STUDIES ON ANIONIC TRANSPORT IN YEAST MITOCHONDRIA AND PROMITOCHONDRIA. SWELLING IN AMMONIUM PHOSPHATE, GLUTAMATE, SUCCINATE AND FUMARATE SOLUTIONS

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1. Introduction

The transport of some anionic components into mitochondria appeared to be mediated by a series of exchange-diffusion carriers, localized in the internal membrane. Moreover, rat liver mitochondria have been shown to contain at least eight specific carriers [1–4]. One of the most useful tools, in identifying some of these carriers was the osmotic swelling of mitochondria suspended in the isotonic ammonium salt of the anion to be tested [5]. For instance, rat liver mitochondria spontaneously swelled in ammonium phosphate or glutamate, suggesting an anion–hydroxyl exchange. But, with succinate or malate, phosphate was necessary before swelling occurred. However, only the existence of specific inhibitors is a probe for a mediated-carrier transport.

With yeast mitochondria and promitochondria, Kolarov et al. [6] noted likewise a swelling in ammonium phosphate, but they reported that succinate uptake did not require phosphate as it did for malate: these data suggested the presence of a succinate carrier distinct of the malate carrier.

In order to determine if, in yeast mitochondria (aerobically grown cells) and promitochondria (anaerofically grown cells), the phosphate uptake was carrier mediated, we studied the mersalyl effects on the swelling. We also examined the glutamate, succinate and fumarate transport in these organelles.

2. Material and methods

A diploïd wild strain *Saccharomyces cerevisiae* (Yeast Foam) was used. The cells were grown aerobically in the complete medium (1% Yeast extract, 0.1% potassium phosphate, 0.12% ammonium phosphate, 2% galactose) and harvested late in the logarithmic growth phase. For the anaerobic growth, this medium was complemented with 0.34% Tween 80 and 0.0012% ergosterol. Before harvesting anaerobically grown cells, cycloheximide was added to a final concentration of 25 μg/ml. For other conditions concerning anaerobiosis see ref. [7].

Mitochondria and promitochondria were isolated from protoplasts following the Kovac's procedure [8] and suspended in the standard medium: 0.6 M mannitol, 2 mM EDTA, 10 mM Tris–maleate, pH 6.8.

All the experiments of swelling were performed in presence of antimycin (0.5 μg/ml) and oligomycin (4 μg/ml). The other conditions are given in the legends.

3. Results and discussion

3.1. *Phosphate transport*

It is well known that phosphate transport in mammalian mitochondria is inhibited by thiol-reagents [9–11]. To establish accurately the presence of a phosphate-carrier in yeast mitochondria and promitochondria, we tested the mersalyl action on swelling in ammonium phosphate solutions and its dependence towards several factors as the concentration of phosphate solutions, the pH and the amount of mersalyl required.

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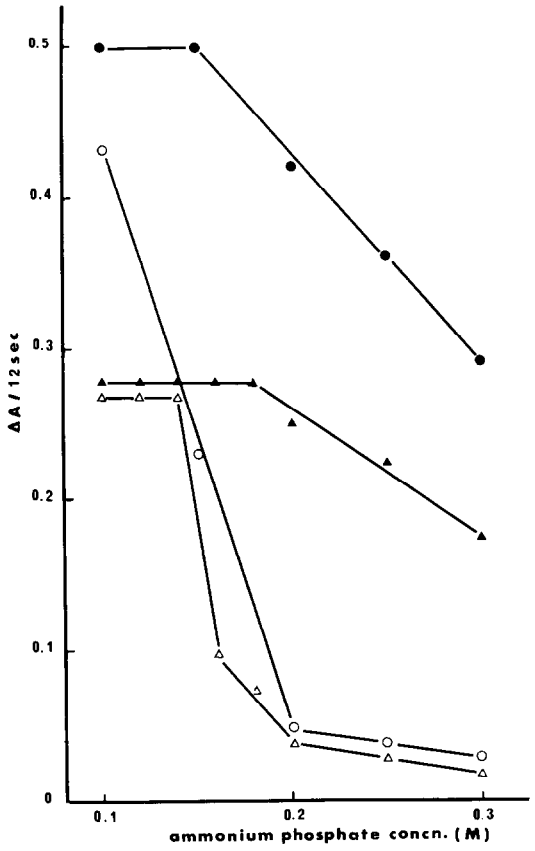

Fig. 1. Effect of ammonium phosphate concentration on mitochondrial and promitochondrial swelling: action of mersalyl. Mitochondria or promitochondria (0.63 mg protein) were or were not pre-incubated with mersalyl (30 nmoles mg protein^{-1}) for 30 sec in the standard medium, then suspended in 1 ml of ammonium phosphate pH 6.8 at different molarities. Swelling was monitored at 546 nm using an Eppendorf photometer and the extent measured during the first 12 sec. (●—●) mitochondria, (▲—▲) promitochondria incubated without mersalyl; (○—○) mitochondria, (△—△) promitochondria pre-incubated with mersalyl.

As shown in fig. 1, yeast mitochondria and promitochondria swelled in ammonium phosphate solutions of various molarities. The extent and the rate of swelling decreased when the phosphate concentration increased. In both cases the inhibition by mersalyl was almost complete when the phosphate concentration attained a value of 0.2 M. Volume changes observed with mersalyl pre-incubated organelles, suspended in low molarity ammonium phosphate solutions, were due to an osmotic response as we can expect with non permeant anions [1]. This point was verified with ammonium chloride solutions (not shown).

The inhibitory action of mersalyl was found to be pH dependent: yeast mitochondria were pre-incubated with or without mersalyl for 30 sec in the standard medium, then suspended in 0.2 M ammonium phosphate solutions adjusted to different pH values. In each case, the swelling inhibition was measured. As shown in fig. 2, this inhibition decreased when the pH increased. A more pronounced effect of the pH was found with yeast promitochondria. The decrease of the inhibitory action of mersalyl at alkaline pH might be due to an alteration of the membrane causing an electrogenic phosphate diffusion phenomenon.

Therefore, the swelling in 0.2 M ammonium phosphate at pH 6.8 was a suitable system to test transport sensitivity to mersalyl. As shown in fig. 3A (mitochondria) and fig. 3B (promitochondria) the inhibition was complete with 20 nmoles of mersalyl per mg of protein. This value is in agreement with those previously reported with mammalian mitochondria [10–13]. It can be concluded, therefore, that the anaerobiosis did not affect the phosphate-

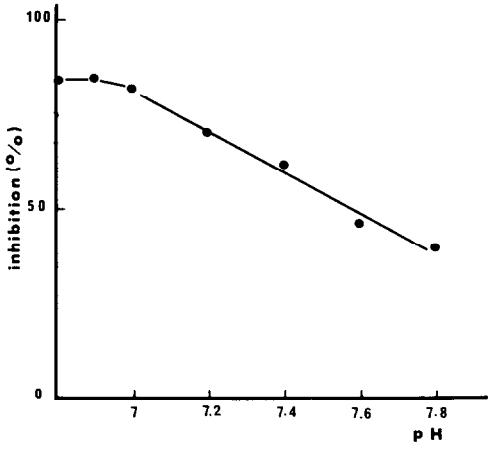

Fig. 2. pH dependence on the inhibition of mitochondrial swelling in 0.2 M ammonium phosphate. Mitochondria were pre-incubated with or without mersalyl (20 nmoles mg protein^{-1}) in the standard medium, pH 6.8, for 30 sec; then swelling was followed in 1 ml of 0.2 M ammonium phosphate at different pH values.

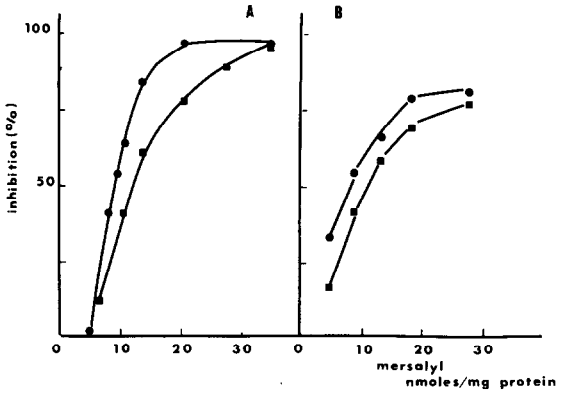

Fig. 3. Mitochondrial (A) and promitochondrial (B) swelling in 0.2 M ammonium phosphate or succinate: sensitivity to mersalyl. Mitochondria or promitochondria (0.8 mg protein) were pre-incubated with different amounts of mersalyl for 30 sec in the standard medium (cf. methods). They were: (●—●) suspended in 1 ml of 0.2 M ammonium phosphate, pH 6.8 (Swelling was monitored and the inhibition calculated as in fig. 1), and (■—■) suspended in 1 ml of 0.2 M ammonium succinate, pH 6.8, with 20 mM phosphate added after 1 min. The inhibition was calculated during the first 12 sec following the phosphate addition.

carrier synthesis. These results are in agreement with the observations that promitochondria are differentiated in regard to the partial reactions of the phosphorylation system involving phosphate ions [14,15].

3.2. *Glutamate transport*

In rat liver mitochondria it was found that the glutamate transport is inhibited by N-ethylmaleimide and not by mersalyl [4,16], suggesting a glutamate-hydroxyl exchange mediated by a carrier other than the one catalysing the phosphate transport. Likewise, yeast mitochondria swelled spontaneously in 0.2 M ammonium glutamate at pH 6.8, but this uptake was inhibited by mersalyl. Furthermore, it was not possible to discriminate between the thiol-groups implicated in phosphate and glutamate transport because in both cases the amount of mersalyl required for a full inhibition was the same (20 nmoles per mg protein). However, the possibility of a net uptake of glutamate by a glutamate–phosphate exchange coupled to the phosphate–hydroxyl exchange (inhibited by mersalyl) was eliminated by the two following observations: 1) Phosphate did not stimulate the rate of glutamate entry (fig. 4). 2) In phosphate-depleted mitochondria the glutamate uptake occurred at a normal level.

As shown in fig. 4B, the promitochondria swelled in ammonium glutamate. This swelling was inhibited by mersalyl though a rapid residual volume change was always observed. Nevertheless, it can be concluded that the glutamate carrier is preserved in promitochondria.

3.3. *Succinate and fumarate transport*

Yeast mitochondria did not swell when they are suspended in 0.2 M ammonium succinate at pH 6.8 ; swelling occurred only after addition of phosphate (fig. 4a). This change of volume was inhibited by mersalyl and *n*-butylmalonate (fig. 3A and 4A) indicating a carrier mediated succinate–phosphate exchange coupled to the phosphate–hydroxyl exchange as for mammalian mitochondria [1–3]. As reported by Kolarov et al. [6] we noted in some cases a spontaneous swelling insensitive to mersalyl and *n*-butylmalonate; this observation may be related to an alteration of the mitochondrial membrane. As shown by these authors, swelling was not observed in ammonium fumarate in spite of a phosphate addition.

With promitochondria a spontaneous swelling was always observed in 0.2 M ammonium dicarboxylate solutions. However in ammonium succinate, phosphate induced an additional swelling sensitive to mersalyl and *n*-butylmalonate (fig. 3B and 4B) pointing out the coupling between the phosphate and dicarboxylate exchange. In contrast with mitochondria, a phosphate-dependent swelling was also observed when succinate was replaced by fumarate. This uptake was inhibited by mersalyl and *n*-butylmalonate (fig. 4B) suggesting a fumarate–phosphate exchange catalysed by the dicarboxylate-carrier.

From this study it can be concluded that the anaerobiosis did not affect qualitatively the phosphate and glutamate transport. The only difference between mitochondria and promitochondria was the possibility for the latter to carry out a fumarate–phosphate exchange. This finding opens the question about the significance of this transport in the metabolic pathways of the anaerobically grown yeast.

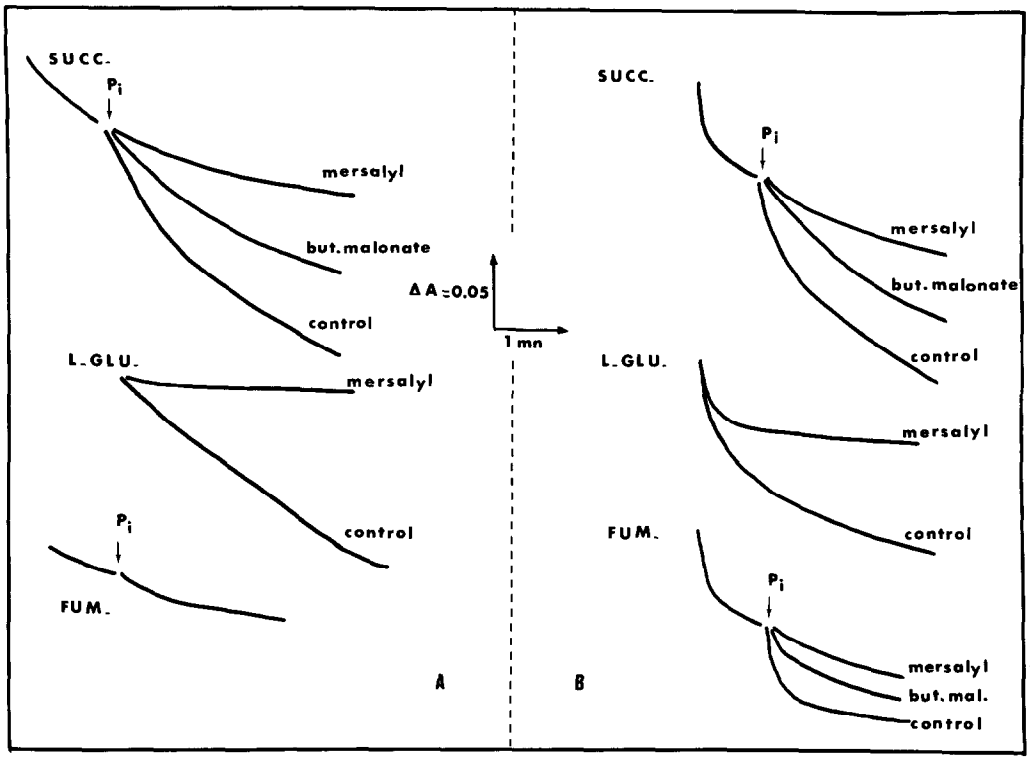

Fig. 4. Swelling of mitochondria (A) and promitochondria (B) in ammonium salts of organic acids. Mitochondria or promitochondria (0.8 mg protein) were added to 1 ml of 0.2 M ammonium salt, pH 6.8, as indicated on curves and the swelling recorded as in fig. 1. When *n*-butylmalonate (20 mM) or mersalyl (20 nmoles per mg of protein) were used, they were added before the organelles.

Acknowledgements

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