower than the value maintained in the absence of opening. It is likely that the K^+/H^+ exchange, which is spontaneously active in yeast mitochondria [13], even under phosphorylation conditions [8], prevents an unregulated accumulation of K^+ via the K^+-conducting pathway. A possible co-regulation of these two systems is under investigation.

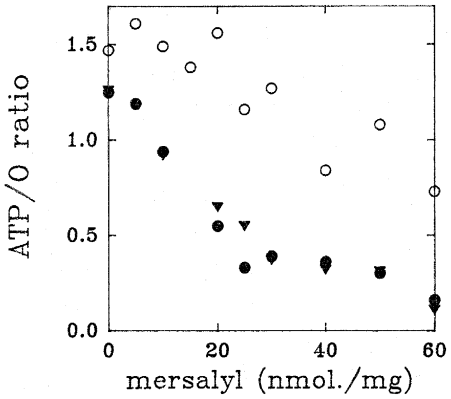

Fig. 2. Effect of mersalyl on the ATP/O ratio. Experimental conditions identical to Table 1 (5 mM phosphate) except that mitochondria were preincubated for 3 min at 4°C in the presence of the indicated amounts of mersalyl. ○, without KCl; ●, +0.1 M KCl; ▼, +0.1 M KCl+7 μM decavanadate (equivalent orthovanadate).

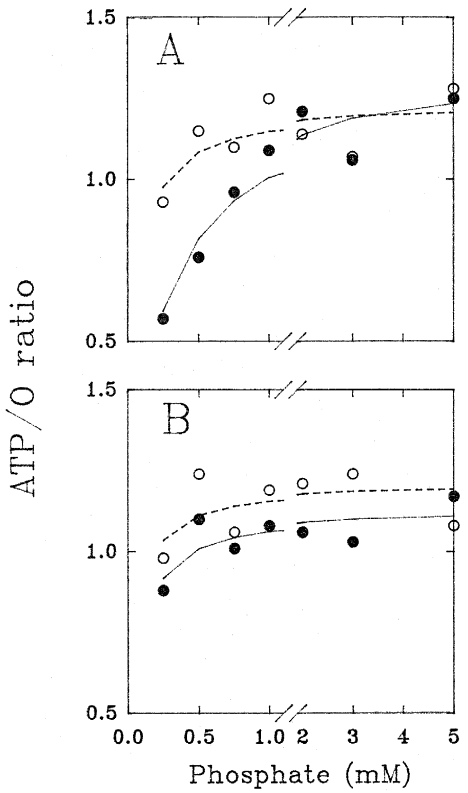

Fig. 1. Phosphate-dependence of the ATP/O ratio. Experimental conditions identical to Table 1, in the absence (A) or in the presence (B) of 7 μM decavanadate (equivalent orthovanadate). ○---○, without KCl; ●—●, +0.1 M KCl.

It should be noted, however, that the observations reported herein cannot, to date, be extrapolated to the in vivo situation since the low phosphate concentration was far below the phosphate concentration of yeast cells under 'normal' conditions (see e.g., Refs. [14,15]). Additionally, these results cannot be taken as arguments to question previous interpretations of certain aspects of the regulation of oxidative phosphorylation on isolated yeast mitochondria, which were observed in the absence of K^+ and at high phosphate concentration (e.g. [16,17]). Most of all, our data strengthen these observations since they unambiguously demonstrated that no uncoupling occurred under these 'standard' conditions whereas an uncoupling occurred under the particular conditions where the K^+-transport pathway was activated.

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