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THE DISCRIMINATION BETWEEN Rb^+ AND K^+ BY *ESCHERICHIA COLI* IS CHANGED AFTER BACTERIOPHAGE T7 INFECTION

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Rb^+ and K^+ have similar chemical properties. They share the uptake systems in *Escherichia coli* and can replace each other inside the cell. These common features led to experiments in which the radioactive isotope ^{86}Rb was used to trace intracellular K^+ fluxes. However, the *E. coli* pumps discriminate between these two ions and one should thus be cautious using $^{86}Rb^+$ as a tracer for K^+. We now report that T7 infection alters the degree of discrimination in such a way that changes of intracellular Rb^+ do not reflect changes of K^+. It has been observed that shortly after infection the $^{86}Rb^+$ level was strongly reduced (Ponta, H., Altendorf, K.-H. and Schweiger, M. (1976) Mol. Gen. Genet. 149, 145–150). In contrast, determination of the K^+ content showed no change directly after infection (Kuhn, A., Jütte, H. and Kellenberger, E. (1983) J. Virol. 47, 540–552). The efflux of ^{86}Rb was only evident when Rb^+ was used in trace amounts. In media conditions under which intracellular K^+ was mainly replaced by Rb^+, $^{86}Rb^+$ efflux was not observed.

A virus-induced drop in the intracellular ionic strength might have a regulatory function during infection, producing an optimal ionic environment for viral enzymes and concomitantly disfavouring host enzymes [1]. Judging from $^{86}Rb^+$ fluxes, Ponta et al. [2] concluded that shortly after T7 infection *Escherichia coli* B loses a substantial amount of its intracellular K^+. This observed change of $^{86}Rb^+$ fluxes was taken to support the hypothesis of a phage-induced change in the ionic strength of the *E. coli* cell. However, Rhoads et al. [3] showed that all K^+-transport systems of *E. coli* discriminate between K^+ and Rb^+. Therefore, Rb^+ might not be a good analog to estimate changes in the cellular K^+ content. In this respect, we have investigated whether K^+ and Rb^+ concentrations are changed after T7 infection by comparing $^{86}Rb^+$ fluxes and the cellular contents of Rb^+ and K^+.

The intracellular level of Rb^+ and K^+ in *E. coli* was determined in media containing different Rb^+ concentrations. The concentration of K^+ in these media was always kept at about 1 mM, in order to repress the expression of the Kdp-transport system which codes for the high-affinity K^+-transport system [4]. Under our media conditions the K^+ as well as the Rb^+ are transported by the Trk system that codes for the low-affinity K^+-transport system [5]. This pump discriminates between the two ions about 10-fold and preferentially takes up K^+ [3].

For estimation of $^{86}Rb^+$ content (Amersham International) (2 mCi/mmol), fresh overnight cultures were diluted 1000-fold into tryptone medium containing 1 μCi $^{86}Rb^+$/ml and grown for at least five generations. At certain times, before or after infection, 0.5 ml of culture was deposited on a 0.22 μm membrane filter (Millipore GSWP) and washed with 4 ml of the corresponding nonradioactive

medium. The dried filters were then put into Insta-Gel (Packard) and assayed for radioactivity.

The effect of T7 infection on the intracellular levels of K^+ and $^{86}Rb^+$ was analyzed. As shown in Fig. 1, when only traces of Rb^+ were present in the media (approx. 0.5 μM) the $^{86}Rb^+$ content of cells rapidly decreased upon phage infection. However, the intracellular K^+ content remained unchanged ($\pm 4\%$) suggesting that *E. coli* B had enhanced discrimination of K^+ over Rb^+ after infection by T7. This could have been achieved either by an increase in the Rb^+ efflux or by inhibition of Rb^+ uptake shortly after infection. To test these possibilities, we investigated the efflux of $^{86}Rb^+$ from prelabelled cells with an experimental approach by which we were able to exclude interference by uptake of tracer. This was accomplished using a continuous filtration technique whereby the lost solutes are continuously washed away and are no longer available for uptake [6]. Fig. 2 shows the efflux from uninfected *E. coli* B, which was observed after removal of extracellular fluid by washing with 12 ml medium. Shortly after infection, the $^{86}Rb^+$ efflux was considerably enhanced, explaining the lowering of the Rb^+ content described above. On the other hand,

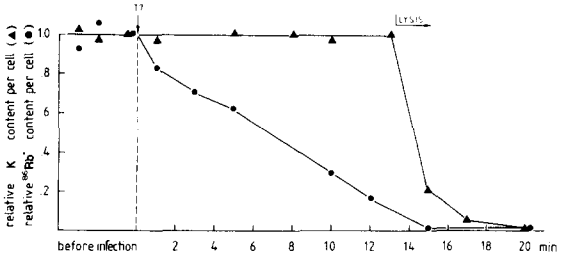

Fig. 1 $^{86}Rb^+$ vs. K^+ content at conditions with low intracellular Rb^+ concentration. *E. coli* B were grown at 37°C in tryptone medium containing 0.5 μM RbCl to a density of $2 \cdot 10^8$ cells/ml and then infected by T7 (five phages per cell). At the times indicated on abscissa 2-ml portions were deposited on membrane filters (Millipore GSWP, 0.22 μM) under suction. The cells were washed with 4 ml of aerated, prewarmed, isotonic 50 mM LiCl/0.2% glucose/7% sucrose. The $^{86}Rb^+$ content was analyzed radiochemically (●) and the K^+ content chemically (▲). For the chemical analysis, the cells were treated with 1 M HNO_3 for 30 min. The solution was then analyzed by a Unicam SP 2900 atomic absorption spectrometer at 766.5 nm. The values were calculated per cell and transformed to intracellular concentration, taking a cellular water volume of $1.22 \cdot 10^{-12}$ ml [6].

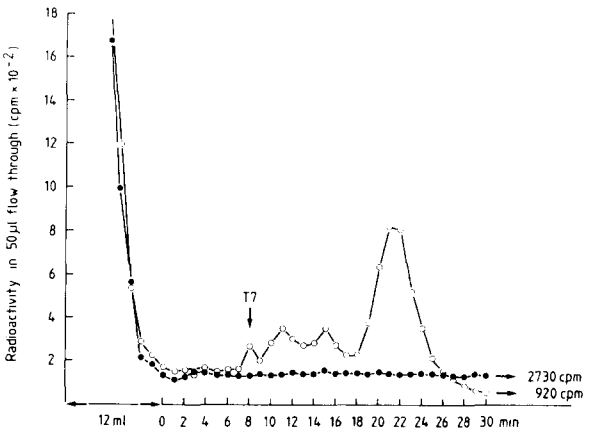

Fig. 2 $^{86}Rb^+$ efflux of uninfected (●) and T7-infected cells (○) monitored by continuous filtration. Fresh overnight cultures of *E. coli* B were diluted 10^{-3}-fold into tryptone medium containing 1 μM/ml ^{86}RbCl (1 mCi/mmol) and grown to a density of $1 \cdot 10^8$ cells per ml at 37°C. Then 1 ml of the culture was deposited in a Millex-GS filter and continuously washed with aerated, non-labelled medium (1 ml/min) at 37°C. The fractionated flow-through was then analyzed for radioactivity.

it has already been shown [2,7] that uptake of $^{86}Rb^+$ into uninfected and T7-infected cells occurs at about the same rate. Therefore, although the properties of the K^+ pumps might not be affected upon T7 infection, the permeability of the membrane is changed in such a way that Rb^+ is lost preferentially over K^+.

What might stimulate the host cell to change certain efflux properties of its membrane? One could assume that high intracellular concentrations of Rb^+ have a toxic effect on the propagation of the phage. We tested this by increasing the concentration of Rb^+ in the culture medium to about 15 mM RbCl (Table I). Quantitation of both [Rb^+] and [K^+] showed that about 60% of the intracellular K^+ was replaced by Rb^+. The sum of the Rb^+ and K^+ concentrations corresponded to the value we obtained for the K^+ content alone (290 mM) when the cells were grown in Rb^+-free tryptone. Under growing conditions with high Rb^+ concentrations the cells still strongly discriminated Rb^+ from K^+. Nonetheless, T7 phage infection under these growing conditions resulted in a normal propagation cycle with cellular lysis and a productive burst. Therefore, we excluded the possibility that there was any toxic effect of Rb^+ on

TABLE I

DISCRIMINATION BETWEEN K^+ AND Rb^+ BY *E. COLI* B AND K-12 GROWING IN MEDIA WITH A HIGH RbCl CONCENTRATION ANALYZED BY FLAME PHOTOMETRY

For intracellular concentration, the water volume of *E. coli* B and K-12 has been determined to $1.22 \cdot 10^{-12}$ and $1.26 \cdot 10^{-12}$ ml, respectively [6].

Strain	Cation	Medium concentration (mM)	Intracellular concentration (mM)
B	K^+	0.9	114
	Rb^+	16.7	175
	Rb^+/K^+	18.6	1.5
K-12	K^+	0.6	125
	Rb^+	13.8	166
	Rb^+/K^+	22.6	1.3

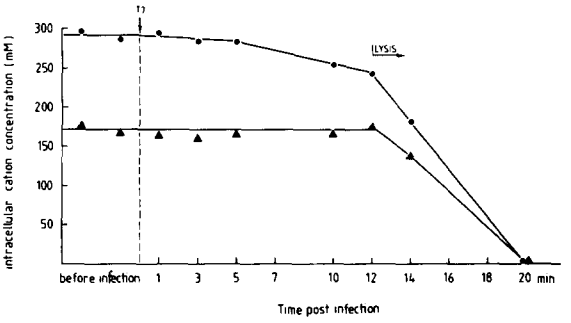

Fig. 3 Rb^+ vs. K^+ content at conditions with high intracellular Rb^+ concentration. *E. coli* B growing in tryptone medium containing 16.7 mM RbCl to a density of $2 \cdot 10^8$ cells per ml were infected by T7 (5 phages per cell). At the indicated times, 2-ml portions were analyzed for K^+ content as described in the legend to Fig. 1. Intracellular Rb^+ (▲) was measured by atomic emission spectroscopy at 780 nm. Spill-over from K^+ was calculated to be 2%. As our samples contained similar amounts of Rb^+ and K^+, spill-over was negligible. The values were calculated per cell and transformed to intracellular concentration, taking a cellular water volume of $1.22 \cdot 10^{-12}$ ml [6]. The sum of intracellular Rb^+ and K^+ (●) corresponded to the K^+ concentrations which had been determined for cells growing in tryptone medium without added RbCl [6].

bacterial or phage growth. In addition, we did not observe a significant change in the Rb^+ and K^+ content (Fig. 3). As soon as Rb^+ is present in a higher concentration inside the cell it behaves like K^+. Based on this result, we concluded that the above changes of membrane permeability are seen only for traces and therefore are very minor. Corroborating this interpretation, the extent of change of Rb^+ efflux was obviously correlated to the ratio of the intracellular composition of K^+ versus Rb^+, which in turn was responding to the outside concentrations of the cations (Table II, Fig. 4). Only when Rb^+ was present in trace amounts was discrimination between K^+ and Rb^+ increased.

In conclusion, the results we obtained here and in our previous paper [6] show that the intracellular K^+ content is not changed after T7 infection. The experiments with $^{86}Rb^+$ used as tracer [2] do

TABLE II

EFFECT OF THE INTRACELLULAR Rb^+ CONCENTRATION ON $^{86}Rb^+$ EFFLUX AFTER T7 INFECTION

The intracellular $Rb^+ + K^+$ (290 mM) was calculated from Table I.

$[Rb^+]_{out}$ [a] (mM)	$[Rb^+]_{in}$ [b] (mM)	$[Rb^+]_{in}/[Rb^+]_{out}$	Contribution of Rb^+ on intracellular $Rb^+ + K^+$ (%)	Loss of $^{86}Rb^+$ 10 min after T7 infection (% of initial $^{86}Rb^+$)
10	170	17	59	8
1	86	86	30	22
0.100	11	110	4	29
0.003	0.300	100	0.1	37
0.001	0.100	121	0.03	50

[a] Rb^+ concentration of medium achieved by adding RbCl.

[b] Calculated concentration inside the cell according to the distribution of $^{86}Rb^+$.

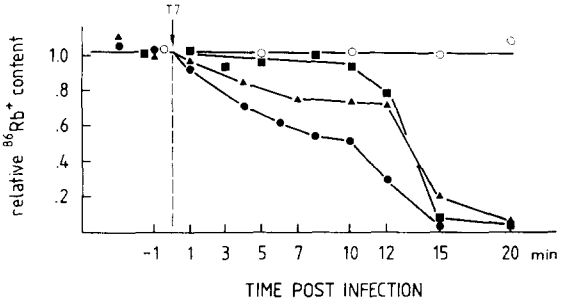

Fig. 4 $^{86}Rb^+$ content after T7 infection where Rb^+ differently contributes to the intracellular K^+ content. Fresh overnight cultures of *E. coli* B were diluted 10^{-3}-fold into tryptone medium containing 1 μM (○, ●) 100 μM (▲) and 10 mM RbCl (■). Additionally, all cultures contained 1 μCi/ml $^{86}Rb^+$. When the cultures reached a density of $(2-3)\cdot10^8$ cells/ml, phage T7 was added at a multiplicity of infection of 5, except for the control (○). Intracellular radioactivity was assayed after washing the cells with 4 ml non-labelled medium.

not reflect the state of intracellular K^+. The hypothesis that the phage induces changes in the levels of intracellular ions [2,7,8] is therefore not supported by our results. A change in the concentrations of intracellular ions other than K^+ has to be investigated. We have analyzed for intracellular K^+, Mg^{2+} and polyamine content and observed that T7 induced no substantial change for any of these ions [6]. Most likely, the ionic strength in the infected cell is kept unchanged. Conserving the intracellular concentration of small solutes might therefore be an important precondition for normal phage development [9].

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