EFFECT OF LITHIUM ON PROLINE TRANSPORT BY WHOLE CELLS OF ESCHERICHIA COLI

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SUMMARY

The rate of proline transport by whole cells of Escherichia coli in the medium containing a monovalent cation but not an energy source was enhanced approximately 2.5-fold by 50 mM Li^+, whereas K^+, Na^+, Rb^+ and Cs^+ were ineffective. The stimulatory effect of Li^+ was saturated at 5 mM and was due to an increase in the Vmax for proline without affecting the Km. Proton conductors inhibited proline transport in the absence of Li^+ and more strongly in the presence of Li^+. Valinomycin did not affect the transport in itself or in the presence of Li^+, but inhibited markedly when it was present with K^+. Gramicidin also induced a strong inhibition of the transport only in the presence of either Li^+, K^+ or Na^+.

There were recent several reports on Na^+-dependent transport processes of amino acids (1-4) and melibiose (5) in microbial cells, in which the Km for the solute was shown to be reduced while the Vmax remained constant (2-5). These are examples of the co-transport system of the solute and Na^+. However, Na^+-stimulated proline transport system in membrane vesicles of Mycobacterium phlei has the same Km for proline and the increased Vmax (6).

The stimulatory effect of K^+ was also reported in several transport processes (3, 7-9) and the possible mechanisms of the K^+ effect vary: a K^+ gradient acts at least partly as a driving force of citrate (7) and glycine (8) transport, and K^+ affects the accumulation process of aminoisobutyrate (3) and glutamate (9) after the entry into the cell.

Although Li^+ was reported to stimulate the accumulation of aminoisobutyrate in a marine pseudomonad as found with K^+ (2), this action was later attributed specifically to K^+ (3). Proline transport by membrane vesicles of M. phlei was enhanced about 10-fold by Li^+ as well as Na^+ (6). However, the role of Li^+ in this system was not described and other examples of Li^+ effect on the transport in microorganisms have not been reported.

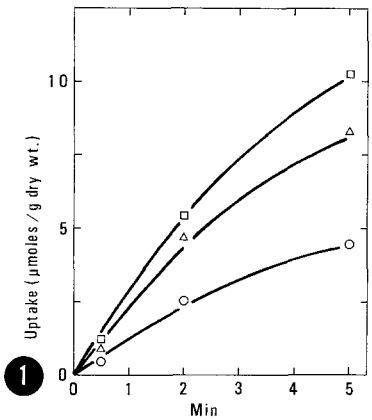
This paper describes the stimulatory effect of Li^+ specific for proline transport by whole cells of E. coli K12 under the conditions in which no energy source was added.

MATERIALS AND METHODS

E. coli K12 was used throughout this study and grown aerobically on a minimal medium (10) containing 0.2% glucose as the carbon source. Cells harvested at 0.3 of the absorbancy at 560 nm were washed three times in a medium composed of 0.25 M sucrose, 10 mM Tris-HCl buffer, pH 7.5, and 10 mM $MgCl_2$ and then resuspended in the same medium supplemented with 100 µg/ml chloramphenicol to give 0.25 of the absorbancy at 560 nm (0.18 mg dry weight of cells per ml).

The cell suspensions with or without cations and other supplements were preincubated for 5 min at 37° with constant shaking and then ^{14}C-proline (25.5 mCi/mmole) was added at 10 µM. At time intervals shown in the text, one ml of the cell suspensions was quantitatively collected on a Millipore filter (0.45 µ), washed once with 10 ml of the medium used for washing the cells, and then counted as described previously (11).

Valinomycin and carbonylcyanide m-chlorophenylhydrazone (CCCP) were products of Calbiochem. and gramicidin was obtained from Sigma. All other compounds were analytical grade.



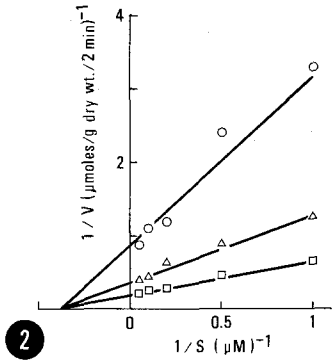


Fig. 1. Effect of Li^+ on proline transport by whole cells of E. coli K12. Experimental conditions used for the assay of transport activity were described in the text. Both effectors, 50 mM LiCl and 22 mM glucose, were added to the transport medium 5 min prior to initiation of the uptake. O, None; □, LiCl; △, glucose.

Fig. 2. Kinetics of proline transport in the presence and absence of Li^+. Cell suspensions prepared as described in the text were preincubated for 5 min at 37° in the presence and absence of Li^+. Varying concentrations (1-20 μM) of ^{14}C-proline (25.5 mCi/mmole) were added to the reaction medium to determine the uptake for 2 min at 37°. The reciprocals of the rate of proline transport were plotted according to Lineweaver and Burk (14). O, None; △, 5 mM LiCl; □, 50 mM LiCl.

RESULTS

The rate of proline transport by intact cells of E. coli K12 was enhanced approximately 2.5-fold by 50 mM Li^+ (Fig. 1) and K^+, Na^+, Rb^+, and Cs^+ were ineffective at 50 mM. Glucose added to the cell suspension at 22 mM also stimulated the transport approximately 75% as effective as that of Li^+. Proline transported into the cell in the presence of 50 mM Li^+ recovered as proline more than 95% when analyzed by paper chromatography of the cell extracts (11, 12).

The steady state level of proline transported was calculated to be 6.8 mM and 2.8 mM in the presence and absence of 50 mM Li^+ (Fig. 1), respectively, when water space of E. coli

cells grown on minimal medium is taken as 2.55 ml per g dry weight (13). These were 680-times and 280-times the level of proline added to the medium, respectively.

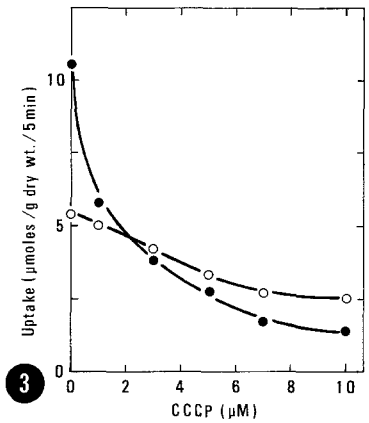
The stimulatory effect of Li^+ on proline transport was saturated at 5 mM of this cation when the concentrations were varied.

The rate of proline transport in the presence and absence of Li^+ was determined as a function of proline concentrations (Fig. 2). Li^+ was found to increase the Vmax but not affect the Km value for proline, suggesting that the symport of proline with Li^+ is not the case.

It has been reported that transports of some amino acids by Streptococcus faecalis (15) lacking cytochromes and of β-galactosides by E. coli under anaerobic conditions (16) are inhibited by uncouplers of oxidative phosphorylation and evidence was presented to indicate that these uncouplers accelerate proton translocation across a variety of membranes (15, 17).

CCCP inhibited proline uptake about 12.5% and 55.4% at 1 μM and 10 μM, respectively, when Li^+ was absent and this inhibition by CCCP was more markedly induced in the presence of 50 mM Li^+ (Fig. 3). The other uncoupler, 2, 4-dinitrophenol (DNP), also resulted in the same type of the inhibition as found with CCCP and a low concentration of DNP such as 20 μM inhibited the transport almost completely so that no further increase in the transport activity was observed by addition of 50 mM Li^+.

Valinomycin (18) and gramicidin (18,19), which are known to be ionophores stimulating cation translocation, were then used in order to know the mechanism of this cation effect.



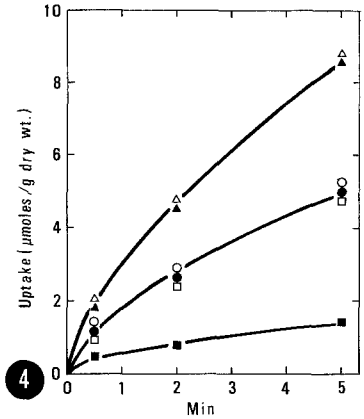


Fig. 3. Effect of CCCP on proline transport. CCCP at indicated concentrations was contained in the transport medium with or without 50 mM LiCl and preincubated for 5 min at 37°, followed by 5-min incubation with 10 μM ^{14}C-proline at 37°. O, None; ●, LiCl.

Fig. 4. Effect of valinomycin on proline transport. All supplements were added to the transport medium during preincubation time at following concentrations: valinomycin, 1 μM; LiCl, KCl and NaCl, 50 mM each. Proline transport under these conditions was started by adding 10 μM ^{14}C-proline and was determined as described in the text. O, None; ●, valinomycin; △, Li^+; ▲, Li^+ plus valinomycin; □, K^+; ■, K^+ plus valinomycin. Na^+ alone and Na^+ plus valinomycin gave the same time course of the uptake as that of the non-supplement (data not illustrated).

Although proline transport was not affected either by 1 μM valinomycin or 50 mM K^+ alone, the presence of valinomycin plus K^+ reduced markedly the transport. However, the stimulatory effect of Li^+ were not altered in the presence of this ionophore (Fig. 4). Na^+ in the presence and absence of valinomycin did not alter the rate of the transport.

Gramicidin also resulted in a marked inhibition of proline transport by whole cells of E. coli only when was present with either Li^+, K^+ or Na^+, though data obtained with Na^+ were not illustrated in Fig. 5.

DISCUSSION

Proline transport in microorganisms has been extensively

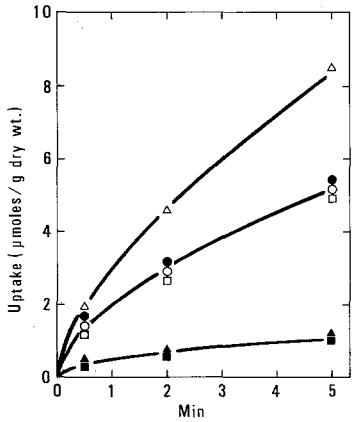


Fig. 5. Effect of gramicidin on proline transport. Experimental conditions were exactly same as described in the legend of Fig. 4 except for use of 1 μM gramicidin instead of valinomycin. O, None; ●, gramicidin; △, Li^+; ▲, Li^+ plus gramicidin; □, K^+; ■, K^+ plus gramicidin. With Na^+ alone and Na^+ plus gramicidin, time courses of the transport were same as found with K^+ and K^+ plus gramicidin, respectively, though the data were not illustrated.

studied with intact cells as well as membrane preparations. This paper is assumed to be the first one describing the effect of monovalent cations on proline transport by E. coli cells at least so far as the effect of Li^+ concerns.

A possibility of the symport of proline and Li^+ was excluded by the kinetic data (Fig. 2), though the saturable nature of the Li^+ effect may suggest an interaction of Li^+ with component(s) of the cellular membrane.

Evidence was presented to indicate that proline uptake is driven by a high-energy membrane state in the cell of E. coli (20, 21), which is resulted from the oxidation of the respiratory chain but not from the hydrolysis of ATP by ATPase. It is still unknown whether this energized state of the membrane as the driving force for the transport is a result of a proton gradient provided by oxidation of respiratory substrates. However, there is ample evidence which indicates that the

proton gradient postulated by Mitchell (22, 23) is involved in the active transport of many solutes (8, 15, 16, 24-27).

Active proline transport found in whole cells of E. coli was inhibited by low concentrations of uncouplers to the level at which no longer stimulation of the transport by 50 mM Li^+ was detected (Fig. 3). These results may support the postulated hypothesis that proton movement may play a role in the transport processes (25) and Li^+ may affect in some manner the proton translocation, though alternative explanations are certainly possible.

It is not evident from the data presented in this paper whether proline transport depends at least partly on the electrical potential, although valinomycin plus K^+ inhibited markedly the transport (Fig. 4). There was not determined that Li^+ influx is occurred and electrogenic, and there is no evidence to suggest that Li^+ transport system is involved in the circulation of protons and other cations as discussed by Harold (25). These problems are now under investigation.

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