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Rubidium transport in *Neurospora crassa*

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Rb^+ transport in low-K^+ cells of *Neurospora crassa* is biphasic, transport at millimolar Rb^+ being added to a transport process which saturates in the micromolar range. Both processes exhibit Michaelis-Menten kinetics, but in the micromolar phase the kinetic parameters depend on the K^+ content of the cell (the lower the K^+ content the lower the K_m and the higher the V_{max}). Normal-K^+ cells, suspended in a buffer with millimolar K^+, do not present Rb^+ transport in the micromolar range. Millimolar transport in these cells presents kinetics which depend on the K^+ in buffer (the higher the K^+ the higher the K_m), although the K^+ content of the cells is constant. Na^+ inhibits competitively Rb^+ transport in low-K^+ and normal-K^+ cells, but, even when the differences between the Rb^+ K_m values are more than three orders of magnitude, the apparent dissociation constant for Na^+ is the same, and millimolar, in both cases.

Introduction

In all forms of life, cells require K^+, and accumulate K^+, generally, against significant transmembrane concentration gradients. In cells of higher plants, fungi, and algae, the existence of a cell wall that can support high turgor presures determines the ability of growing in very diluted media. In these media, most substrates must move against high concentration gradients, K^+ being a good example of this situation, because of the high K^+ acitivity in the cellular water.

Until recently literature has suggested that higher plants and fungi were considerably different in their K^+ requirements and transport. Whereas higher plants thrive and transport K^+ at concentrations in the growing medium in the micromolar range (even lower than 1 μM K^+) [1,2], it was thought that both *Saccharomyces cerevisiae* [3] and *Neurospora crassa* [4] required 0.2–0.5 mM K^+ for growth, and had transport systems that presented half-maximum rates in the millimolar range [5,6]. Recent investigations, however, have shown that *S. cerevisiae* grows at micromolar K^+, provided that neither NH_4^+ nor Na^+ are at millimolar concentrations, and has a transport system that saturates in the micromolar range [7]. In addition to the micromolar transport, *S. cerevisiae* has another K^+ transport mode opperating at millimolar K^+, both resembling system 1 and system 2 in higher plants [1,2]. In low-K^+ cells of *Neurospora*, K^+ transport in the micromolar range has been demonstrated (Rodríguez-Navarro, A., Blatt, M.R. and Slayman, C.L., unpublished data), but the possibility of two transport modes (or systems), as in *S. cerevisiae* [7] or plants [1,2] had not been investigated.

In the present paper we report the characteristic of Rb^+ transport in *Neurospora* both in the micromolar and millimolar ranges, and discuss these results in connection with K^+ and Rb^+ transport in higher plants.

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Materials and Methods

Preparation of cells

Wild-type RL21a of *Neurospora crassa* was used throughout this work. The general methods of handling the cells have been described previously [4]. $3 \cdot 10^6$ conidia were inoculated per ml of a medium of the following composition: 15 mM $(NH_4)_2HPO_4$; 8.4 mM citric acid; 0.81 mM $MgSO_4$; 0.68 mM $CaCl_2$; 2% saccharose, plus trace elements and biotine as previously described [8]. The pH was adjusted to 5.8 with NH_4OH. To this medium, KCl was added at two different levels, 0.25 mM and 37 mM, to prepare low-K^+ cells and normal-K^+ cells, respectively.

For low-K^+ cells, cultures were incubated until the external K^+ dropped to 10–15 μM, which took about 12 h. At this moment, the size of the cells was similar to 10-h-old normal cultures, and the K^+ content was about a half of normal. For normal-K^+ cells cultures were incubated during 10 h in 37 mM K^+ medium. In both cases, cells were removed by filtration, washed with water, and suspended at 0.5–1.0 mg (dry weight) $\cdot$ ml^{-1} in 10 mM 4-morpholineethanesulphonic acid, 0.1 mM $MgCl_2$, 1% glucose, brought to pH 5.8 with $Ca(OH)_2$. Low-K^+ cells were suspended in the buffer without added K^+, and normal -K^+ cells, except in experiments of Fig. 1, in buffer with 0.5, 1.0 or 2.0 mM K^+.

In some experiments, low-K^+ cells were harvested before external K^+ dropped to 15 μM. By this procedure, we obtained cells with various K^+ contents (cf. Table I). These cells were used as those incubated up to 15 μM external K^+.

Rb^+ transport

Cells were allowed to equilibrate in buffers for 5 to 15 min, and then Rb^+ (RbCl was used unless otherwise stated) was added. At times, samples were taken, the cells washed with water, transferred to a new filter and washed again. After acid extraction, cations were analyzed by atomic absorption spectrophotometry.

Initial rates of uptake were determined from the plots of Rb^+ content versus time. Five samples were taken in five minutes and usually, the five points were in a straight line.

Cation contents expressed in nmol $\cdot$ mg^{-1} were occasionally expressed as millimolar by dividing by 2.5 [4].

Results

Rb^+ transport

When examined in a broad range of Rb^+ concentrations (10 μM to 100 mM), the initial rate of Rb^+ uptake (zero-trans influx) showed an apparent saturation in the micromolar range, but when Rb^+ reached the millimolar range there was a further increase in rate, and subsequent saturation (Fig. 1). The actual relationship between Rb^+ transport rate and concentration depended on the K^+ status of the cells. In washed cells from high-K^+ media, transport was slower than in cells harvested from low-K^+ media, in which the K^+ content was below the normal content. In this last case, the lower the K^+ content of the cells, the higher the Rb^+ transport. From the Rb^+ taken up, only a small proportion was in exchange with the internal K^+ (less than 25% for Rb^+ above 15 μM); thus the total cation content of the cells increased significantly.

Analyses of the initial rates of Rb^+ uptake between 15 μM and 200 μM Rb^+, did not show significant deviations from a Michaelis-Menten kinetics, and K^+ showed a linear competitive inhibition on Rb^+ transport (Fig. 2) (apparent dis-

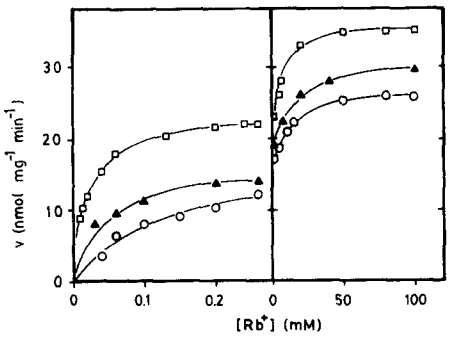

Fig. 1. Initial rates of Rb^+ uptake as a function of the Rb^+ concentration. (○) Cells grown at high K^+ (37 mM) washed and transferred to K^+-free buffer (192 mM K^+ content). (▲) Cells grown at 0.25 mM K^+ and harvested when the external K^+ was 50 μM (140 mM K^+ content). (□) Cells grown at 0.25 mM K^+ and harvested when the external K^+ was 15 μM (116 mM K^+ content). Initial rates of uptake were determined as described in text.

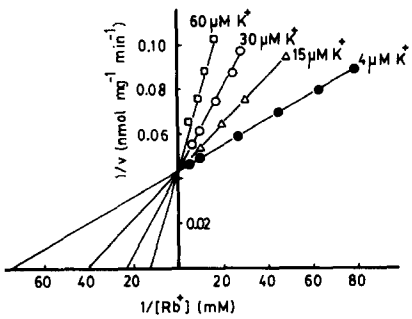

Fig. 2. Double-reciprocal plot of Rb^+ transport in low-K^+ cells at several K^+ concentrations up to 0.2 mM Rb^+. Experiments were carried out as in the upper curve of Fig. 1, cells with 116 mM K^+ content. 4 μM K^+ is the average K^+ concentration, in K^+-free buffer after Rb^+ addition to the cells.

sociation constants, 6 μM for Rb^+ and 5 μM for K^+). The data of Fig. 1 could be fitted to a rate equation resulting from the addition of two processes, both following Michaelis-Menten equations (Table I) [1]. Although the kinetics might be also explained by a multiphasic process [9] or by two Michaelis-Menten terms plus a linear term [10], a dual system gave enough precision for our porposes and we did not try to make a more complex kinetic study.

In higher plants, the transport of Rb^+ in the millimolar range showed a much lower activity when Rb^+ sulfate was used instead of Rb^+ chloride [1]. We investigated whether the anion had a similar effect in *Neurospora*, with negative results.

In yeast, the micromolar transport of K^+ and Rb^+ has been investigated in cells grown in the absence of NH_4^+ using L-arginine phosphate medium [7]. In *Neurospora*, this approach was impossible because arginine inhibited Rb^+ transport at low Rb^+, and it also inhibited growth at low K^+ (below 100 μM K^+) (not shown).

Millimolar Rb^+ transport

In higher plants, the second phase of Rb^+ transport (as in Fig. 1) has been proposed to correspond to the activity of a millimolar transport system [1], although other interpretations have been given [11]. In *Neurospora*, the two phases of Rb^+ transport were characteristic only of low-K^+ cells. Normal-K^+ cells which have not been washed (only centrifugated and suspended) did not show the phase of micromolar Rb^+ transport. Similarly, neither washed normal-K^+ cells nor low-K^+ cells exposed to K^+ for a few minutes showed micromolar transport. Therefore we studied Rb^+ transport in the millimolar range in normal-K^+ cells. Cells were grown in high K^+ and, after washing, transferred to the buffer with different amounts of K^+; once the steady state had been reached (10 min), Rb^+, or Rb^+ and K^+, was added. In all cases, Rb^+ transport did not appreciably deviate from a Michaelis-Menten kinetics, and K^+ showed a linear competitive inhibition. Table II summarizes the kinetic parameters of Rb^+ transport for cells in steady state in different K^+ concentrations. The apparent dissociation constant of Rb^+

TABLE I

KINETIC PARAMETERS FOR Rb^+ TRANSPORT IN LOW-K^+ CELLS, AND WASHED CELLS WITH A NORMAL-K^+ CONTENT

Kinetic parameters were calculated from data in Fig. 1 using the formula [1]:

$$v = \frac{V_{max_1}[Rb^+]}{K_{m_1}+[Rb^+]} + \frac{V_{max_2}[Rb^+]}{K_{m_2}+[Rb^+]}$$

Internal K^+ (mM)	K_{m_1} (mM)	V_{max_1} (nmol·mg^{-1}·min^{-1})	K_{m_2} (mM)	V_{max_2} (nmol·mg^{-1}·min^{-1})
116	0.006	22	12.5	16.7
140	0.040	16	12.0	14.8
192	0.111	15	13.3	10.5

TABLE II

KINETIC PARAMETERS FOR Rb^+ TRANSPORT IN NORMAL-K^+ CELLS IN STEADY-STATE WITH 0.5, 1 .0 AND 2.0 mM K^+

Parameters of transport were obtained from double reciprocal plots of Rb^+ transport. Cells were grown in 37 mM K^+ and after washing preincubated in buffer with 0.5, 1.0, 2.0 mM K^+. Initial rates of uptake were determined as described in text, after the addition of Rb^+ or Rb^+ plus K^+.

$[K^+]_0$ (mM)	$[K^+]_i$ (mM)	Rb^+ K_m (mM)	K^+ K_i (mM)	V_{max} (nmol·mg^{-1}·min^{-1})
0.5	182±38	4.0	0.9	11.8
1.0	186±19	10.5	3.5	11.1
2.0	182±16	15.0	4.5	11.5

and K^+ for the porter varied with the K^+-status of the cells. The higher the external K^+ (cells in steady state), the higher the apparent dissociation constants. The V_{max} of the process did not varied with the K^+-status in normal-K^+ cells. Interestingly, although Rb^+ transport was affected by the external K^+ level in which the cells were in steady state, we did not find significant differences in the K^+ content of the cells. This suggests that internal K^+ is not the regulator of Rb^+ (K^+) transport in normal-K^+ cells. In these experiments, most of the Rb^+ taken up was in exchange with K^+, and total cation content did not increase significantly.

Effect of Na^+ on Rb^+ transport

Na^+ inhibited Rb^+ transport competitively in both micromolar and millimolar modes (Fig. 3). The apparent dissociation constant of the porter for Na^+ was millimolar in both modes of transport (14 mM in micromolar cells and 15 mM in millimolar cells in presence of 0.5 mM K^+).

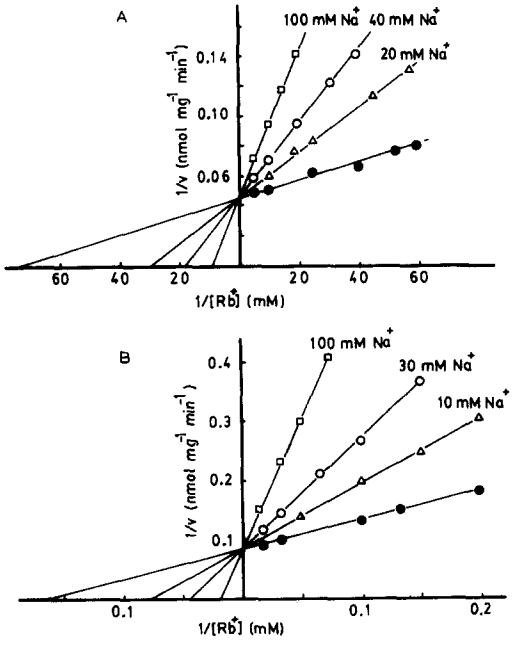

Fig. 3. Effect of Na^+ on Rb^+ transport. Double reciprocal plot of Rb^+ transport at several Na^+ concentrations in low-K^+ cells (116 mM K^+ content) (A), and normal-K^+ cells in 0.5 mM K^+ (B). Experiments were carried out as in Figs. 1 and 2 in presence of the indicated concentrations of Na^+.

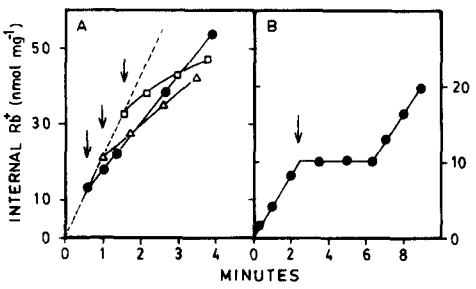

Fig. 4. Effect of glucose addition on the Rb^+ transport of carbon-starved cells. (A) Low-K^+ cells (116 mM K^+ content) were suspended in K^+-free, glucose-free buffer, and aerated for three hours. The cells were then transferred to fresh buffer (no glucose), preincubated 5 min and RbCl added (50 μM) at time zero. In three experiments, glucose (1 mM) was added at minutes 0.5 (●), 1.0 (△), 1.5 (□) (arrows). When glucose was added at time zero, uptake was not different from control (no glucose) experiment. (B) Normal-K^+ cells were carbon-starved for three hours in 0.5 mM K^+ buffer (no glucose) and suspended in fresh buffer. After 5 min of preincubation, RbCl (50 mM) was added (time zero) and 2.1 minutes later glucose (1 mM) was added.

Energy requirements

Rb^+ transport, in the micromolar and millimolar modes, was sensitive to cyanide. The use of salicylhydroxamic acid with cyanide to inhibit the cyanide-insensitive oxidases [12] did not increase inhibition. In presence of cyanide, the inhibition of Rb^+ transport was similar in low-K^+ cells and in normal-K^+ cells, even when, in millimolar cells, transport was downhill (50 mM external Rb^+ would equilibrate to 125 nmol · mg^{-1} in cells, or more considering the activity coeficient). In both cases, cyanide induced a significant K^+ loss, which was even higher than the Rb^+ gain.

To investigate the effect of depolarization of the membrane on Rb^+ transport, we added glucose to carbon-starved cells [13]. In low-K^+ cells, the effect was a clear inhibition when glucose was added after Rb^+, but, surprisingly, the inhibition was lower if glucose was added soon after Rb^+, and no inhibition resulted if added simultaneously. In normal-K^+ cells, addition of glucose at any time produced a low transient inhibition of Rb^+ uptake with a rapid recovery of the normal rate (Fig. 4).

Discussion

Present results show that Rb^+ transport in *Neurospora* has the following characteristics: (i) in

low-K^+ cells and washed cells with a normal-K^+ content, Rb^+ transport had two phases, which could be sufficiently explained by the addition of two Michaelis-Menten equations; (ii) in normal-K^+ cells (unwashed cells) there was only one phase showing an apparent dissociation constant three orders of magnitude higher than that of the first phase in low-K^+ cells; (iii) K^+ and Rb^+ competed for transport, and both had dissociation constants of the same order; (iv) Na^+ was also a competitive inhibitor of Rb^+ transport, but its apparent dissociation constants were similar in low-K^+ and normal-K^+ cells, comparable to that of Rb^+ in normal-K^+ cells; (v) micromolar transport took place with a low Rb^+/K^+ exchange, but transport in normal-K^+ cells was mainly a Rb^+/K^+ exchange.

The most important conclusion from present results is that $Rb^+(K^+)$ transport in *Neurospora* compared perfectly with transport models in higher plants [2], even with reference to the dependence on the K^+-status of the cells (compare Fig. 1 with results in Ref. 14). Regarding the two modes of transport (micromolar and millimolar transport), micromolar transport was observed in K^+–starved cells obviously with the function of replenishing the cell to the normal K^+ content. In contrast the low affinity transport, observed in normal-K^+ cells, only had to provide the K^+ required for growth. At this point, a pertinent question is whether the millimolar transport in normal-K^+ cells is the second phase of transport in low-K^+ cells. This question cannot be answered with the present results, but the insensitivity of the second phase to the K^+-status of the cell (Table I) might indicate that this phase is not the expression of the millimolar K^+ transport system. In fact, in normal-K^+ cells, at 0.5 mM K^+, the apparent K_m for Rb^+ is 4 mM (Table II), three times lower than the apparent K_m of the second phase in low-K^+ cells (Table I). For a K^+ transport system, a more active transport would be expected in low-K^+ cells. The second phase could be the Na^+ transport system, as proposed in plants [15,16]. However, because Na^+ inhibits Rb^+ transport and has the same affinity for the carrier both in low-K^+ and normal-K^+ cells (Fig. 3), the existence of only one system for K^+ and Na^+ transport cannot be ruled out. This system could have several modes of operation.

Previous works with *Neurospora* [4,5] reported a K_m of 11.8 mM for K^+ uptake, which is not consistent with the present results. Those experiments were carried out in absence of Ca^{2+}, and the lack of this cation may affects transport in *Neurospora* as it does in plants [17,18].

Acknowledgements

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