UPTAKE AND EXTRUSION OF K^+ REGULATED BY EXTRACELLULAR pH IN *ESCHERICHIA COLI*

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1. Introduction

Potassium ion is an essential monovalent cation for growth of *Escherichia coli*. In growing cells of *E. coli*, the intracellular $[K^+]$ is ~200 mM [1,2]. This internal $[K^+]$ is not affected by the external $[K^+]$ [1,2]. For the uptake of K^+, *E. coli* cells possess 4 distinct transport systems, namely TrkA, TrkD, TrkF and Kdp systems [3]. Of those, the TrkA and the Kdp systems are quantitatively more important. The TrkA system is a constitutive system and the Kdp system is a repressible system. Thus, when cells are grown in the media containing high $[K^+]$, the TrkA system is responsible for the majority of K^+ uptake. However, in [4] *E. coli* was reported to possess another K^+-transport system, namely the K^+/H^+ antiport system. Judging from the polarity of electrochemical gradient of H^+ [5], extrusion of K^+ from cells via the K^+/H^+ antiport system should occur under appropriate conditions. Here, we report that K^+ is extruded from cells when external pH is alkaline. At neutral or acidic pH values, K^+ is taken up by cells.

2. Materials and methods

2.1. *Organism and growth*

Escherichia coli K12 strain RA11 was grown in minimal salt medium [6] supplemented with 1% bactotryptone. Cells were harvested in the late exponential phase of growth, washed 3 times with buffer A (20 mM morpholinopropane sulfonic acid-(MOPS)–choline and 135 mM choline chloride, pH 7.2), suspended in this buffer at ~50 mg protein/ml. These cells were used in the experiments in fig.2,3. For K^+ depletion, cells were treated as in [7], and used in the experiments illustrated in fig.1.

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2.2. *Transport assay*

Potassium ion transport was monitored with a K^+ electrode (Radiometer, Copenhagen). The procedure is essentially that for the measurement of Na^+ transport with a Na^+ electrode in [8].

2.3. *Protein determination*

Protein contents were determined by the method in [9].

3. Results

The uptake of K^+ by K^+-depleted cells is shown in fig.1. The initial velocity of K^+ uptake was 62 ng ions . min^{-1} . mg $protein^{-1}$ under standard assay conditions. Under anaerobic conditions no transport of K^+ was detected (not shown). Carbonyl cyanide *p*-trifluoromethoxyphenylhydrazone(FCCP) completely inhibited K^+ transport. No K^+ uptake was also observed with heat-treated cells. Thus K^+ uptake energized by respiration was detected with a K^+ electrode.

We tested the effect of pH on K^+ transport. In these experiments, normal cells (K^+ not depleted) were used, with the assumption that if there is extrusion activity under certain conditions, K^+ must be present in cells to measure such parameter. As shown in fig.2, cells took up K^+ at $pH < 7.7$. The highest uptake activity was observed at pH 7.0. When external pH was 7.4–7.7, extrusion of K^+ was observed in addition to uptake. The uptake component was

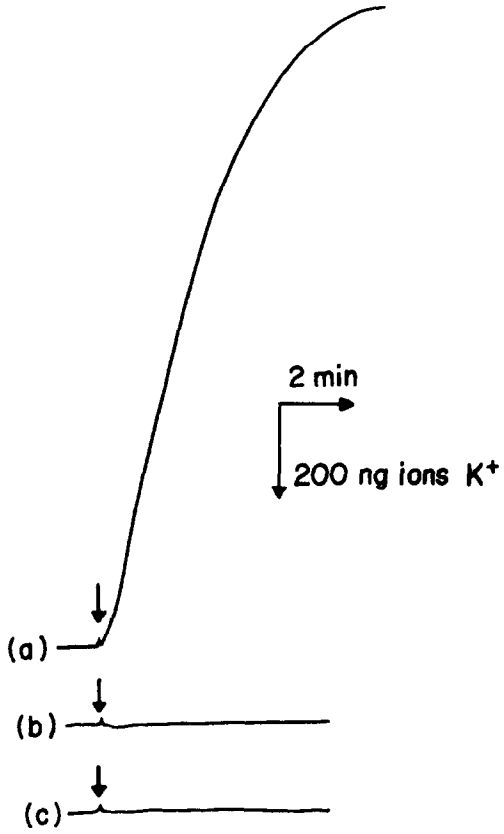

Fig.1. Energy-dependent uptake of K^+. Assay mixture (9.9 ml) consisting of 50 mM MOPS–Tris (pH 7.2), 50 μM KCl, 100 mM choline chloride and 5 mM Tris–lactate was placed in a plastic vessel. Transport reaction was initiated at time points indicated by arrows by addition of 0.1 ml cells (4.6 mg cell protein). (a) K^+-depleted cells; (b) K^+-depleted cells in the presence of 10 μM FCCP; (c) K^+-depleted cells were then incubated at 60°C for 30 min (heat-treated cells). An upward deflection indicates a decrease in $[K^+]$ in the external medium.

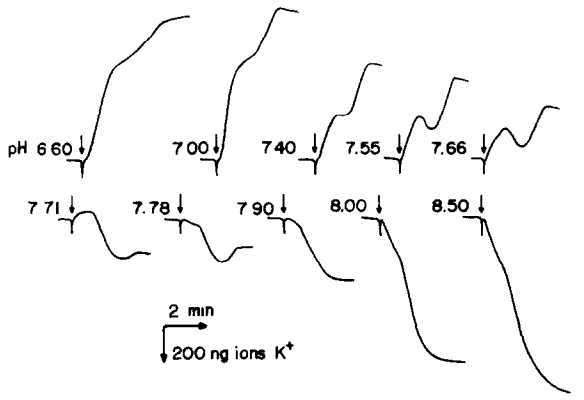

Fig.2. Effect of pH on K^+ transport. Cell suspension (0.2 ml, 10 mg cell protein) was diluted in 9.8 ml buffer containing 2 mM MOPS–Tris, 50 μM KCl, 100 mM choline chloride and 5 mM Tris–lactate. Values of pH were adjusted with tetramethylammonium hydroxide as indicated. The assay vessel was maintained under anaerobic conditions by passing a continuous stream of water-saturated N_2 gas. Respiration was initiated at time points indicated by arrows by adding 5 μl 6% H_2O_2.

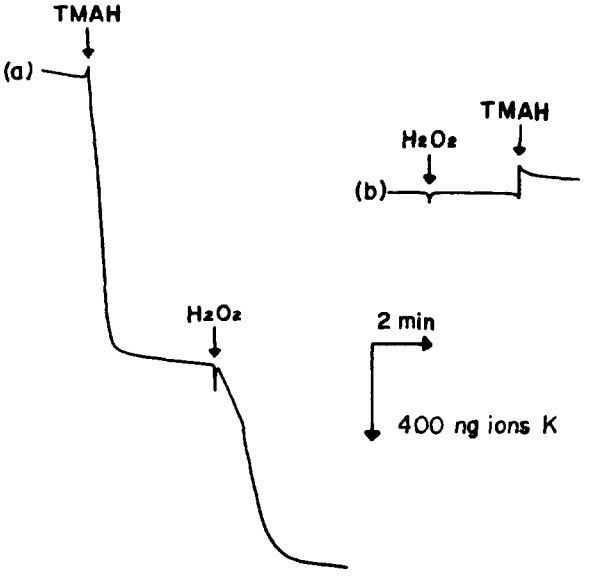

Fig.3. Efflux of K^+ induced by alkalinization of external medium. (a) Cells were incubated at pH 7.4 as under fig.2. At time points indicated, tetramethylammonium hydroxide-(TMAH) (40 μl 1 M solution) was added to alkalinize the medium (pH 8.0), and H_2O_2 (5 μl 6% solution) was added to initiate respiration. (b) Cells were omitted from the assay mixture, and effect of H_2O_2 (5 μl 6% solution) and TMAH (40 μl 1 M solution) on the electrode was tested.

weaker at higher pH values, and the extrusion component was stronger. At pH >7.7, only extrusion was observed. Both uptake and extrusion of K^+ were energy dependent. Extrusion of K^+ observed at pH 8.0 was partially sensitive to FCCP (not shown). In these experiments we supplied energy by respiration. Respiration itself was not affected by pH at 6.5–8.5 (not shown).

Cells kept at pH < 7.7 contain much K^+. We tested whether or not K^+ efflux from such cells took place when external pH was suddenly changed to alkaline in the absence of respiration. As shown in fig.3, a sudden change of external pH from 7.4–8.0 by addition of tetramethylammonium hydroxide-

(TMAH) caused rapid efflux of K^+ from cells under anaerobic condition. Respiration which was initiated by addition of H_2O_2 elicited further extrusion of K^+. Control experiments shown in fig.3 indicate that H_2O_2 gave no effect on pK tracing in the absence of cells. The effect of TMAH on K^+ electrode was not significant with regard to the magnitude and the direction of pK change.

4. Discussion

Here we report that transport pattern of K^+ in *E. coli* cells depends on medium pH. At pH < 7.7, uptake of K^+, which is thought to be mediated mainly by the TrkA system, was observed as was expected. On the other hand, extrusion of K^+ was observed at pH > 7.7. According to [10], respiring cells maintain an internal pH more alkaline than that of the medium at pH < 7.65. This can be accomplished by respiratory- or ATP-driven H^+ extrusion [5]. When medium pH is >7.65, cells maintain an internal pH more acidic than the medium. This implies a mechanism for uptake of H^+ at higher pH values. The involvement of the K^+/H^+ antiporter in the maintenance of intracellular pH during growth at alkaline pH was postulated [4]. Our results that respiration-dependent, FCCP-sensitive, K^+ extrusion was observed only at pH > 7.7 (fig.2) support this possibility. Furthermore, H^+ uptake driven by respiration, but not extrusion, was detected under alkaline conditions (unpublished). It should be pointed out that the K^+/H^+ antiport system is inactive at pH < 7 and reaches its maximal activity at pH ~8 [4]. Sudden alkalinization of external medium from pH 7.4–8.0 casued efflux of K^+ (fig.3). When a H^+ gradient of this polarity is imposed, H^+ leaves cells down its concentration gradient. This H^+ efflux is expected to raise internal pH, which would be unfavorable for cell physiology. Then K^+, which is abundant in cytosol, will be exchanged with H^+ by means of the K^+/H^+ antiport system to keep internal pH neutral. Now we are testing if the K^+ extrusion activity observed under alkaline conditions is due to the K^+/H^+ antiporter. A mutant was isolated in [11] which can not grow at pH 8.3. This mutant lacks the K^+/H^+ antiporter. Thus it seems clear that the K^+/H^+ antiport system regulates intracellular pH under alkaline conditions in *E. coli*. However in [12] a non-alkalophilic mutant of *Bacillus alcalophilus* was isolated which lacks the Na^+/H^+ antiport system. This mutant possesses normal K^+/H^+ antiport activity (Mandel, Guffanti and Krulwich (1980) J. Biol. Chem. in press). Thus the Na^+/H^+ antiport system is responsible for the regulation of intracellular pH in *B. alcalophilus*.

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