Role of Lithium Ions in Proline Transport in *Escherichia coli*

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Mechanisms of Li⁺ stimulation of proline transport were studied in cells of *Escherichia coli* 7 and NR70, a mutant of strain 7 lacking adenosine triphosphatase (EC 3.6.1.3). An electrochemical potential difference of Li⁺ induced in an inward direction of energy-depleted cells caused a transient uptake of proline, depending on the driving force provided. When proline was added to unbuffered cell suspensions under anaerobic conditions, the medium was found to be acidified only in the presence of Li⁺ but not in the presence of Na⁺ or K⁺. This acidification was abolished by the addition of a permeant anion, SCN⁻, to the medium containing Li⁺, but this was not demonstrated with cells of a mutant strain deficient in a carrier protein specific for proline. These results support the assumption that proline is taken up by a mechanism of Li⁺-proline cotransport in *E. coli.*

With regard to the mechanism of microbial active transport of organic solutes mediated by membrane carriers, evidence has been accumulated that these solutes move across the cell membrane by coupling with monovalent cations. Sodium ion was found to be cotransported with thiomethylgalactoside (TMG) (8, 13, 14, 16) and glutamic acid (3, 7, 9, 15). In the transport of TMG, Li⁺ replaced Na⁺ and was assumed to be cotransported with substrate (8, 13, 16).

The previous papers of the authors described the stimulation by Li⁺ of proline transport in cells of a wild strain of *Escherichia coli* K-12, which was rather specific for Li⁺ (5, 6). However, the mechanism of Li⁺ stimulation was not elucidated. This report describes further the properties of Li⁺ effect and presents evidence for Li⁺-proline cotransport in cells of *E. coli.*

MATERIALS AND METHODS

*Bacteria.* *E. coli* 7 and NR70 were provided by B. P. Rosen. Strain NR70 is a mutant of strain 7 lacking adenosine triphosphatase (EC 3.6.1.3) (10). *E. coli* ML308 *pro* is a spontaneous mutant of ML308 defective in proline transport (4) and was made available to this study by F. M. Harold. These strains were grown aerobically at 37°C on a minimal medium (2) supplemented with 0.2% glucose as the carbon source with exception of 0.4% glucose for growth of strain NR70. Cells were harvested at an optimal density of 0.35 absorbance units at 560 nm.

*Transport assay method.* Harvested cells were washed twice, suspended in 10 mM Tris-hydrochloride buffer (pH 7.5) containing 10 mM MgCl₂ and used for assay of [¹⁴C]proline transport as described previously (5, 6). A final concentration of 10 μM [¹⁴C]proline was used in the assay.

*Assay of transport induced by artificial driving forces in energy-depleted cells.* Experiments for proline transport were conducted essentially in the same manner as described for TMG transport (16) driven by an artificially produced electrochemical potential difference for Na⁺. One portion of cells was grown in minimal medium containing 0.1 M LiCl, which would be high in cellular Li⁺ concentration. Another portion of cells was grown in minimal medium in the absence of Li⁺ to reduce intracellular Li⁺. These two types of cells were treated with Li⁺ (0.1 M)-containing and Li⁺-free buffer of 10 mM Tris-maleate (pH 8.0), respectively, during the subsequent energy depletion and washing steps. All cells were energy depleted by exposure to 5 mM dinitrophenol (DNP) for 1 h at 37°C and then washed three times.

Both the high-Li⁺ and low-Li⁺ cells after energy depletion were divided into two groups; one group was washed in 10 mM Tris-maleate buffer (pH 8.0) and the other was washed in 10 mM Tris-maleate buffer (pH 6.0). This washing the high-Li⁺ cells were treated with 0.1 M Li⁺-containing buffer (pH 6 or 8), and the low-Li⁺ cells were treated with Li⁺-free buffer (pH 6 or 8). To each of the four groups were added 2 mM KCN and 5 μM carbonylcyanide-m-chlorophenylhydrazone (CCCP), and they were incubated for 10 min at 37°C. Cells were then diluted 100-fold into a solution consisting of 0.1 M LiCl, 10 mM Tris-maleate buffer (pH 8.0), 2 mM KCN, 5 μM CCCP, and 10 μM [¹⁴C]proline and then incubated continuously at 37°C.

The final cell density was 0.35 absorbancy units at 560 nm (0.25 mg, dry weight, per ml). Samples (1 ml) were taken at indicated intervals, filtered, washed, and counted.

*Measurements of pH under anaerobic conditions.* The experimental conditions were essentially similar to the methods of West and Mitchell (17) and of Tsuchiya et al. (16). Cells (4 mg, dry weight) of strain 7 and ML308 *pro* were washed three times with either 120 mM KCl or 110 mM KCl containing 10 mM LiCl or NaCl, and suspended in 2 ml of the
same solution, respectively, containing 100 μg of carbonic anhydrase. The cell suspension was incubated at 37°C under anaerobic conditions until extracellular pH declined gradually and reached a steady state of about pH 6.4. It took about 1 h. An anaerobic 0.5 M proline solution, 20 μl, was then added to the cell suspension to follow changes in extracellular pH under anaerobic incubation at 37°C. When necessary, KSCN was added to the medium at 30 mM.

Chemicals. CCCP was obtained from Sigma Chemical Co., St. Louis, Mo., and L-[U-¹⁴C]proline (290 mCi/mmol) was from Radiochemical Centre, Amersham, England. Other chemicals used were of the analytical grade.

RESULTS

Stimulation of proline transport by Li⁺ in strains 7 and NR70. Previous studies with a wild strain of E. coli K-12 revealed that proline transport energized by endogenous energy was stimulated 7.2-fold by LiCl and 2.4-fold by NaCl (6). This result was confirmed with strain 7 and its ATP-negative mutant, strain NR70 (Fig. 1; Table 1). The addition of Li⁺ to the uptake medium stimulated the initial rate of proline entry as well as the steady state achieved. Sodium ion at 10 mM was found to enhance the uptake by 50% of the Li⁺ effect, but K⁺ was ineffective.

Proline accumulation driven by an electrochemical potential difference of Li⁺. To test whether or not Li⁺-proline cotransport might be involved in the mechanism of Li⁺ stimulation, we designed experimental conditions in which H⁺ and Li⁺ would move in opposite directions.

Cells of strain NR70 used as control were treated throughout with 0.1 M LiCl at pH 8 and suspended finally in the same medium. This control, in which no pH gradient or chemical gradient was present, accumulated proline very slightly and reached a low steady state quickly (Fig. 2). The level of the control was calculated to be 25.9 μM, which was 2.6 times greater than the outside concentration, when the cellular water space was taken as 2.55 ml/g, dry weight, of cells (12). This control level was similar to that of Na⁺-TMG cotransport (16).

When only the chemical gradient of Li⁺ (ΔpLi) was induced by incubating the cells at pH 8 in the absence of Li⁺ and, by subsequent dilution into a medium containing 0.1 M Li⁺, proline transport was enhanced 2.6-fold at the maximal level from the control and was 6.8 times greater than the concentration in the medium (Fig. 2).

In the next experiment cells were equilibrated at pH 6.0 by incubation for 10 min in the presence of CCCP and then diluted 100-fold into a medium at pH 8, while the concentration of Li⁺ was maintained to be equal on both the inside and outside of the cell. It would be expected that this condition would result in outward movement of protons via CCCP to generate a membrane potential, making the inside negative. This would provide an electrical driving force (Δψ) for the inward movement of Li⁺, and actually a 3.5-fold increase in proline accumulation compared

![Fig. 1. Effect of LiCl on proline transport by strains 7 and NR70. Cell suspension in 10 mM Tris-hydrochloride buffer (pH 7.5) and 10 mM MgCl₂ with or without 10 mM LiCl was preincubated for 5 min at 37°C. [¹⁴C]proline was added at a final concentration of 10 μM, and 1-ml samples after incubation for periods indicated were taken, filtered, washed, and counted (5, 6). Symbols: ○, Strain 7 with Li⁺; △, strain 7 without Li⁺; A, strain NR70 with Li⁺; Δ, strain NR70 without Li⁺.](http://jb.asm.org/)

### Table 1. Effect of monovalent cations on proline transport by strains 7 and NR70

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Fig. 2. Proline transport induced by artificial driving forces in energy-depleted cells of strain NR70. Experimental details are given in the text. Membrane potential ($\Delta \psi$), interior negative, was induced by providing a pH gradient (inside acidic) in the presence of CCCP. An inwardly directed chemical gradient of Li$^+$ ($\Delta p$Li) was established by adding low-Li$^+$ cells to high Li$^+$ (0.1 M) buffer. Symbols: ○, Control (no driving force); ●, $\Delta \psi$; ◆, $\Delta p$Li; ▲, $\Delta \psi$ and $\Delta p$Li.

with the control (9.1 times the outside concentration) was observed under this condition (Fig. 2).

Finally, conditions were arranged to provide both electrical and chemical forces ($\Delta \psi + \Delta p$Li) for the inward movement of Li$^+$. This was accomplished by preincubating the cell at pH 6.0 in the presence of CCCP but without preloading with Li$^+$. Dilution of the cells into the medium containing 0.1 M LiCl at pH 8 resulted in a 14-fold accumulation of proline (5.4 times that of the control).

Measurements of external pH changes by the addition of proline. It was demonstrated that transient acidification of the external medium resulted from Na$^+$-TMG cotransport (16). To test whether the same phenomenon would be the case in Li$^+$-stimulated proline transport, we followed the change in the external pH of an anaerobic cell suspension of strain 7 in the presence of monovalent cations (Fig. 3). The suspension was bubbled continuously with nitrogen gas until pH changes reached a steady state. The addition of an anaerobic proline solution resulted in an enhanced acidification in the presence of LiCl but not in the presence of NaCl or KCl (Fig. 3).

The addition of KSCN to a cell suspension containing LiCl abolished the proline-induced acidification (Fig. 4b). This could be explained by a reduction due to the entry of lipid-soluble SCN$^-$, of the membrane potential caused by Li$^+$-proline cotransport, which resulted in a decrease in proton exit. The addition of proline to a cell suspension of ML308 proT lacking a proline-carrier protein failed to cause acidification of the medium in the presence of LiCl (Fig. 4c).

Effect of PCMBS on the stimulatory effect of Li$^+$. It was demonstrated with cells of E. coli K-12 that the addition of 0.5 mM p-chloromercuribenzenzene sulfonate (PCMBS) to the cell suspension completely abolished the effect of Li$^+$ to stimulate proline transport without affecting the basal transport in the absence of Li$^+$ (6). This result was confirmed with cells of strains 7 and NR70 (data not shown), although the effective concentration of PCMBS was different: 0.5 mM in strain 7 and 0.2 mM in strain NR70.

DISCUSSION

Recent studies on transport of amino acids and sugars have revealed that Na$^+$ or Li$^+$ is
on the finding that carbon sources with Li⁺ synergistically enhanced the transport of proline (6), it was assumed that a Li⁺ gradient on the transport could be generated by proton-lithium (or sodium) antiport which was demonstrated in *E. coli* by West and Mitchell (17) and others (1, 11). The experiment was thus designed to provide conditions such that proton and Li⁺ would move in opposite directions as described for the Na⁺ (or Li⁺)-TMG cotransport system (8, 16).

The results shown in Fig. 3 and 4 were consistent with the assumption that proline would enter the cell with Li⁺ to reduce the membrane potential (inside positive), resulting in exit of H⁺. This was supported by the facts that the presence of SCN⁻, a lipid-soluble permeant anion, inhibited proton exit and that proton exit in the presence of Li⁺ was not detectable with cells of ML308 proT lacking a proline carrier protein (Fig. 4).

In this paper, evidence was presented indicating the presence of a proline:Li⁺ symport and an H⁺:Li⁺ antiport. A problem raised from our results is that, while Na⁺ stimulated proline uptake 50% of that observed with Li⁺, no pH change of the medium containing Na⁺ was found on proline addition. Since an H⁺:Na⁺ antiport system is present in *E. coli* cells (1, 11, 18), an acidification of the medium containing Na⁺ should be detected when proline is added. The reason why this was not observed in cells of strain 7 is not known at the present time. One of possible assumptions is that the stimulating effect of Li⁺ on proline transport was highly reproducible with any type of *E. coli* K-12 cell, but that of Na⁺ was sometimes undetectable. The other explanation might be possible if the *Kₐ* for Li⁺ is assumed to be simply fortuitously lower than Na⁺. With regard to the physiological significance of Na⁺ versus Li⁺, it is perhaps still possible that Na⁺ rather than Li⁺ is the ecologically significant cation. Further studies are therefore required to elucidate the physiological significance of the monovalent cations on proline transport in *E. coli*.

**LITERATURE CITED**


