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Closure of the Yeast Mitochondria Unspecific Channel (YMUC) Unmasks a Mg^{2+} and Quinine Sensitive K^+ Uptake Pathway in *Saccharomyces cerevisiae*

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The K^+ uptake pathways in yeast mitochondria are still undefined. Nonetheless, the K^+-mediated mitochondrial swelling observed in the absence of phosphate (PO_4) and in the presence of a respiratory substrate has led to propose that large K^+ movements occur in yeast mitochondria. Thus, the uptake of K^+ by isolated yeast mitochondria was evaluated. Two parallel experiments were conducted to evaluate K^+ transport; these were mitochondrial swelling and the uptake of the radioactive K^+ analog $^{86}Rb^+$. The opening of the yeast mitochondrial unspecific channel (YMUC) was regulated by different PO_4 concentrations. The high protein concentrations used to measure $^{86}Rb^+$ uptake resulted in a slight stabilization of the transmembrane potential at 0.4 mM PO_4 but not at 0 or 4 mM PO_4. At 4 mM PO_4 swelling was inhibited while, in contrast, $^{86}Rb^+$ uptake was still observed. The results suggest that an energy-dependent K^+ uptake mechanism was unmasked when the YMUC was closed. To further analyze the properties of this K^+ uptake system, the Mg^{2+} and quinine sensitivity of both swelling and $^{86}Rb^+$ uptake were evaluated. Under the conditions where the unspecific pore was closed, K^+ transport sensitivity to Mg^{2+} and quinine increased. In addition, when Zn^{2+} was added as an antiport inhibitor, uptake of $^{86}Rb^+$ increased. It is suggested that in yeast mitochondria, the K^+ concentration is highly regulated by the equilibrium of uptake and exit of this cation through two specific transporters.

KEY WORDS: Permeability transition; K^+ transport; K^+ channels; yeast mitochondria; YMUC.

INTRODUCTION

In the cell, K^+ is the most abundant cation, both in the cytoplasm and within the mitochondrial matrix, where its concentration has been estimated at 140 mM (for a review, see Brierley *et al.*, 1994). K^+ is perhaps the main mitochondrial osmoregulator, and the volume of the matrix is modified in response to the K^+ movements across the mitochondrial inner membrane. In mammalian mitochondria, the uptake of K^+ was first proposed to occur through a 53 kDa uniporter (Diwan *et al.*, 1988). More recently, a mitochondrial K_{ATP} channel was identified which is similar to the plasma membrane uptake system (Inoue *et al.*, 1991); the K^+ uniport activity seems to have little dependence on pH although it exhibits high sensitivity to adenine nucleotides (Beavis *et al.*, 1993). The mitochondrial and the plasma membrane K_{ATP} channels are both closed by ATP or glybenclamide (Garlid, 1996) and probably by ruthenium red (Kapus *et al.*, 1990). The exit of K^+ is catalyzed through an antiport with protons, as predicted by Mitchell (1961) and characterized by Brierley (1978). These two K^+ transport systems control the concentration of K^+ within isolated mitochondria (Garlid, 1980). In turn, mitochondrial K^+ has large effects on mitochondrial volume, thus regulating diverse processes, such as oxidative phosphorylation and fatty acid catabolism (Halestrap, 1989; Nicholls *et al.*, 1972). The release of K^+ is activated when mitochondrial Mg^{2+} or nucleotides are depleted, or when the pH is increased (Jezek *et al.*, 1990). It has been proposed that the recycling of K^+ needs to be highly regulated in order to avoid the depletion of both the transmembrane potential and the pH gradient (Garlid, 1988).

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Mitochondria are regarded as perfect osmometers (Chappel and Crofts, 1966; Roucou *et al.*, 1995). The swelling method has been used to study many mitochondrial transport systems including the K^+/H^+ antiporter (Beavis *et al.*, 1993; Brierley *et al.*, 1994). Using this method, several features of the mammalian K^+/H^+ antiporter have been defined (Garlid, 1988): it is inhibited by Mg^{2+} (Jung and Brierley, 1986), by dicyclohexylcarbodiimide (DCCD) (Martin *et al.*, 1986) and by quinine (Brierley and Jung, 1988; Nakashima and Garlid, 1982). In addition, it has been demonstrated that quinine exhibits a partial inhibitory effect on the K^+ uniporter (Diwan, 1986).

The mitochondrial swelling method also allowed the characterization of the K^+/H^+ antiporter in mitochondria from *Saccharomyces cerevisiae*, which is similar to the antiporter from mammalian mitochondria, except that in yeast inhibition by Mg^{2+} is not detected and transport is active at physiologic pH (Roucou *et al.*, 1995; Welihinda *et al.*, 1993). In addition it was demonstrated that the yeast K^+ antiporter is sensitive to Zn^{2+} or Cu^{2+} (Manon and Guérin 1992, 1995).

The use of electrophysiological techniques has revealed the existence of high-conductance, unspecific ionic channels in the internal membrane of yeast mitochondria (Ballarin and Sorgato, 1995, 1996). These unspecific channels have been characterized physiologically and have been tentatively identified as the equivalent to the permeability transition pore (PTP) from mammalian mitochondria (Zoratti and Szabó, 1995). Both the PTP and the yeast mitochondrial unspecific channel (YMUC) (Manon *et al.*, 1998) allow the passage of solutes with a MW of 1 to 1.5 kDa (Jung *et al.*, 1997; Zoratti and Szabó, 1995). However, while the PTP is opened by PO_4 and Ca^{2+} and closed by cyclosporine (Bernardi *et al.*, 1994; Bernardi and Petronilli, 1996), the YMUC is closed by PO_4 and is not affected by Ca^{2+} or cyclosporine (Jung *et al.*, 1997). In yeast mitochondria, ATP induces the opening of the YMUC and thus, the collapse of the $\Delta\Psi$ when K^+ is present and PO_4 is absent, suggesting that yeast mitochondria accumulate K^+ when the YMUC is open (Manon and Guérin, 1998).

It was decided to evaluate the correlation between osmotic swelling and $^{86}Rb^+$ uptake in order to better define the K^+ uptake mechanisms in yeast mitochondria. As described in the literature (Manon *et al.*, 1998), addition of different concentrations of PO_4 resulted in the closure of the YMUC. In contrast, protein concentrations in the range used in our experiments exhibited only mild effects on the permeability status on mitochondria. Under the conditions where the YMUC remained closed, a K^+ uptake system was unmasked, which probably is the equivalent to the mammalian K^+ uniporter and which is Mg^{2+} and quinine-sensitive. The uptake of $^{86}Rb^+$ detected under these conditions did not lead to modification in the light scattering properties of the mitochondrial suspension.

MATERIALS AND METHODS

Materials

All chemicals were of the best quality commercially available. Mannitol, MES, KCl, RbCl, $MgCl_2$, Quinine, Mersalyl and FCCP, were from Sigma Chemicals (St. Louis, MO). $^{86}RbCl$ was acquired from NEN Life Science Products, Inc. (Boston, MA). The yeast *Saccharomyces cerevisiae* was a commercial baker's strain (La Azteca, S.A).

Isolation of Yeast Mitochondria

Yeast cells were incubated in a rich liquid medium (De Kloett *et al.*, 1961) under aeration (3 L/min) for 8 h and starved overnight in distilled water under aeration. After incubation, cells were harvested and suspended in cold, isotonic, isolation medium containing 0.6 M mannitol, 0.1% bovine serum albumin, 5 mM MES, pH 6.8 (TEA). The suspension was mixed with an equal volume of glass beads (0.45 mm diameter) and disrupted in a Braun cell homogenizer (Melsungen, Germany) and mitochondria were isolated from the homogenate by differential centrifugation as described before (Peña *et al.*, 1977). The concentration of mitochondrial protein was determined by biuret (Gornal *et al.*, 1949). In the experiments reported here, 2 μL/mL of 10% oxygen peroxide were added in order to ensure the availability of oxygen throughout the experiment.

Mitochondrial Swelling

The variations in mitochondrial volume were followed by measuring the decrease in optical density at a wavelength of 540 nm in an Aminco DW2000 spectrophotometer in split mode. The spectrophotometer was equipped with a magnetic stirrer.

Transmembrane Potential

Safranine-O was used to measure the transmembrane potential following the change in absorbance at 511–533 nm in an DW2000 Aminco spectrophotometer in dual mode (Akerman and Wikström, 1976).

$^{86}Rb^+$ Transport

The uptake of rubidium was measured after preincubating mitochondria for 1 min in 0.6 M mannitol, 5 mM MES, pH 6.8 (TEA) plus the indicated concentrations of PO_4, pH 6.8 (TEA), $MgCl_2$, quinine and $ZnCl_2$. Then, $^{86}RbCl$ was added to the final concentration indicated in each figure. After a second minute of incubation, 20 mM ethanol was added and 100 μL aliquots were taken at the indicated times. These samples were transferred to a nitrocellulose filter (0.45 mm pore) previously humidified and mounted on a high-vacuum Millipore multifilter. The filters were then washed using a buffer containing 0.6 M mannitol, 5 mM MES, 80 mM KCI, pH 6.8 (TEA) and transferred to scintillation vials, then 5 mL of scintillation liquid were added and radioactivity was measured in a Beckman LS6500 scintillation counter using the phosphate window.

RESULTS

Mitochondrial Permeability Regulation by Inorganic Phosphate (PO_4)

In the absence of inorganic phosphate, yeast mitochondria swell in the presence of K^+ (Velours *et al.*, 1977). Under the conditions employed in this study, swelling was reevaluated, as a high concentration of mitochondria (3 mg/mL) was used in all experiments; in agreement with the literature, in the absence of PO_4 and in the presence of 20 mM KCI (Results not shown) or RbCl (Fig. 1) optical density decreased, indicating mitochondrial swelling because of ion fluxes through an open unspecific pore (Castrejón *et al.*, 1997; Manon *et al.*, 1998). This effect was energy-dependent, as indicated by the lack of swelling in the presence of the uncoupling agent FCCP (Fig. 1).

In order to further evaluate a possible effect of protein concentration on the opening state of the YMUC, the transmembrane potential was measured in the presence of different PO_4 concentrations and increasing protein (Fig. 2). In the absence of PO_4, the transmembrane potential was depleted within 1 min at all the mitochondrial concentrations tested, although an initial increase in the potential was observed which was higher as protein increased from 0.5 to 3 mg protein/mL (Fig. 2(A)). When the same experiment was performed at 0.4 mM PO_4 increasing protein resulted in a partial stabilization of the transmembrane potential. From 0.5 to 2.0 mg protein/mL, the potential still decreased to minimal values after 40 s, while at 3.0 mg protein/mL, depletion of the potential was

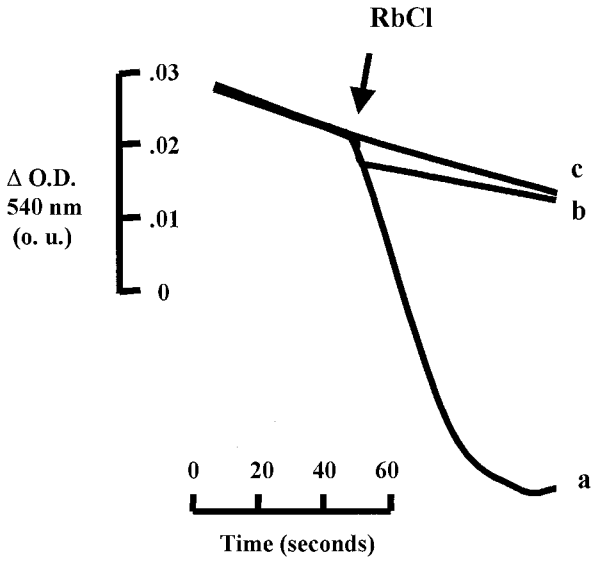

Fig. 1. Rubidium-mediated swelling of yeast mitochondria – Reaction mixture: 0.6 M mannitol, 0.5 mM MES; pH 6.8 (TEA), 20 mM ethanol, 2 μL/mL H_2O_2 (10%), mitochondria 3 mg protein/mL. Where indicated concentrations used were (a) 20 mM RbCl, (b) 20 mM RbCl plus 6 μM FCCP, and (c) 0 RbCl. The arrow indicates rubidium addition. Swelling was determined spectrophotometrically at 550 nm, using an Aminco DW2000 spectrophotometer in split mode. Final volume was 2 mL. (Experiment was carried out at room temperature).

only partial (Fig. 2(B)). Transmembrane potential depletion was not due to lack of oxygen, because oxygen peroxide was added in all experiments in sufficient quantity to last throughout the experiment. This was evaluated by oxymetry using a Clark electrode (Results not shown). At 4 mM PO_4, the transmembrane potential was stable at all the protein concentrations tested (Results not shown).

When increasing concentrations of PO_4 were added to the reaction mixture, the extent of mitochondrial swelling in response to the addition of 20 mM RbCl (Fig. 3) or KCl (Results not shown) decreased proportionally; swelling was almost completely inhibited at 1.0 mM PO_4. In this regard, different groups have reported that at 0.4 mM PO_4, the YMUC remains open most of the time, while at 4 mM PO_4, the YMUC is closed (Manon and Guérin, 1997).

To further characterize the effect of PO_4 on K^+ uptake by yeast mitochondria, the uptake of $^{86}Rb^+$ was evaluated in the absence of PO_4, in the presence of 0.4 mM PO_4, which is known to allow for K^+ mediated swelling, and at 4 mM PO_4, where no swelling is observed. At 0.4 mM PO_4 the YMUC is mostly opened, while at 4 mM PO_4 the YMUC seems to be closed (Jung *et al.*, 1997; Velours *et al.*, 1977). In the absence of PO_4 no uptake of $^{86}Rb^+$ could be detected (Fig. 4, trace a). In contrast, in the presence of 0.4 mM PO_4, mitochondria accumulated

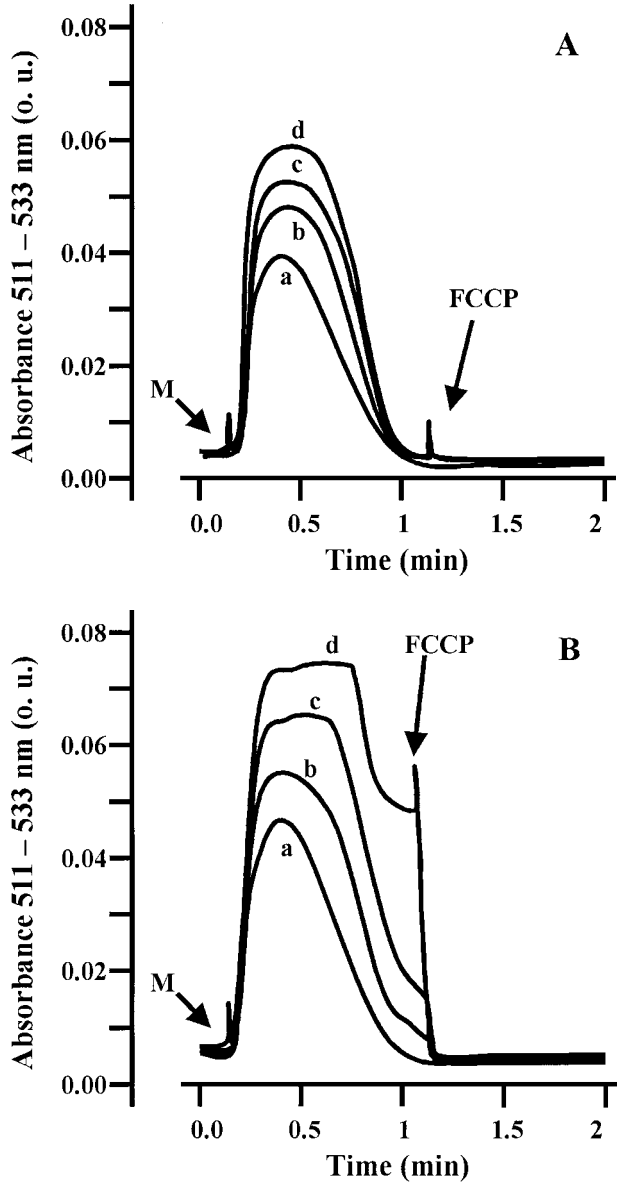

Fig. 2. Effect of protein concentration on the mitochondrial transmembrane potential – Experimental conditions were as in Fig. 1, except for 10 μM safranine-O and different concentrations of mitochondrial protein as follows: (a) 0.5 mg/mL, (b) 1 mg/mL, (c) 2 mg/mL, (d) 3 mg/mL. Final volume was 2 mL. Mitochondria (M) or 6 μM FCCP were added where indicated. (A) 0 and (B) 0.4 mM PO_4. PO_4 was added from either a 0.1 M or a 1 M stock solution, pH 6.8 (TEA).

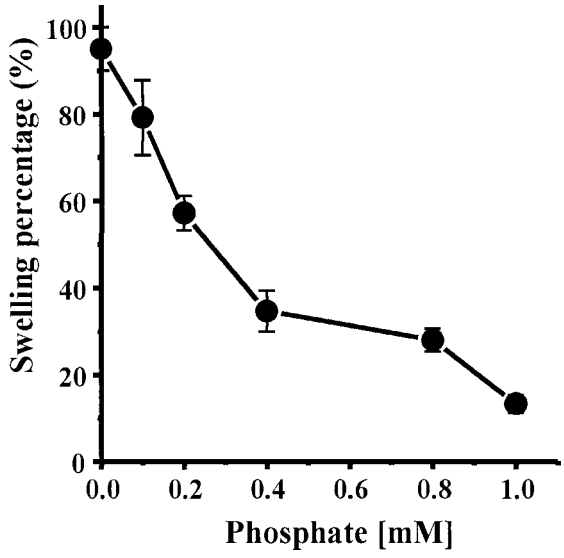

Fig. 3. Effect of PO_4 on the Rb^+-mediated swelling of yeast mitochondria – Experimental conditions were as in Fig. 1, except that RbCl was 20 mM and PO_4 concentrations were as indicated. PO_4 was added from either a 0.1 M solution or a 1 M stock solution, pH 6.8 (TEA). Data are expressed as percentages of the swelling in the absence of PO_4. Data are the means of four determinations ± standard deviation.

approximately 10 nmoles of $^{86}Rb^+$ (mg protein)$^{-1}$ in 2 min (Fig. 4, trace b). At 4 mM PO_4, the initial rate of uptake was similar to that observed at 0.4 mM PO_4; however, at 4 mM PO_4, $^{86}Rb^+$ uptake slowed down after 30 s such that at 30 s, 5 nmoles $^{86}Rb^+$(mg protein)$^{-1}$ were accumulated by mitochondria and only a small increase in uptake was observed after 2 min of incubation (Fig. 4, trace c). In all cases, the addition of FCCP inhibited the uptake of $^{86}Rb^+$ (Results not shown). Taken together, the results indicate that PO_4 inhibits the K^+ mediated swelling of mitochondria, while in contrast it promotes the uptake of the

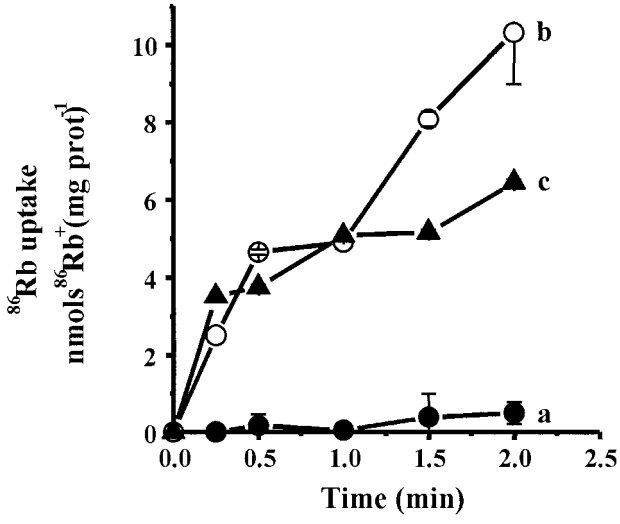

Fig. 4. Effect of PO_4 on the uptake of $^{86}Rb^+$ by yeast mitochondria– Experimental conditions were as in Fig. 1, except for the addition of 20 mM ^{86}RbCl, 2 μL/mL H_2O_2 (10%). Final volume was 1 mL. PO_4 concentrations were (a) 0, (b) 0.4 mM, and (c) 4 mM. PO_4 was added from either a 0.1 M solution or a 1 M stock solution, pH 6.8 (TEA). Data are the means of six determinations ± standard deviation.

radioactive K^+ analog $^{86}Rb^+$ i.e, there was no correlation between both parameters. In addition, the $^{86}Rb^+$ uptake experiments indicated that there is an energy-dependent system for K^+ uptake, which may be studied in the presence of the PO_4 concentrations known to close the YMUC.

Regulation of Mitochondrial Permeability by Mg^{2+} and Quinine

In mammalian mitochondria, Mg^{2+} and quinine have been reported to inhibit the uptake of K^+. In an effort to further characterize the K^+ uptake system in yeast mitochondria, the effects of Mg^{2+} and quinine on the Rb^+-mediated swelling of yeast mitochondria were tested in the presence of 0.4 mM PO_4 where an active K^+-or Rb^+-mediated swelling has been reported. It was observed that swelling was inhibited by increasing concentrations of Mg^{2+}, reaching 50% inhibition at 1 mM Mg^{2+} and stabilizing at a 70% inhibition at 2–8 mM Mg^{2+} (Fig. 5(A)). When mitochondrial swelling was determined in the presence of increasing quinine concentrations, swelling decreased linearly with quinine concentrations, reaching 55% inhibition at 0.5 mM quinine (Fig. 5(B)).

In order to assess the correlation between the swelling experiments and monovalent cation uptake, the uptake of $^{86}Rb^+$ was measured at 0.4 and 4 mM PO_4 and in the presence of increasing concentrations of Mg^{2+} or quinine (Fig. 6). In the presence of increasing concentrations of Mg^{2+}, the uptake of $^{86}Rb^+$ decreased both in the presence of 0.4 mM PO_4 (Fig. 6(A), trace a) and 4 mM PO_4 (Fig. 6(A), trace b). The maximal inhibition was reached at both PO_4 concentrations at 2 mM Mg^{2+}, although the Mg^{2+} mediated inhibition of uptake was higher at 4 mM PO_4, reaching 1.62 nmoles $^{86}Rb^+$ (mg protein)$^{-1}$, than at 0.4 mM PO_4 where the uptake was 4.01 nmoles $^{86}Rb^+$ (mg protein)$^{-1}$. Quinine also inhibited the uptake of $^{86}Rb^+$, although, as expected from the swelling experiments, the effects were lower than with Mg^{2+}. In addition, the quinine-mediated inhibition of $^{86}Rb^+$ uptake was higher in the presence of 4 mM PO_4 (Fig. 6(B), trace b) than in the presence of 0.4 mM PO_4 (Fig. 6(B), trace a). The maximal inhibition of $^{86}Rb^+$ uptake was reached in the presence of 0.5 mM quinine and 4 mM PO_4, where uptake was 2.3 nmoles $^{86}Rb^+$ (mg protein)$^{-1}$. Thus, in the experiments using increasing concentrations of different Mg^{2+} or quinine, the results obtained from the mitochondrial swelling and the $^{86}Rb^+$ uptake experiments did correlate, indicating that both molecules inhibited the uptake of monovalent cations by yeast mitochondria. In this regard, the K^+ uptake system from yeast mitochondria would be similar to its mammalian counterpart.

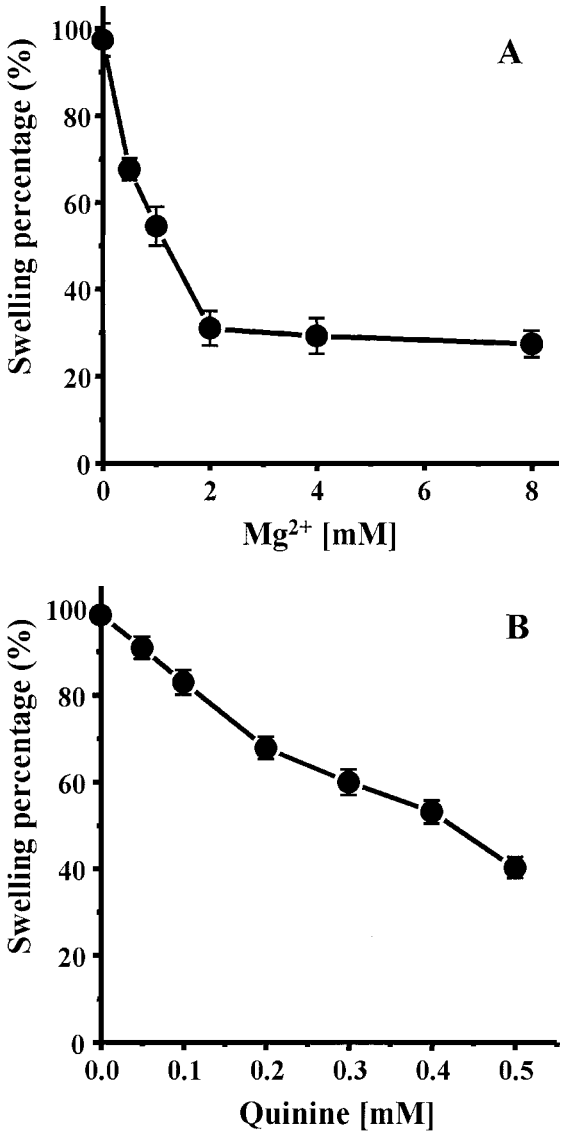

Fig. 5. Effect of Mg^{2+} and Quinine on the Rb^+-mediated swelling of yeast mitochondria – Reaction mixture used was the same as in Fig. 1, except that PO_4 concentration was 0.4 mM and either (A) different magnesium concentrations were added as indicated from a 1M $MgCl_2$ stock solution or (B) different quinine concentrations were added from a 50 mM stock solution in dimethyl formamide. Data are expressed as percentages of maximum swelling. Data are the means of four determinations ± standard deviation.

Effect of Zn^{2+} on the Uptake of $^{86}Rb^+$ by Yeast Mitochondria

The rate of $^{86}Rb^+$ uptake by yeast mitochondria was far from the equilibrium predicted by the Nerst equation. This may be due to the slow rate of transport and/or to the establishment of an equilibrium where the

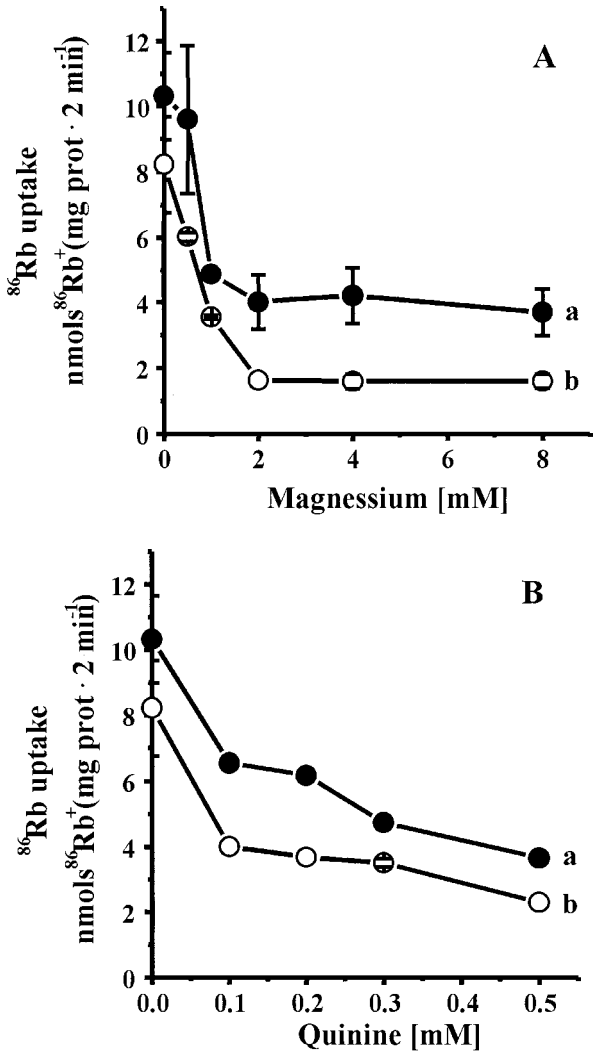

Fig. 6. Effect of Mg^{2+} or Quinine on the uptake of $^{86}Rb^{+}$ by yeast mitochondria – Experimental conditions were as in Fig. 3 except that (A) different magnesium concentrations were added as indicated from a 1M $MgCl_2$ stock solution and (B) different quinine concentrations were added from a 50 mM stock solution in dimethyl formamide. PO_4 concentrations were (a) 0.4 mM or (b) 4 mM. Data are expressed as the uptake of $^{86}Rb^{+}$ after 2 min. Data are the means of six determinations ± standard deviation.

antiporter is actively expelling $^{86}Rb^{+}$. To explore this possibility, the uptake of $^{86}Rb^{+}$ was measured in the presence of 0.5 mM Zn^{2+}, which inhibits the antiporter in yeast mitochondria (Manon and Guérin, 1992, 1995) (Fig. 7). In the presence of 4 mM PO_4, the addition of 0.5 mM Zn^{2+} resulted in an increase in the total amount of $^{86}Rb^{+}$ uptake. Thus, the inhibition of the antiporter resulted in displacement of the equilibrium of $^{86}Rb^{+}$ movements in mitochondria allowing for enhanced uptake and suggesting that this monovalent

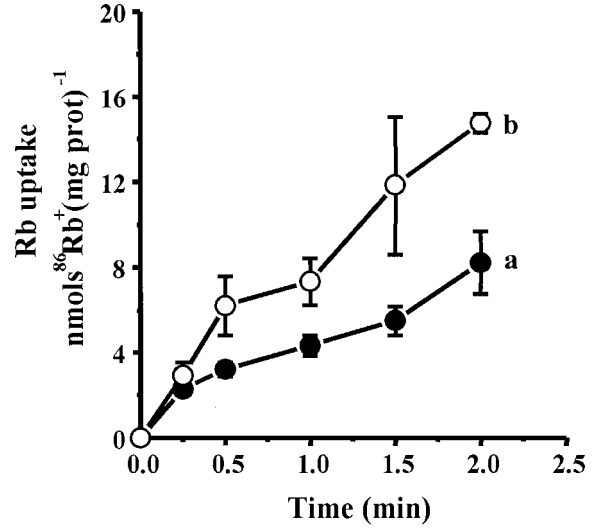

Fig. 7. $^{86}Rb^{+}$ transport by yeast mitochondria—*Effect of* Zn^{++} – Experimental conditions were as in Fig. 4. Except that PO_4 concentration was 4 mM. $ZnCl_2$ was added from a 0.1 mM stock solution to a final concentration of (a) 0 or (b) 0.5 mM. Data are the means of six determinations ± standard deviation.

cation uptake system is active under our experimental conditions.

DISCUSSION

Two different K^{+} uptake systems were detected in yeast mitochondria. These were the yeast mitochondrial unespecific channel (YMUC) and a second pathway, which was detectable under conditions where the YMUC was closed. The second pathway is probably a uniporter for K^{+} and Rb^{+}, as the uptake of $^{86}Rb^{+}$ was inhibited after 30 s under the conditions where the YMUC was closed and counter-ion movements were inhibited (Fig. 4, trace c). Another evidence favoring an electrogenic uniport mechanism was the dependence of $^{86}Rb^{+}$ transport on the $\Delta\Psi$, i.e. when $\Delta\Psi$ was depleted in the presence of an uncoupler or because of the absence of PO_4, no uptake of $^{86}Rb^{+}$ was observed. The swelling data did not always correlate with the $^{86}Rb^{+}$ uptake results, i.e. at 4 mM PO_4, no swelling was detected while the uptake of $^{86}Rb^{+}$ proceeded for the first 30 s and then reached a plateau. The lack of swelling probably results from the specificity of the $^{86}Rb^{+}$ uptake system which does not allow for counterion movements and from its inhibition after 30 s; i.e. both the PO_4-mediated lack of swelling and $^{86}Rb^{+}$ uptake inhibition may be due to the impermeability of the membrane to chloride once the YMUC is closed by 4 mM PO_4.

Both Mg^{2+} and quinine inhibited the uptake of monovalent cations in yeast mitochondria. Mg^{2+} was a better

inhibitor than quinine. The $^{86}Rb^+$ uptake sensitivity to both Mg^{2+} and quinine increased when the YMUC was closed, while it was enhanced by Zn^{2+}, suggesting that the slow $^{86}Rb^+$ uptake was probably counteracted by $^{86}Rb^+$ efflux through the antiporter (Villalobo *et al.*, 1981). It was not possible to follow the uptake of $^{86}Rb^+$ for longer than 2 min because of the possibility of O_2 depletion, even in the presence of added hydrogen peroxide. The highest $^{86}Rb^+$ uptake was observed at the low PO_4 concentration (0.4 mM), where the YMUC seems to be mostly opened, but does not seem to be as unspecific as in the absence of added PO_4. The Mg^{2+} and quinine sensitivity suggests that the K^+ uptake system in yeast mitochondria is qualitatively similar to the mammalian uniporter (Beavis *et al.*, 1993).

In mammalian mitochondria, PTP opening results in $\Delta\Psi$ depletion and Ca^{2+} efflux; it has been suggested that Ca^{2+} detoxyfication may be a physiological function of the PTP in cells that are not programmed to die (Crompton and Costi, 1990). In yeast mitochondria, the opening of the YMUC also results in depletion of the $\Delta\Psi$ and efflux of K^+ and probably other cations. These results suggest a general physiological role for unspecific mitochondrial pores, which may be involved in cation detoxification (Crompton and Costi, 1990). Furthermore, whether yeast undergo a process resembling apoptosis or not, is still controversial and ion detoxification would be a likely alternative as a putative function of mitochondrial unspecific channels (Manon *et al.*, 1998).

In the absence of PO_4, where the YMUC is open and the transmembrane potential is depleted, K^+-mediated swelling has been observed, (Castrejón *et al.*, 1997; Manon and Guérin, 1997). In the absence of PO_4, no $^{86}Rb^+$ accumulation was observed, even though swelling was present. Under these conditions, the YMUC was completely open, probably allowing for rapid permeation of molecules, leaving only proteins inside the matrix and evoking swelling as a result of oncotic pressure. Another possibility, is that $^{86}Rb^+$ was taken up leading to swelling, but the filtration process involving washing filters with cold Rb^+ resulted in the exit of $^{86}Rb^+$ through the open YMUC.

In the presence of 0.4 mM PO_4, the transmembrane potential was lost partially only if K^+ was added to the reaction mixture, suggesting that the unspecific pore became rather selective at this PO_4 concentration. Under this condition, both swelling and $^{86}Rb^+$ uptake correlated. At 4 mM PO_4, the data were still different, as no swelling was detected, while the uptake of $^{86}Rb^+$ was only slightly less than at 0.4 mM PO_4. At 4 mM PO_4, the unspecific channel is closed and thus the uptake of K^+ seemed to be catalyzed by a different system, which was less active than the YMUC. At 4 mM PO_4, $^{86}Rb^+$ did not promote swelling, probably due to the exit of K^+ through the reported K^+/H^+ antiporter (Jung *et al.*, 1997), which would be active in view of the high transmembrane potential detected at this PO_4 concentration.

Thus, in yeast, the K^+ recycling pathways seem to resemble mammalian mitochondria, in that they keep matrix K^+ at levels similar, or lower than those found in the cytosol (Brierley *et al.*, 1994; Garlid, 1996). In mammalian mitochondria, K^+ enters the matrix through a uniport that opens when ATP is depleted and is energized by the transmembrane potential (Beavis *et al.*, 1993). Once in the matrix, K^+ is expelled to the cytoplasm throughout a K^+/H^+ uniport (Brierley *et al.*, 1984, 1994). In yeast, the K^+/H^+ antiporter has already been described (Jung *et al.*, 1997; Villalobo *et al.*, 1981), while the uptake system described here would serve an equivalent function to that of the mammalian uniporter. When the YMUC was closed, the uptake of $^{86}Rb^+$ increased its sensitivity to the mammalian K^+ uptake inhibitors, Mg^{2+} and quinine (Fig. 6).

Under our experimental conditions, the rate of $^{86}Rb^+$ uptake was slow, and thus it was not possible to reach equilibrium before the transmembrane potential collapsed, once oxygen was depleted, even in the presence of oxygen peroxide. Nonetheless, addition of the antiporter inhibitor Zn^{2+} (Manon and Guérin, 1992, 1995) led to higher uptake, suggesting that the uptake of $^{86}Rb^+$ was counter balanced by an active exit of $^{86}Rb^+$ through the uniporter.

A $^{86}Rb^+$ uptake system was observed in yeast mitochondria under conditions where the unspecific pore is closed. This may be the system used by mitochondria to take up K^+ when in the presence of a high membrane potential. This transporter resembles the mammalian uniporter in its energy dependence as well as in the sensitivity to Mg^{2+} and to quinine.

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