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DISCRIMINATION BETWEEN Rb^+ AND K^+ BY *ESCHERICHIA COLI*

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Summary

1. The K^+ requirement of *Escherichia coli* is only partially fulfilled by Rb^+. The molar growth yield on Rb^+ was about 5% of that on K^+ and the growth rate in Rb^+-supplemented media is lower than in K^+-supplemented media.

2. $^{86}Rb^+$ is not a satisfactory tracer for net K^+ influx by any of the four K^+ transport systems of *E. coli*. The high-affinity Kdp system (K_m = 2 μM) is poorly traced by $^{86}Rb^+$. It discriminates against a $^{86}Rb^+$ tracer at least 1000-fold. The two moderate affinity systems, the high-rate TrkA system (K_m = 1.5 mM) and the moderate rate TrkD system (K_m = 0.5 mM), discriminate against a $^{86}Rb^+$ tracer by approximately 10-fold and 25-fold, respectively. $^{86}Rb^+$ is preferred by the low-rate TrkF system and overestimates its K^+ influx by 40%.

Introduction

Because the half life of the K^+ congener $^{86}Rb^+$ is 40 times greater than that of $^{42}K^+$ (19.5 days vs. 12.6 h), $^{86}Rb^+$ has become widely adopted as a convenient substitute for the measurement of K^+ fluxes in cells. Nevertheless, the validity of using $^{86}Rb^+$ as a radioactive tracer for K^+, or the ability of chemical Rb^+ to satisfy a cell's K^+ requirement, has not been adequately demonstrated for many biological systems including the bacterium *Escherichia coli*. In those systems which have been examined, Rb^+ substitution for K^+ yields a range of results. Some species of the lactic acid bacteria exhibit equivalent molar growth yields on K^+ and Rb^+ while others show no growth stimulation by Rb^+ [1]. Yeast transport Rb^+ at about half the rate that they transport K^+ [2]. Both Rb^+ and K^+ are transported equally well by the Na^+ and K^+ transporting adenosine triphosphatase ((Na^+ + K^+)-ATPase) of animal cell plasma membranes [3,4]. Our interest in K^+ transport in *E. coli* led us to investigate the extent to which $^{86}Rb^+$ accurately traces K^+ fluxes in this organism.

K^+ serves an osmoregulatory function in *E. coli* [5]. The multiplicity of K^+

transport systems in *E. coli* attests to the importance of this function. K^+ transport is mediated by four transport systems which have been resolved by genetic and kinetic techniques [6—8]. The high affinity Kdp system (K_m = 2 μM) is sensitive to cold osmotic shock [9] which releases periplasmic proteins involved in transport [10]. This system is repressed by growth on high K^+ (>0.5 mM for wild-type strains) and is absent in strains carrying mutations in any one of 4 linked *kdp* genes. The ability of *E. coli* to grow at maximum rates in very low external K^+ concentrations is due to derepression of this system. Two constitutive moderate affinity systems, the TrkA system (K_m = 1.5 mM) and the TrkD system (K_m = 0.5 mM), are apparently coded for by single genes — *trkA* and *trkD*, respectively. The TrkA system, with a *V* at 37°C of 550 μmol/g min, is responsible for the majority of K^+ uptake during growth in media containing high K^+. The *V* of the TrkD system is 40 μmol/g min at 30°C. Both of the moderate affinity systems are resistant to osmotic shock (ref. 9 and Woo, A. and Rhoads, D.B., unpublished). In strains lacking all three saturable K^+ transport systems by mutation, a low rate of K^+ uptake linearly dependent on the external K^+ concentration occurs by a system referred to as TrkF. Mutations in this system have not been identified to date.

We find that Rb^+ only partially fulfills the K^+ requirement of *E. coli*. $^{86}Rb^+$ is not a satisfactory tracer for any of the K^+ transport systems of *E. coli*. The Kdp system is very poorly traced by $^{86}Rb^+$. Both the TrkA and the TrkD systems mediate $^{86}Rb^+$ uptake, but the tracer reflects only about 10% and 4% of the K^+ fluxes mediated by these systems, respectively. $^{86}Rb^+$ overestimates the TrkF system by 40%.

Materials and Methods

Bacterial strains. The five strains used in this work, all *Escherichia coli* K-12, are listed in Table I along with their genotype and K^+ transport systems present.

Media and growth of bacteria. Phosphate-buffered media, described earlier [6,7], are referred to by K^+ concentration in mM; e.g., K115 contains 115 mM K^+. KO, to which no K^+ is added, contains about 20 μM contaminating K^+. K-free medium is prepared by incubating a K^+-limited wild-type strain in KO plus glucose for 30 min, then removing the bacteria by filtration. Magnesium maleate medium [8] was used in several experiments because it contains less contaminating K^+. Cells were grown at 37°C in the presence of 11 mM glucose. Derepression of the Kdp system was accomplished by transferring growing cells to KO medium until cells were K^+ limited for 1 h. Cell growth was monitored by turbidity measurements at 610 nm with a Bausch and Lomb Spectronic 20 colorimeter.

Transport studies. Transport measurements were made in K^+-depleted cells. K^+ efflux is very low during the initial phase of K^+ uptake by such cells [11] so net K^+ influx is essentially identical to the unidirectional K^+ influx. Cells were grown in phosphate-buffered media, harvested by centrifugation, washed twice with KO medium, and depleted of K^+ by a 30 min incubation at 37°C in KO medium containing 10 mM 2,4-dinitrophenol. These K^+-depleted cells were then washed twice with Buffer A (75 mM $NaPO_4$, pH 7, 0.4 mM $MgSO_4$, and 0.15 mM chloramphenicol) and suspended in the same buffer containing 11

TABLE I

BACTERIAL STRAINS

Strain	K^+ transport system(s) present	Relevant genotype	Reference
FRAG-1	All	wild type	6
TK1001	TrkA TrkF	*kdpABC5 trkD1*	8
TK1110	TrkD TrkF	*kdpABC5 trkA405*	
TAD109	Kdp TrkF	*trkA405 trkD1*	9
TK405m	TrkF	*kdpABC5 trkA405 trkD1*	Similar to TK401 (8)

mM glucose. Uptake was initiated by the addition of KCl (KPO_4, pH 7, was used to achieve K^+ concentrations of 10 mM or more) containing a trace of $^{86}RbCl$. Timed cell samples were collected on 0.45 μm Millipore filters, washed with ice-cold 0.4 M glucose containing 10 mM Tris · Cl, pH 7, and 0.4 mM $MgSO_4$, dried and counted in a liquid scintillation spectrometer. Filters were removed from the scintillation vials, rinsed of scintillation fluid by dipping them successively in two beakers of petroleum ether, and analyzed for K^+ by flame photometry. Assay of the Kdp system was done by the medium depletion method [8]. Transport rates are expressed as μmoles taken up per minute and gram dry weight, the latter determined from turbidity measurements and a calibration curve.

Chemicals. RbCl was prepared from RbOH (purity = 99.9%, Apache Chemicals, Rockford, Ill.) by neutralization with HCl, dried to constant weight and stored in plastic. $^{86}Rb^+$ and $^{42}K^+$ were obtained from New England Nuclear Corp., Boston, Mass.

Results

The ability of Rb^+ to fulfill the K^+ requirement of *E. coli* was determined by examining the growth of wild-type strain FRAG-1 on these two cations. The growth yields shown in Fig. 1 demonstrate that although a high concentration of Rb^+ can stimulate the growth of *E. coli*, the growth achieved is only a fraction of that achieved on low concentrations of K^+. Cells from this experiment were analyzed for K^+ content to determine whether the Rb^+-stimulated growth could be accounted for by K^+ contamination. Assuming that essentially all K^+ is intracellular in K^+-limited overnight cultures, cell K^+ content and amount of growth can be used to calculate the initial K^+ contamination in each culture. Cells grown in the absence of added cation have 260 μmol K^+/g consistent with a 4 μM K^+ contamination in this culture while cells grown to a considerably higher density in the presence of 10 mM RbCl have 50 μmol K^+/g consistent with 6 μM K^+ contamination. The increased growth supported by RbCl cannot be accounted for by K^+ contamination. When an overnight culture of FRAG-1 grown in Mg maleate medium plus 10 mM RbCl is diluted 10-fold with the same medium, another round of growth ensues similar to the first round but is not accompanied by a further reduction in the intracellular K^+ content. *E. coli* K-12 is not unique among enteric bacteria in exhibiting a low growth yield on

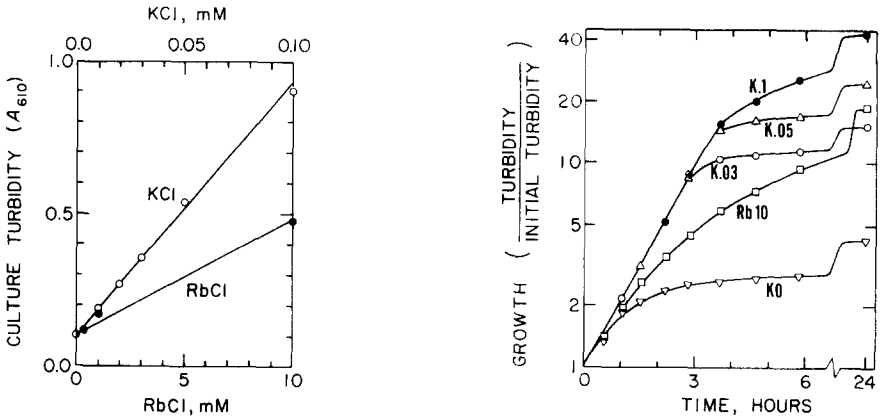

Fig. 1. Growth yield of strain FRAG-1 on KCl and RbCl. Strain FRAG-1 was grown in magnesium maleate medium containing 2 mM KCl and 11 mM glucose. Cells were collected by centrifugation, washed twice with Mg maleate medium, and inoculated at a density of $3 \cdot 10^7$ cells per ml into fresh medium containing 11 mM glucose and various amounts of KCl (○) or RbCl (●). Cells were incubated with shaking for 22 h at 37°C, and an additional 11 mM glucose was added. 2 h later medium turbidity was measured at 610 nm in a Spectronic 20. Note the different scale for the two salts.

Fig. 2. Growth of FRAG-1 on KCl and RbCl. These data are from the experiment of Fig. 1. ▽, no addition; ○, 30 μm KCl; △, 50 μM KCl; ●, 100 μM KCl; □, 10 mM RbCl.

Rb^+ as compared to K^+ since we find that both the distantly related *E. coli* strain ML 308-225 and a *Salmonella typhimurium* strain have a similar pattern of growth yield in Rb^+-supplemented media (data not shown).

Strain FRAG-1 also exhibits a lower growth rate when grown on Rb^+ than when grown on K^+. Wild-type cells growing on limiting K^+ grow at a maximum rate until the extracellular K^+ is exhausted, at which point the turbidity plot inflects [12]. The growth data in Fig. 2 show that the growth rate on 10 mM RbCl is never as great as the rate when only K^+ is present, and decreases progressively with time.

The ability of Rb^+ to interfere with K^+ utilization was tested in three strains, each of which depends on a different K^+ transport system for K^+ uptake during growth (Table II). Strain TK1001, which relies mainly in the TrkA system for K^+ uptake, grows at a half-maximal rate in 0.34 mM K^+ in Mg

TABLE II

EFFECT OF RbCl ON THE GENERATION TIMES OF K^+ TRANSPORT MUTANT STRAINS

Experimental protocol is described in the legend to Fig. 1. Growth was monitored by turbidity measurements at 45-min intervals.

Strain	K^+ transport system	K^+ concn. in medium (mM)	RbCl concentration (mM)			
			0	10	30	80
TAD109	Kdp	0.1	63 *	67		
TK1001	TrkA	0.2	152		400	
		20	60		60	
TK405m	TrkF	20	188			310
		100	69			

* Values are generation times in minutes.

maleate medium [8]. In 0.2 mM K^+, a concentration at which growth rate is very sensitive to K^+ concentration, the generation time of strain TK1001 is increased 2.5-fold by the addition of 30 mM RbCl (Table II). The inhibitory effect of RbCl is reversed when the K^+ concentration is raised to 20 mM. Similarly, strain TK405m which requires very high K^+ to support rapid growth exhibits a 70% increase in the generation time on 20 mM K^+ when a four-fold excess of RbCl is added. In strain TAD109 which uses the Kdp system at low K^+ concentrations, no significant effect of 10 mM RbCl was seen in medium containing 100 μM KCl. A sensitive test of Rb^+ inhibition in this strain was not possible by this type of growth experiment because addition of K^+ to well above the 2 μM K_m of the Kdp system was necessary to prevent K^+ limitation.

The utility of $^{86}Rb^+$ as a K^+ tracer was tested in experiments in which transport of both was measured simultaneously in a strain where K^+ uptake was solely, or very largely due to action of a single K^+ transport system. Figure 3 shows an experiment in which K^+-depleted Kdp-derepressed cells of strain FRAG-1 were incubated in the presence of $^{42}K^+$ and $^{86}Rb^+$. It can be seen that the cells took up over 99% of the $^{42}K^+$ in 4 min, while less than 3% of the $^{86}Rb^+$ was taken up even after 25 min. Estimations of the concentration gradients achieved were made from cell samples taken at 25 minutes. $^{42}K^+$ was concentrated 6 · 10^6-fold while $^{86}Rb^+$ was concentrated no more than 5000-fold. Thus $^{86}Rb^+$ is a very poor tracer for K^+ with the high-affinity Kdp system.

Table III shows net K^+ uptake rates in K^+-depleted cells via the moderate affinity systems measured by a $^{86}Rb^+$ tracer as compared to the rates measured by flame photometry of chemical K^+. The K^+ uptake rates determined by the $^{86}Rb^+$ tracer were calculated by dividing the initial rate of uptake of radioactivity by the cpm of $^{86}Rb^+$ per μmol of K^+ in the medium. Comparison of this value with the value derived from chemical K^+ measurements yields an estimation of the accuracy of $^{86}Rb^+$ as a K^+ tracer. It is clear that for both the TrkA and the TrkD systems, the $^{86}Rb^+$ tracer method underestimates the K^+ influx. The underestimation varies from 5- to 13-fold for the TrkA system and from 23- to 30-fold for the TrkD system. The results of a similar experiment with the low affinity TrkF system are shown in Table IV. Here, at all three K^+ concen-

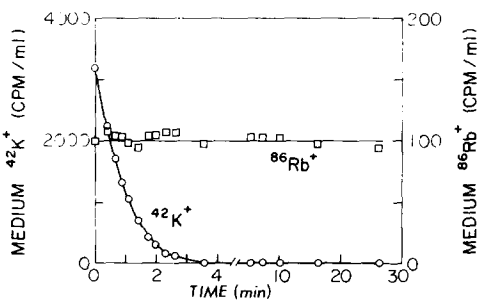

Fig. 3. Uptake by the Kdp system. Strain FRAG-1 was K^+ limited in KO medium, depleted of K^+ by DNP treatment, and suspended in K-free medium plus 11 mM glucose at 18°C and a density of 27 μg cells per ml. At zero time uptake was initiated by the addition of 4.5 μM ^{42}KCl (3200 cpm/ml) and 0.67 nm ^{86}RbCl (100 cpm/ml). Medium samples obtained by rapid filtration were counted immediately and 13 days later when $^{42}K^+$ had decayed by greater than 7 orders of magnitude. $^{86}Rb^+$ remaining in the medium (□) was determined by decay correcting the latter counts and $^{42}K^+$ remaining (○) was calculated by subtracting the $^{86}Rb^+$ counts from the immediate counts.

TABLE III

MEASUREMENT OF NET K^+ UPTAKE RATES BY THE TrkA AND TrkD SYSTEMS WITH $^{86}Rb^+$

Cells were grown in K115, depleted of K^+ by 2,4-dinitrophenol, and assayed for K^+ uptake at the indicated K^+ concentrations in the presence of 2 μM $^{86}RbCl$ (600 000 cpm/ml). K^+ uptake by the chemical K^+ method was determined by flame photometry. K^+ uptake by the $^{86}Rb^+$ method was determined by assuming that $^{86}Rb^+$ is a perfect tracer for K^+ (see Text).

Strain	Measurement method	K^+ concentration (mM)		
		0.1	0.5	1.7
TK1001 (TrkA)	Chemical K^+	26 *	94	160
	$^{86}Rb^+$	5.4	12	12
TK1110 (TrkD)	Chemical K^+	7	14	18
	$^{86}Rb^+$	0.3	0.5	0.6

* Values are initial rates of uptake in μmol/g min at 30° C.

TABLE IV

MEASUREMENT OF NET K^+ UPTAKE RATES BY THE TrkF SYSTEM WITH $^{86}Rb^+$

Strain TAD109 grown in K115 medium to repress the Kdp system was used. The experimental protocol was the same as described in Table III. $^{86}RbCl$ was added to 10 μM ($6.4 \cdot 10^6$ cpm/ml).

Measurement method	K^+ concentration (mM)		
	10	30	100
Chemical K^+	0.8 *	2.2	9.0
$^{86}Rb^+$	1.2	3.2	12.5

* Values are initial rates of uptake in μmol/g min at 30° C.

trations tested, the $^{86}Rb^+$ tracer method overestimates K^+ influx. On the average, this overestimation is by a factor of 1.4.

Discussion

The experiments presented in this paper demonstrate that a) the K^+ requirement of *E. coli* can only partially be fulfilled by Rb^+ (Fig. 1), b) Rb^+ acts as a low-affinity inhibitor of cell growth (Table II), and c) $^{86}Rb^+$ does not accurately trace any of the K^+ transport systems in *E. coli* (Fig. 3, Tables III and IV). Lester [13] has presented evidence that 1.5 mM RbCl can fulfill the K^+ requirement in *E. coli*. However, he did not rule out K^+ contamination of the RbCl by direct analysis of cell K^+ content. Our results show that at this low concentration of Rb^+, *E. coli* growth is barely stimulated. Much higher RbCl concentrations are required to significantly stimulate growth, and this growth cannot be accounted for by K^+ contamination in the Rb^+ used in this work. The finding that Rb^+ stimulated bacterial growth suggests that Rb^+ can serve a function similar to that served by K^+, albeit with less efficiency. Our inability to reduce cell K^+ content below 50 μmol/g by growth in Rb^+-supplemented media could be due to an absolute requirement for at least some K^+ even in the presence of Rb^+. This postulated K^+ requirement not completely satisfied by

Rb^+ could be in osmoregulation or in the activation of cell enzymes. Our data do not distinguish between these possibilities.

Three of the four K^+ transport systems of *E. coli* are traced by $^{86}Rb^+$ during net K^+ uptake but $^{86}Rb^+$ uptake does not provide an accurate quantitation of K^+ influx by any of the systems. We have used the accuracy with which $^{86}Rb^+$ traces K^+ uptake for each K^+ transport system as a measurement of the discrimination of each system between $^{86}Rb^+$ and K^+. Discrimination by each system was approximately proportional to each system's apparent affinity for K^+. The high-affinity Kdp system discriminated against the $^{86}Rb^+$ tracer by at least 1000-fold. The moderate affinity systems exhibit an intermediate discrimination toward the $^{86}Rb^+$ tracer — about 10-fold for the TrkA system and about 25-fold for the TrkD system. The low-affinity TrkF system takes up $^{86}Rb^+$ preferentially to K^+. This phenomenon leads to a 40% overestimate of K^+ influx by this system using the $^{86}Rb^+$ tracer method. Discrimination between $^{86}Rb^+$ and K^+ for uptake may be only apparent if K^+ is retained while $^{86}Rb^+$ rapidly leaves the cell as soon as it is taken up. Discrimination in the efflux process is not likely to account for our results since the uptake measurements were made with K^+-depleted cells which have a low K^+ efflux [11] and would not be presumed to have a high initial rate of Rb^+ efflux. Furthermore, $^{86}Rb^+$ efflux does not appear to be rapid since the initial uptake slopes are linear for all three K^+ transport systems that mediate significant $^{86}Rb^+$ uptake. We conclude that for *E. coli*, chemical Rb^+ is a poor substitute for K^+ and $^{86}Rb^+$ is not a satisfactory tracer for K^+ fluxes.

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