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Uptake of Li^+ by Ehrlich Ascites Tumor Cells Induced by Neutral Amino Acids

TOMOFUSA TSUCHIYA,* HIROSHI KANAZAWA and MASAMITSU FUTAI

Department of Microbiology, Faculty of Pharmaceutical Sciences, Okayama University, Tsushima-naka, Okayama 700, Japan

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Uptake of Li^+ by Ehrlich ascites tumor cells induced by amino acid influx was measured with an Li^+-selective electrode. Alanine and serine induced the largest influxes of Li^+, while cysteine, glycine, α-aminoisobutyric acid, proline and threonine elicited Li^+ uptake to lesser extents. These amino acids also induced Na^+ uptake. It seems that the A system is responsible for Li^+-amino acid cotransport. Some kinetic properties of the Li^+ uptake were investigated.

Keywords——Ehrlich ascites tumor cell; Li^+ uptake; neutral amino acid; Li^+-electrode; Li^+-amino acid cotransport

Several transport systems for neutral amino acids in Ehrlich ascites tumor cells have been reported.[1,2] The A system and the ASC system are Na^+-dependent systems, and transport many neutral amino acids. The two systems differ in several respects: one difference is that Na^+ can be replaced by Li^+ for the A system, but not the ASC system.[3] Thus, the A system seems to be the sole Li^+-dependent (and also Na^+-dependent) transport system in Ehrlich cells, although Li^+-dependency of the ASC system has been observed in other animal cells.[4,5] Li^+ was found to be taken up when various neutral amino acids were added to Ehrlich cells.[3] Thus Li^+ is thought to be a co-substrate of the A system of the Ehrlich cells.

We developed an Li^+-selective electrode to analyze Li^+ transport in microbial cells,[6] and demonstrated that Li^+ uptake was induced by transport of substrates in two transport systems of *Escherichia coli*, the melibiose system,[6] and the proline system.[7] With this Li^+-electrode, it is possible to measure rapid Li^+ uptake by cells, and since radioactive Li^+ is not available commercially, this procedure seems to be the best available for kinetic analyses.

In this work, using an Li^+-electrode, we evaluated the specificity and kinetic properties of Li^+ uptake induced by influx of amino acids into Ehrlich cells.

Experimental

Ehrlich Ascites Cells——Ehrlich ascites tumor cells were maintained in mice. About 2×10^5 cells in phosphate-buffered saline were injected into each mouse, and cells were harvested 7 to 9 d later, washed three times with 100 mM 4-morpholinepropanesulfonic acid–Tris, pH 7.5, containing 1 mM $MgSO_4$, and suspended in the same buffer.

Measurement of Li^+ Uptake——The Ehrlich cells (about 5×10^7 cells) were incubated at 37 °C in 3 ml of 100 mM 4-morpholinepropanesulfonic acid–Tris buffer, pH 7.5, containing 1 mM $MgSO_4$. LiCl was added at a final concentration of 0.5 mM unless otherwise stated. An Li^+-selective electrode[6] and reference electrode (K801, Radiometer, Copenhagen, Denmark) were put into the assay mixture. The two electrodes were connected to a pH meter (PHM80, Radiometer), and the Li^+ concentration in the assay mixture was recorded. A solution of amino acid (40 μl of 1.5 M or 60 μl of 1.0 M) was added to the cell suspension with stirring to induce Li^+ uptake. Records were calibrated by addition of known amounts of Li^+.

Results and Discussion

The A system is an alanine-preferring system.[1] Thus, we first tested whether alanine

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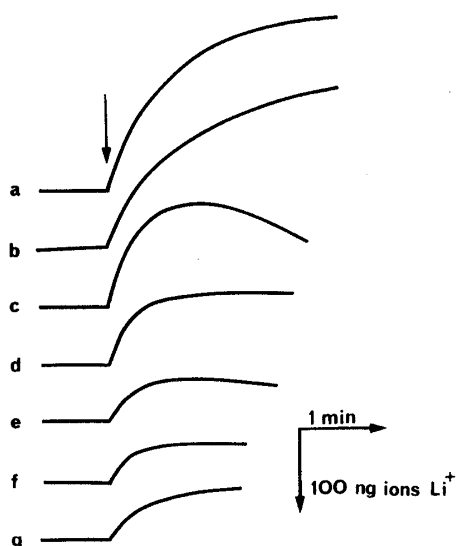

Fig. 1. Amino Acid Specificity of the Induction of Li^+ Uptake

Ehrlich cells (8×10^7) were incubated in 3 ml of 100 mM 4-morpholinepropanesulfonic acid–Tris, pH 7.5, containing 0.5 mM LiCl and 1 mM $MgSO_4$ at 37 °C. At the time indicated by an arrow, an amino acid was added at 20 mM (final concentration) with stirring. Changes in Li^+ concentration of the assay mixture were monitored with an Li^+-selective electrode. Upward deflection indicates uptake of Li^+. The amino acids added were: a, alanine; b, serine; c, cysteine; d, glycine; e, α-aminoisobutyric acid; f, proline; g, threonine.

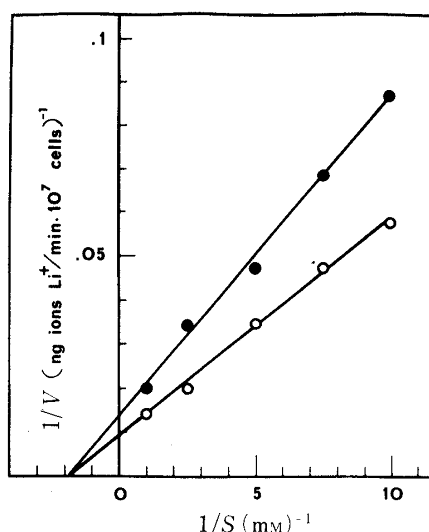

Fig. 2. Lineweaver–Burk Plot of Amino Acid-Induced Li^+ Uptake

The initial velocity of Li^+ uptake by Ehrlich cells induced by alanine (○) or serine (●) was measured at various concentrations of Li^+. Assay conditions were as for Fig. 1 except for the Li^+ concentration and amino acid concentration (40 mM).

induced Li^+ uptake. A low concentration (0.1 mM or less) of the amino acid induced Li^+ uptake in *E. coli*,[7] but not in Ehrlich cells. This suggests that Ehrlich cells do not have a very high affinity system for Li^+-amino acid cotransport. Thus, higher concentrations of alanine were added to the cell suspension. As shown in Fig. 1, considerable Li^+ was taken up when 20 mM alanine was added. The initial velocity of Li^+ uptake was about 35 ng ions/min · 10^7 cells with 0.5 mM Li^+. We next tested the effects of various neutral amino acids on Li^+ transport. The amino acids tested, which are known to be substrates of the A system, all induced Li^+ uptake (Fig. 1). These results indicate that Li^+ was transported as a co-substrate *via* the A system. Alanine and serine induced the most uptake of Li^+, followed by cysteine, glycine, α-aminoisobutyric acid, proline and threonine. These results are consistent with the findings obtained by Christensen and Handlogten[3] by flame photometry that serine and alanine induced the most uptake of Li^+, although the order of effectiveness observed here is not identical with that in their report. All the amino acids listed above also elicited Na^+ uptake, as determined with an Na^+-selective electrode (data not shown).

Since alanine and serine induced the most Li^+ uptake, the kinetic properties of Li^+ uptake induced by these amino acids were investigated. The effect of the concentration of these amino acids on Li^+ uptake was tested, and the half-maximal concentrations of alanine and serine for induction of Li^+ uptake were found to be 13 and 17 mM, respectively, with 0.5 mM Li^+ (data not shown).

The effect of Li^+ concentration on the Li^+ uptake was then tested. A sub-saturating concentration of alanine or serine (40 mM) was added to the cell suspension to induce Li^+ uptake in the presence of various concentrations of Li^+, and the initial velocities of Li^+ uptake were measured. With both alanine and serine the K_m of Li^+ uptake was about 0.5 mM (Fig. 2); the V_{max} values were 113 and 74 ng ions Li^+/min · 10^7 cells, respectively.

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Christensen and Handlogten[3] reported that 40 to 80 mM Li^+ resulted in half-maximal uptakes of serine and alanine by Ehrlich cells. If Li^+-amino acid cotransport is stoichiometric, then it would be reasonable to suppose that the K_m for Li^+ uptake and the concentration of Li^+ giving half-maximal uptake of amino acids would be similar. However, we found that the K_m was 0.5 mM, whereas the concentration of Li^+ for half maximal uptake of amino acids was 40 to 80 mM, although the assay conditions were not the same. Since these values differ by two orders of magnitude, it seems that there are two K_m values for Li^+. Unfortunately, it is impossible to measure Li^+ uptake at Li^+ concentrations of above 10 mM with an Li^+-selective electrode, and it seems to be very difficult to measure Li^+ uptake at low concentrations of Li^+ by the flame photometric method.[3] However, by the electrode method, we were able to cover the lower concentration range of Li^+, and observed high affinity Li^+ uptake.

In microbial cells, intracellular Li^+ inhibits glycolysis by acting on pyruvate kinase, and also inhibits growth.[8] At high concentrations, Li^+ is also toxic to animal cells, although its site of action is unknown.[9] Since a large amount of Li^+ is taken up by Ehrlich cells together with amino acids, the intracellular Li^+ concentration may become very high, and this should be toxic. It is known that both amino acid transport and sugar transport are higher in transformed cells than in untransformed cells.[10,11] Therefore, when Li^+ is administered much more Li^+ may be taken up by transformed cells or tumor cells than by normal cells, so that Li^+ may be useful in the therapy of some types of cancer.

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