The respiration-driven active sodium transport system in *E. coli* does not function with lithium

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Abstract Comparison of respiration-driven active transport of alkali cations from *E. coli* cells loaded with Na^+ or Li^+ showed that Li^+ could not be expelled from the cells like Na^+. K^+ accumulation, which was fast in Na^+-loaded cells, was strongly inhibited in Li^+-loaded cells, despite high membrane potential and respiratory rate. When Li^+-loaded cells were placed into medium containing Na^+ instead of Li^+, Li^+/Na^+ exchange took place initially, while K^+ accumulation was delayed. Only after almost all inside Li^+ was replaced by Na^+ did active Na^+ and K^+ transport commence. These data confirm that it is a distinct active sodium transport system (AST) with $Na^+,K^+/H^+$ antiporter activity, and not the Na^+/H^+ antiporters, that is responsible for active Na^+ transport in *E. coli* [Verkhovskaya, M.L., Verkhovsky, M.I. and Wikström, M. (1996) Biochim. Biophys. Acta 1273, 207–216]. In contrast to the Na^+/H^+ antiporters, the AST system is inhibited by Li^+.

Key words: Na^+ transport; Na^+/Li^+ selectivity; *E. coli*

1. Introduction

It has been generally accepted that the respiration-dependent sodium gradient in *E. coli* is generated by the activity of two Na^+/H^+ antiporters, the products of the *nhaA* and *nhaB* genes (see [1] for a review). The *nhaA* and *nhaB* genes both confer resistance to Li^+ [2,3], which is toxic for *E. coli* [4]. Thus, these gene products were considered to extrude Li^+ as well as Na^+ from the cells. Earlier, it was shown that Li^+, like Na^+, is efficient in dissipating ΔpH generated by respiration in sub-bacterial vesicles from *E. coli* [5]. Later, this was found to be due to functioning of the *nhaA* and *nhaB* gene products [3]. The *nhaB* gene product was alone responsible for dissipation of ΔpH generated by respiration in membrane vesicles from an *nhaA* gene-deleted mutant, Na^+ and Li^+ being equally effective dissipators [6]. Finally, the purified NhaA protein catalyses Na^+/Li^+ as well as Na^+/Na^+ exchange in reconstituted liposomes [7]. All these results prove that the two well-characterised Na^+/H^+ antiporters can also operate with Li^+.

We recently suggested that the major respiration-linked Na^+ transport in *E. coli* is not catalysed by the NhaA or NhaB antiporter as previously thought, but by an active sodium transport system (AST), which possesses $Na^+,K^+/H^+$ antiporter activity [8]. Here we present results showing that Li^+ cannot be actively extruded from respiring *E. coli* cells like Na^+ which strongly corroborates our proposal.

2. Materials and methods

The *E. coli* strain GR70N was a gift from Dr. R.B. Gennis, University of Illinois. The cells were grown aerobically overnight at 37°C in synthetic salt medium, pH 7.6, containing sodium succinate, and harvested in the middle of the exponential growth phase. Then the cells were loaded with Na^+ or Li^+ using the diethanolamine treatment [9]. Loaded cells were concentrated to 30–40 mg protein/ml and kept on ice.

Transport of alkali cations was initiated by dilution of cell suspensions into aerated, stirred Na^+ (or Li^+) medium, containing 150 mM NaCl (or LiCl), 50 mM tricine-NaOH (or LiOH), pH 8.5, 5 mM $MgSO_4$, 10 mM KCl, 2 mM dithiotreitol (DTT) and 200 μM ubiquinone-1 (Q-1). Then the cell suspension was filtered (Millipore, 45 μm), the filter was washed with 5 ml 0.5 M mannitol solution, and the content of alkali cations on the filter was measured by flame photometry. Measurements and calculations of electric potential (Δψ) in EDTA/Tris treated cells, using the tetraphenylphosphonium cation (TPP^+) and a TPP^+-selective electrode, were performed as described previously [8].

3. Results and discussion

Na^+-loaded cells diluted into Na^+ medium, containing K^+ and respiratory substrates, showed fast respiration-driven extrusion of Na^+ and uptake of K^+ (Fig. 1A), as described earlier [8]. However, neither significant Li^+ extrusion nor K^+ uptake were observed in Li^+-loaded cells diluted into Li^+ medium that also contained K^+ and respiratory substrates (Fig. 1B). To check possible toxic effects of Li^+, we measured both the rate of respiration and the electric potential across the cell membrane. No significant difference in respiratory rates of Na^+- and Li^+-loaded cells was found using the Q-1/DTT substrate couple. Without added Q-1/DTT the rate of respiration was much slower in Li^+-loaded cells indicating that they were depleted of endogenous substrates. However, after addition of Q-1/DTT the membrane potential was close to −200 mV (in the absence of K^+ which decreases Δψ), independently of whether the cells had been loaded with Na^+ or Li^+, or suspended in Na^+- or Li^+-containing medium.

Sodium transport could be blocked also if the Trk system, which is responsible for uphill K^+ transport, is inhibited by Li^+. This possibility was tested by measuring K^+ uptake under hyperosmotic conditions when Trk is activated (see [10] for a review). The *E. coli* cells were capable of accumulating K^+ as a response to a rapid increase of osmolarity of the medium by addition of sucrose to 0.5 M, in the presence of 100 mM Li_2SO_4 (not shown).

These data show that the sodium efflux system is incapable of extruding Li^+. In such a case the Trk system which transports K^+ cannot function either, despite the high Δψ, because it is osmotically regulated [11], and since Li^+ did not leave the cytoplasm.

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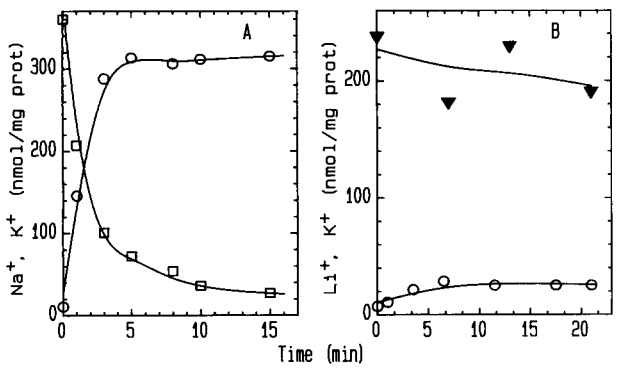

Fig. 1. Transport of alkali cations in Na^+-loaded (A) and Li^+-loaded (B) *E. coli* cells. At zero time the cells were diluted into medium containing 150 mM NaCl (A) or LiCl (B), 50 mM tricine NaOH (A) or LiOH (B), pH 8.5, 5 mM $MgSO_4$, 10 mM KCl, 2 mM DTT and 200 μM Q-1. *Squares*, intracellular Na^+; *circles*, intracellular K^+; *triangles*, intracellular Li^+.

Fast Li^+/Na^+ exchange took place initially when Li^+-loaded cells were suspended in Na^+-containing medium, but K^+ uptake was delayed during this process (Fig. 2). Only after nearly all inside Li^+ had been replaced by Na^+ did both uphill Na^+ efflux and K^+ influx commence (Fig. 2). This suggests that the active Na^+ efflux system is not only incapable of transporting Li^+, but that it is actually inhibited by this cation.

We have recently shown evidence suggesting that active respiration-dependent Na^+ transport in *E. coli* is not catalysed by the electrogenic Na^+/H^+ antiporters NhaA and NhaB, but by a distinct active sodium transport system (AST) that possesses $Na^+,K^+/H^+$ antiporter activity [8]. The present data show that Li^+ is not actively transported, and that it inhibits Na^+ transport, presumably competitively. This excludes that the NhaA and NhaB antiporters are responsible for sodium

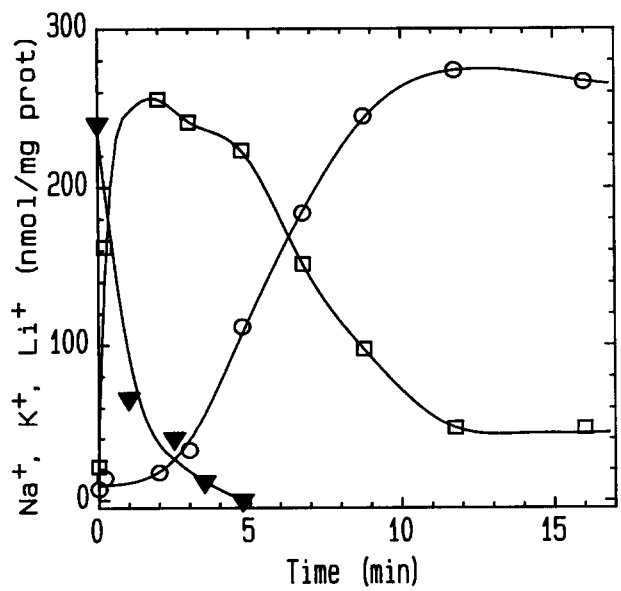

Fig. 2. Uphill transport of Na^+ and K^+ requires replacement of Li^+ with Na^+ in the cytoplasm. At zero time Li^+-loaded cells were diluted into medium containing 150 mM NaCl, 50 mM tricine NaOH, pH 8.5, 5 mM $MgSO_4$, 10 mM KCl, 2 mM DTT and 200 μM Q-1. *Squares*, intracellular Na^+; *circles*, intracellular K^+; *triangles*, intracellular Li^+.

transport because, like some other Na^+-transporting systems [12], they have been shown to operate with Li^+ (see Section 1). On the other hand, many other Na^+ transport systems are inoperative with Li^+, such as the Na^+-translocating NADH-quinone oxidoreductase of *Vibrio alginolyticus* [13,14]. The fast Na^+/Li^+ exchange observed here (Fig. 2) is likely due to the *nhaA* and/or *nhaB* antiporters, which then would explain why they confer resistance to Li^+ [2,3]. The difference in Li^+/Na^+ selectivity between the AST system and the Na^+/H^+ antiporters allows their distinction in vivo, and provides a good basis for future work.

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