Cotransport of proline and Li^+ in *Escherichia coli*

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Uptake of Li^+ induced by the addition of proline to a cell suspension of *Escherichia coli* was detected using an Li^+-selective electrode. This Li^+ uptake was inhibited by L-azetidine 2-carboxylic acid, a competitive inhibitor of the proline transport system. Thus, direct evidence for Li^+-proline cotransport via the proline transport system was obtained. Kinetic parameters of the Li^+ uptake were determined.

Li^+ *Proline* *Cotransport* *Li^+ electrode* *Kinetic parameters* *Temperature effect*

1. INTRODUCTION

The proton-motive force is the driving force for many active transport systems of sugars and amino acids in microorganisms [1]. In many such systems, H^+-solute cotransport takes place. Recent studies, however, revealed that other monovalent cations such as Na^+ and Li^+ are utilized as coupling cations in several systems [2–4]. Na^+-solute and Li^+-solute cotransport mechanisms are found in microbial membranes as well as animal membranes [5,6].

Proline transport in *Escherichia coli* is stimulated by Li^+ [7]. Based on this observation, one of the authors has postulated Li^+-proline cotransport, and obtained results supporting this assumption [8]. We present here direct evidence for Li^+-proline cotransport in *E. coli*. Using a procedure we reported previously [9], we measured Li^+ uptake induced by proline influx into cells. Some properties of the Li^+-proline cotransport system are also described.

2. MATERIALS AND METHODS

2.1. *Organism and growth*

E. coli strain W3133-2, a derivative of K12, was grown in a minimal salts medium supplemented either with 1% Bacto-tryptone (Difco) or with 20 mM glucose at 37°C as in [9]. Cells were harvested at late logarithmic growth phase, washed 3 times with 0.1 M 4-morpholinepropanesulfonic acid (Mops) buffer adjusted to pH 7.0 with Tris, suspended in the same buffer to about 50 mg cellular protein/ml and kept in an ice bath until use.

2.2. *Assays*

An Li^+-selective electrode was constructed, and uptake of Li^+ induced by proline influx into cells was measured as in [9]. Protein was determined as in [10].

3. RESULTS AND DISCUSSION

Li^+ stimulated proline transport in *E. coli* [7], and an artificially imposed Li^+ gradient across the membrane elicited proline uptake [8]. These results suggested the mechanism of Li^+-proline cotransport. The V_{max} value for the proline transport has been reported to be 20–40 nmol·min^{-1}·mg protein^{-1} [11]. If the Li^+-proline cotransport mechanism is present, and if the stoichiometry between Li^+ and proline is not far from 1, then transport of Li^+ together with proline should be detected under appropriate conditions. Since there is no isotope of Li^+ available for laboratory use, measurement of Li^+ transport with an Li^+-selec-

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tive electrode is very useful [9].

The cell suspension was made anaerobic by gassing with N_2, and Li^+ concentration measured with an Li^+-selective electrode. When a small volume of anaerobic proline was added to cells, an immediate fall in Li^+ concentration in the medium was observed (fig.1), indicating proline-induced Li^+ uptake. This result provides direct evidence for Li^+-proline cotransport. Proline-induced Li^+ uptake was not observed when cells were preincubated with proline or L-azetidine 2-carboxylic acid which is a competitive inhibitor of the proline transport system [12]. On the other hand, hydroxyproline which is not a substrate of this system did not affect proline-induced Li^+ uptake. The proline transport system is an inducible system [13,14], and proline transport activity in cells grown on glucose is very low. As expected, Li^+ uptake induced by proline influx was much lower in cells grown on glucose than on tryptone (fig.1). These results strongly support the view that it is the proline transport system, most likely the proline porter I [14], which is responsible for Li^+-proline cotransport. No proton-motive force was detected under these experimental conditions. Furthermore, essentially the same results were obtained when tetrachlorosalicylanilide, a potent proton conductor, was added to the assay mixtures (not shown). Therefore, Li^+ uptake was elicited by passive influx of proline.

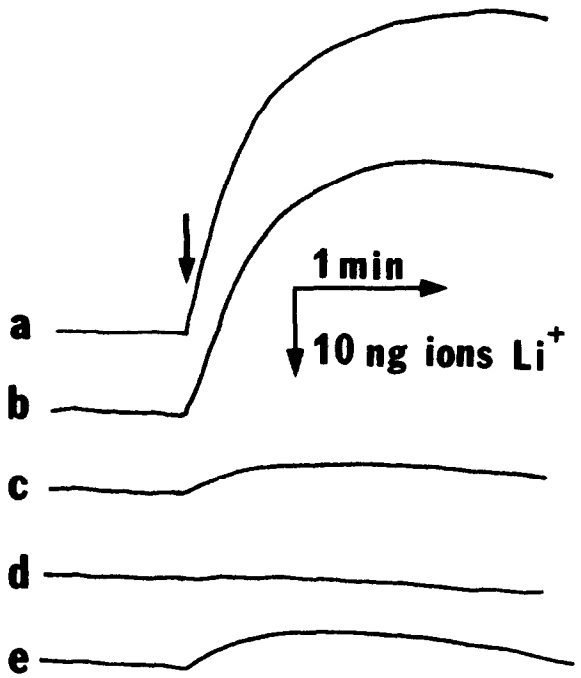

Fig.1. Uptake of Li^+ induced by addition of proline. Cells (10 mg protein/ml) were preincubated in 3 ml of 0.1 M Mops–Tris buffer (pH 7.0) containing 50 μM LiCl under anaerobic conditions at 30°C. Then a solution (3 μl) of 100 mM L-proline was added at points indicated by an arrow. Concentration of Li^+ in the medium was measured with an Li^+-selective electrode. An upward deflection indicates a fall in Li^+ concentration in the assay medium. (a–d) Cells grown on tryptone. (a) Control, (b) cells preincubated with 0.5 mM hydroxy-L-proline for 5 min, (c) cells preincubated with 0.5 mM L-azetidine 2-carboxylic acid, (d) cells preincubated with 0.5 mM L-proline, (e) cells grown on glucose.

A stimulatory effect of Li^+ on proline transport has been reported to be temperature dependent [7]. Li^+ strongly stimulated proline transport at temperatures above 34°C, whereas only weak stimulation was observed below this temperature [7]. It was of interest to test the effect of temperature on proline-induced Li^+ uptake. Li^+ uptake was observed at temperatures between 15 and 44°C (fig.2). A temperature-dependent increase in velocity of Li^+ uptake was observed between 15 and 30°C. A very small increase was observed at temperatures between 30 and 39°C. An Arrhenius plot of the data indicated that a transition point existed at 28°C (not shown).

Because of the high sensitivity and rapid response of the Li^+-selective electrode [9], kinetic analysis of Li^+ uptake was possible. The initial velocity of Li^+ uptake was measured at various concentrations of proline. A Lineweaver-Burk plot of proline-induced Li^+ uptake showed that the K_m for proline was 30 μM and the V_{max} was 2.3 ngion $Li^+ \cdot min^{-1} \cdot mg\ protein^{-1}$ (fig.3). K_m values (0.1–1.0 μM) for energy-dependent proline transport have been reported [11]. The K_m value obtained here (30 μM) is considerably higher than those reported in [11]. It was difficult to observe Li^+ uptake at proline concentrations lower than 5 μM under our experimental conditions.

The V_{max} of Li^+ uptake (2.3 ngion $\cdot min^{-1} \cdot mg\ protein^{-1}$) indicates that a considerable amount of Li^+ is taken up during passive proline transport. Cotransport between H^+ and proline has been suggested [1]. If this is the case, and if the

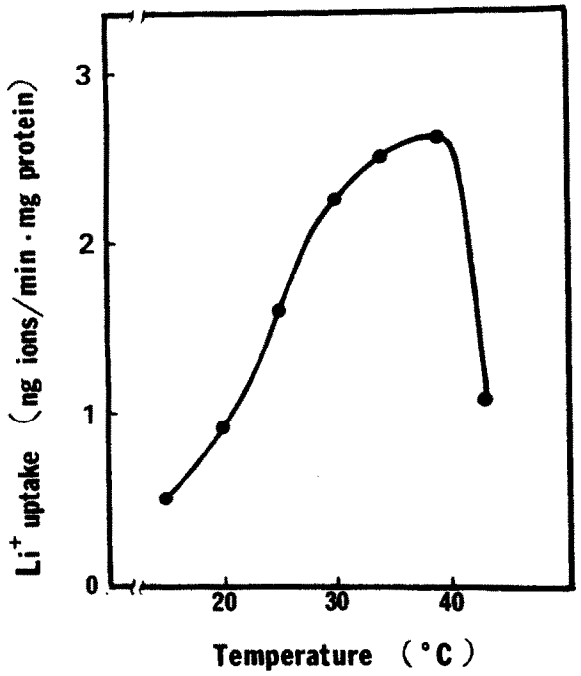

Fig.2. Effect of temperature on Li^+ uptake. Uptake of Li^+ was measured as described in fig.1 at various temperatures.

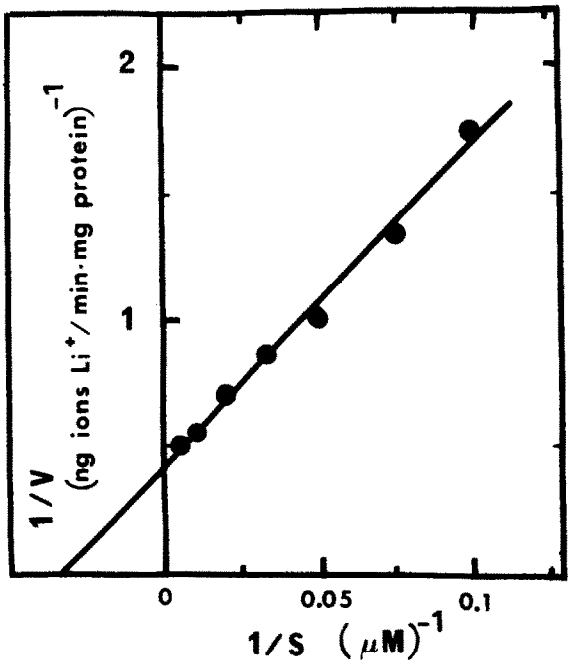

Fig.3. Lineweaver-Burk plot of Li^+ uptake. Uptake of Li^+ was measured as in fig.1. L-Proline was added to the cell suspension at various concentrations to induce Li^+ uptake.

stoichiometry between H^+ and proline is not far from 1, then H^+ uptake induced by passive proline influx should be detected. Thus, we tried to detect such H^+ uptake but were unable to do so under our experimental conditions. As a positive control, H^+ uptake induced by serine influx [15] was detected (not shown). Judging from the detection limit in our assay system, H^+ uptake, if present, induced by proline influx (at saturating proline concentration) is less than 0.02 ngion $H^+ \cdot min^{-1} \cdot mg\ protein^{-1}$. This value is much less than the V_{max} of Li^+ uptake. Therefore, Li^+-proline cotransport is predominant under our experimental conditions. However, we cannot exclude the possibility that H^+-proline cotransport may take place under certain conditions.

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