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A novel antiporter activity catalyzing sodium and potassium transport from right-side-out vesicles of *E. coli*

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Abstract Downhill sodium efflux from right-side-out *E. coli* membrane vesicles was found to be stimulated by negative electric potential, as has been reported earlier [Bassilana et al., Biochemistry 23 (1984) 1015–1022], and in agreement with the concept of electrogenic Na^+/nH^+ antiporters with $n > 1$. However, sodium efflux was much more accelerated by positive electric potential, indicating the operation of another sodium transport system. ΔpH (alkaline inside), created by a pH shift from 8.5 to 6.8 in the medium was found to drive sodium efflux against its concentration gradient, but only when the vesicles had been loaded with *both* Na^+ and K^+. Efflux of K^+ against the concentration gradient was also observed under these conditions. When the vesicles were loaded separately with sodium tricine or potassium tricine, no K^+ efflux and insignificant Na^+ efflux were observed. We propose that there are at least two different mechanisms responsible for Na^+ efflux in *E. coli* vesicles. One is the Na^+/nH^+ antiporter previously described, and the other is a novel $Na^+,K^+/mH^+$ antiporter.

Key words: Na^+ transport; K^+ transport; *E. coli*

1. Introduction

Sodium transport in *E. coli* has been suggested to be catalyzed by electrogenic Na^+/nH^+ antiporters, where $n > 1$ (for review see [1]). The electrogenic antiporter was first proposed by Schuldiner and Fishkes [2] and Beck and Rosen [3] on the basis of measurements of dissipation by Na^+ of respiration-generated ΔpH in membrane vesicles. Careful analysis of downhill Na^+ efflux from *E. coli* membrane vesicles performed by Bassilana et al. [4,5] showed that the efflux was accelerated by $\Delta\Psi$, negative inside, in the pH range 5.5–7.5. Later, two membrane proteins, NhaA and NhaB, were purified, reconstituted into liposomes, and shown to catalyze Na^+/nH^+ exchange [6,7]. However, the activity of these antiporters has been found to be low at the neutral or slightly alkaline pH [7,8] maintained in the *E. coli* cytoplasm [9]. Moreover, a mutant with deletion of the nhaA gene, and an impaired nhaB gene, was competent in sodium transport at neutral pH [10]. This suggests that other systems also participate in sodium efflux. The results presented here show that *E. coli* membranes contain a Na^+ transport system that is strictly dependent on K^+, and which is able to perform uphill Na^+ and K^+ efflux driven by ΔpH.

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Abbreviations: MOPS, 4-morpholinepropanesulfonic acid; MES, 2-[*N*-morpholino]ethanesulfonic acid; tricine, *N*-tris[hydroxymethyl]methylglycine: $\Delta\Psi$, transmembrane difference in electric potential; ΔpH, transmembrane chemical gradient of H^+, ΔpNa, transmembrane chemical gradient of Na^+; TPP^+, tetraphenylphosphonium cation.

2. Materials and methods

2.1. Strains and growth of cells

The *E. coli* strain GR70N was gift by Dr. R.B. Gennis, University of Illinois. Cells were grown aerobically overnight at 37°C in a slightly alkaline (pH 7.5–8.0) medium containing 50 mM succinate [11], and were harvested in the first half of the exponential growth phase.

2.2. Preparation of membrane vesicles

Right-side-out membrane vesicles were prepared according to Kaback [12]. Lysozyme and sucrose were used at final concentrations of 50–100 μg/ml and 30%, respectively, for preparation of the spheroplasts. The process of spheroplast formation was controlled by the osmotic shock test described by Witholt et al. [13]. Several media were used for vesicle formation: (i) 50 mM CH_3COONa, 50 mM CH_3COOK, 20 mM MOPS adjusted to pH 7.5 by equal amounts NaOH and KOH; (ii) 100 mM MOPS adjusted to pH 7.5 with NaOH; (iii) 100 mM MOPS adjusted to pH 7.5 with KOH; (iv) 150 mM MOPS adjusted to pH 7.5 with equal amounts NaOH and KOH. For ΔpH-driven cation transport the vesicles were formed in the medium containing 100 mM tricine adjusted to pH 8.5 with NaOH, or KOH, or equal amounts of KOH and NaOH. Membrane vesicles were finally resuspended in the medium where they were formed at a concentration of 15–30 mg of protein/ml, stored on ice, and used on the day of preparation.

2.3. Na^+,K^+ efflux measurements

For downhill respiration-independent Na^+ efflux, Na^+-loaded membrane vesicles (2–5 μl, 50–80 μg membrane protein) were diluted 200-fold into Na^+-free medium of desired composition. When necessary, sucrose was added to the dilution media to equilibrate the osmolarity of external and internal solutions. After the suspension was incubated at room temperature for various periods of time, it was filtered (Millipore 0.45 μm) and washed once on the filter with 5 ml icecold 0.33 M sucrose.

A pH shift was produced by dilution of 5 μl of vesicles loaded with tricine adjusted to pH 8.5 with NaOH, KOH, or both, into 50 ml medium containing 100 mM MOPS adjusted to 6.5 with KOH, NaOH, or both, to obtain the final pH 6.8. As the concentrations of Na^+ and K^+ in the MOPS solution were significantly lower than in the tricine solution, K_2SO_4 and Na_2SO_4 were added to the MOPS solution to equalize cation concentrations inside and outside of the vesicles. The suspension was stirred at room temperature for various periods; then it was quickly transferred into 1 ml ice-cold 0.33 M sucrose and filtered immediately.

Sodium and potassium were extracted from the filter by washing it with IL-test solution containing detergent (Instrumental Lab. Co) and measured by flame photometry.

3. Results and discussion

Respiration-independent downhill Na^+ efflux from mem-

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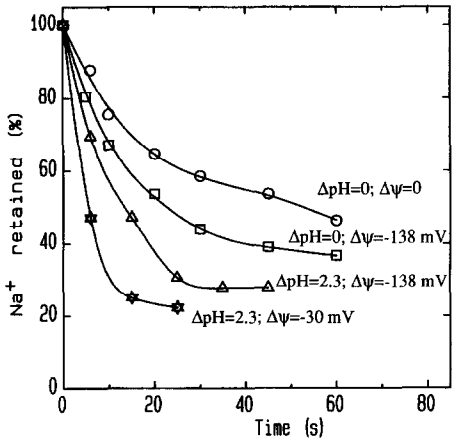

Fig. 1. Dependence of downhill sodium efflux from membrane vesicles loaded with sodium and potassium acetate on the composition of dilution medium. The vesicles containing 50 mM potassium acetate and 50 mM sodium acetate were diluted 200-fold at zero time into different Na-free media, and in the presence of 1 μg/ml valinomycin. *Circles*, 50 mM potassium acetate, 50 mM choline acetate, 20 mM MOPS-choline base, pH 7.5 (only ΔpNa); *squares*, 100 mM choline acetate, 20 mM MOPS-choline base, pH 7,5 (ΔpNa and $\Delta\Psi = -138$ mV); *triangles*, 100 mM MOPS-choline base, pH 7.5, 50 mM sucrose (ΔpNa, $\Delta\Psi = -138$ mV and ΔpH); *asterisks*, as triangles but 10 mM K^+ (as MOPS salt) was added (ΔpNa, $\Delta\Psi = -30$ mV and ΔpH).

brane vesicles loaded with equal amounts of sodium of sodium and potassium acetate, and diluted 200-fold into Na^+-free medium, was monitored (Fig. 1). In the presence of valinomycin, the Na^+ efflux has the lowest rate in potassium acetate medium when the only driving force is the Na^+ concentration gradient (Fig. 1, circles); the rate was faster in choline acetate medium when $\Delta\Psi$ due to K^+ diffusion potential could also drive Na^+/nH^+ antiport (Fig. 1, squares), and the rate was further accelerated when the vesicles were diluted into choline MOPS medium, where ΔpH (alkaline inside) was generated, due to an acetate concentration gradient (Fig. 1, triangles). These findings are in good agreement with the data presented by Bassilana et al. [4,5], and indicate Na^+/nH^+ antiporter activity. However, when the choline MOPS medium was supplemented with 10 mM K^+, the sodium efflux was further accelerated, although $\Delta\Psi$ dropped drastically (Fig. 1, asterisks). This finding could be explained if another Na^+ transport system started to operate, which was initially inhibited by the negative $\Delta\Psi$ due to the K^+ diffusion potential.

For further experiments we used vesicles loaded with 100 mM MOPS adjusted to pH 7.5 by NaOH. We believe that there are two advantages: on the one hand, large anions of the buffer apparently do not participate in sodium transport, which might occur, in principle, with acetate (i.e. acetate, Na^+ co-transport). On the other hand, a high buffer concentration inside the vesicles permits one to exclude any influence of ΔpH that could be created by the activity of a Na^+/nH^+ antiporter, at least on the initial stages of Na^+ transport. When the vesicles were loaded with sodium MOPS and diluted 200-fold into choline MOPS medium, only slow Na^+ efflux was seen (Fig. 2, squares). Sodium efflux was not limited by ΔpH due to the high buffer capacity of the interior, nor by $\Delta\Psi$, because the artificial permeable cation TPP^+ did not stimulate it (not shown). The rate of sodium efflux was strongly accelerated when choline was replaced by K^+ in the dilution medium, and valinomycin accelerated it further (Fig. 2, triangles and circles). Whilst there could be a direct exchange of Na^+ for K^+, the stimulation of Na^+ efflux by valinomycin contradicts it. These findings could be explained if the transport system for Na^+ is electrogenic and creates negative electric potential during Na^+ efflux. If so, it should be stimulated by positive $\Delta\Psi$ due to potassium diffusion potential.

As far as sodium transport systems are described as Na^+/nH^+ antiporters, it was important to check directly if ΔpH could drive uphill sodium transport from membrane vesicles. To create artificial ΔpH (alkaline inside), vesicles were loaded with tricine-NaOH, pH 8.5, and diluted into buffer, pH 6.8, where the sodium concentration was the same as inside the vesicles. However, there was only insignificant sodium efflux against its concentration gradient in such conditions (Fig. 3, open triangles). One may argue that an electrogenic Na^+/nH^+ antiporter with $n > 1$ was stopped by generation of $\Delta\Psi$, positive inside. However, addition of an artificial permeable anion, tetraphenylborate, did not stimulate sodium efflux (not shown). This means that the NhaA and NhaB systems, shown to catalyze $Na^+/2H^+$ and $2Na^+/3H^+$ exchange [6,7], were not active under these conditions. There could be at least two reasons for that: both systems have accelerated activity at alkaline pH [6,7] and could be inhibited by neutral pH outside, and/or these systems could require negative $\Delta\Psi$ for activity.

In contrast, fast efflux of Na^+ did proceed against the concentration gradient when vesicles were loaded with *both* Na^+ and K^+ at pH 8.5, and diluted into medium at pH 6.8, which contained alkali cations at the same concentration as inside, (Fig. 3, closed triangles). Uphill K^+ efflux also occurred, but to a lesser extent (Fig. 3, closed circles). It is difficult to determine whether this ΔpH-driven activity translocates equal amounts of Na^+ and K^+. Apparently, the intrinsic membrane permeability for K^+ is higher than that for Na^+, and downhill influx of K^+ competes with uphill efflux because of the high K^+ concentration outside. No uphill transport of K^+ could be observed under the same pH shift conditions when the vesicles were loaded with K^+ only (Fig. 3, open circles).

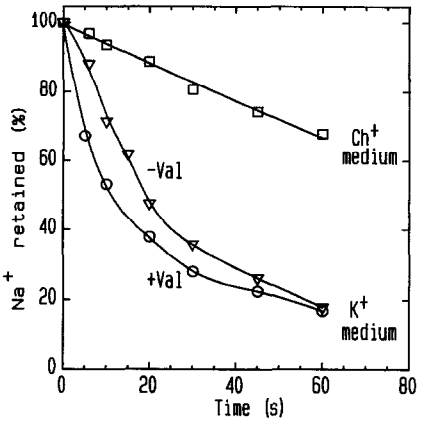

Fig. 2. Effect of potassium on downhill sodium efflux from Na^+-loaded membrane vesicles. The vesicles containing 100 mM MOPS-NaOH, pH 7.5, were diluted 200-fold at zero time into medium containing 100 mM MOPS-choline base, pH 7.5 (*squares*) or 100 mM MOPS-KOH, pH 7.5, in the presence of 1 μg/ml valinomycin (*circles*) or in its absence (*triangles*).

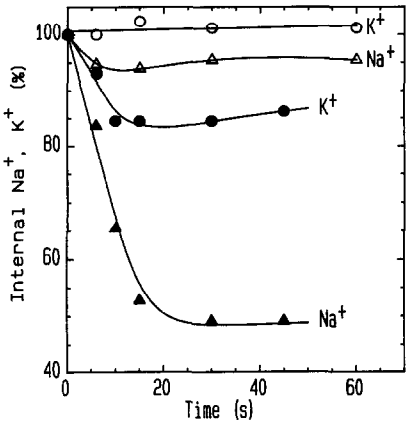

Fig. 3. ΔpH-driven uphill efflux of sodium and potassium. *Open triangles*, internal content of Na^+; membrane vesicles were loaded with 100 mM tricine-NaOH, pH 8.5, and diluted at zero time into the medium, containing the same concentration of Na^+ and 100 mM MES, pH 6.5. *Open circles*, internal content of K^+; membrane vesicles were loaded with 100 mM tricine-KOH, pH 8.5, and diluted at zero time into the medium containing the same concentration of K^+ and 100 mM MES, pH 6.5. *Closed symbols*, membrane vesicles were loaded with 75 mM tricine-NaOH and 75 mM tricine-KOH, pH 8.5, and diluted at zero time into medium containing the same concentration of K^+ and Na^+ and 150 mM MES, pH 6.5. *Closed triangles*, internal content of Na^+. *Closed circles*, internal content of K^+. After dilution pH of the medium shifted to 6.8.

Summarizing, we conclude that apart from the Na^+/nH^+ antiporters described previously (see [1] for review), *E. coli* membranes contain another Na^+ transport system, which also catalyzes sodium/proton exchange. This activity requires K^+ and is stimulated by positive electric potential. As some uphill K^+ efflux could be driven by ΔpH in the presence of Na^+, it is probable that potassium is co-transported with sodium. This suggests that the additional transport system observed here is a $Na^+,K^+/mH^+$ antiporter with $m = 1$.

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