POTASSIUM TRANSPORT IN *ESCHERICHIA COLI*

Evidence for a K^+-transport adenosine-5′-triphosphatase

Leszek WIECZOREK and Karlheinz ALTENDORF
Institut für Biochemie der Pflanzen, Ruhr-Universität Bochum, D-4630 Bochum, FRG

Received 12 December 1978

1. Introduction

Potassium has an important role in the regulation of metabolism in *Escherichia coli* [1]. To meet the K^+ requirement for metabolism under different growth conditions, *E. coli* possesses 4 kinetically and genetically distinct transport systems for K^+ [2]. The Kdp system, a high-affinity transport system is repressible and is operating only when K^+ becomes limiting in the medium [2]. The TrkA, TrkD and TrkF systems are constitutive [2]. Despite the modest affinity for K^+, the TrkA system is responsible for the bulk of K^+ uptake since it has a very high maximum rate [2].

We have demonstrated recently that all 3 structural proteins of the Kdp system, coded by the 3 genes of the *kdpABC* operon, are located in the cytoplasmic membrane [3]. We could not find any evidence for a periplasmic or outer membrane component. The Kdp system is under the control of the positive regulator coded by the *kdpD* gene [4]. It was found previously that the Kdp system is energized by ATP only [5]. It was therefore conceivable that this transport system might exhibit ATPase activity as it is the case for ATP-driven transport systems of animal cells [6].

This article describes the identification of a membrane ATPase associated with K^+ transport in *E. coli*.

2. Materials and methods

2.1. *Bacterial strains and growth conditions*

Strain DG 20/1 (*uncA*) from *E. coli* K12 *Ymel* was kindly supplied by Dr H. U. Schairer [7]. The minimal media employed in this work are identical to those used in [8]. Repressed cells were obtained by growth in media containing 115 mM K^+. Derepression was achieved by growing cells in media supplemented with 50 μM K^+. Glucose (0.2%) was used as carbon and energy source. Cells were grown with agitation at 37°C.

2.2. *Chemicals*

[γ-^{32}P]ATP was purchased from Amersham Buchler and *N,N′*-dicyclohexylcarbodiimide (DCCD) from Sigma. Ouabain was a gift from Professor Dr W. Schoner, Universität Gießen.

2.3. *Preparation of membranes*

Cells were grown to early stationary phase. After harvesting, cells were washed once with 50 mM Tris–SO_4 (pH 7.8), 10 mM $MgCl_2$ and resuspended in the same buffer (1 g wet wt/ml) containing 1 mg DNase/ml suspension. Cells were broken by passage through a Ribi press at 20 000 lb.in^{-2} pressure at 15°C. Cell debris was removed by centrifugation for 10 min at 10 000 × *g* (4°C). Membranes (everted vesicles) were pelleted by centrifugation at 142 000 × *g* for 90 min, washed once with 50 mM Tris–SO_4 (pH 7.8) and resuspended in the same buffer.

2.4. *Assays*

ATPase activity was measured by the determination of phosphate liberated. The assay medium (0.5 ml) contained 50 mM Tris–SO_4 (pH 7.8), 1.5 mM $MgCl_2$, 1.5 mM ATP (Na^+ or Tris-salt) and

an appropriate amount of membrane protein. The reaction was performed at 37°C. The reaction was terminated by the addition of 0.5 ml 10% trichloracetic acid. When the radioactive assay was used (~250 000 cpm [γ-^{32}P]ATP/0.5 ml assay medium), charcoal was added to the reaction mixture to adsorb excess radioactive ATP. After 5 min at room temperature the suspension was passed through a Pasteur pipette containing microfiber glass prefilters (Millipore, type AP 25). Nitrogen pressure was applied to speed up filtration. The ^{32}P liberated was determined by counting the radioactivity in the clear filtrate in a liquid scintillation counter.

In other experiments phosphate released was determined colorimetrically [9]. As indicated in the different tables cations were added as chloride salts.

2.5. *Other methods*

The usual buffers are sufficiently contaminated with K^+ that a concentration well above the K_m (2 μM) of the Kdp system is achieved in the reaction mixture. To demonstrate stimulation of the ATPase activity by K^+ it was necessary in some experiments to use an assay medium deficient in K^+. For this purpose assay medium was inoculated with washed cells from *E. coli* taken from the logarithmic phase of growth and in which the Kdp system was derepressed. After vigorous shaking at 37°C for 30–60 min cells were removed by filtration. This method was very effective in removing traces of K^+ without changing the composition of the assay medium. Even in the presence of Tris–ATP during the pretreatment procedure, *E. coli* cells do not release Na^+ into the assay medium.

Protein concentrations were determined by a modification [10] of the Lowry method with bovine serum albumin as the standard.

3. Results

Since the Kdp transport system for K^+ is energised by ATP and is composed of 3 proteins located in the cytoplasmic membrane it was tempting to speculate that such a transport ATPase should have substrate-dependent ATPase activity. To detect such K^+-stimulated ATPase activity it was first necessary to remove the (Ca^{2+}, Mg^{2+})-dependent ATPase functioning in oxidative phosphorylation. To avoid harsh treatments for removing the F_1 part of the ATPase complex from the membranes, which possibly could also affect K^+-stimulated ATPase activity, we used a mutant strain lacking (Ca^{2+}, Mg^{2+})-stimulated ATPase activity.

It was demonstrated in [2] that the Kdp system is repressed in cells grown in a minimal medium supplement with 115 mM K^+. As shown in table 1 membranes from strain DG 20/1 grown under such repressing conditions exhibit only a negligible ATPase activity which is not further stimulated by K^+. However, in membranes obtained from cells in which the Kdp system is fully derepressed by growth on low K^+ (50 μM), K^+-stimulated ATPase activity is observed. As also indicated in table 1 this ATPase activity is not inhibited by dicyclohexylcarbodiimide. It is also noteworthy to mention that K^+-stimulated ATPase activity was found in everted vesicles (Ribi-press vesicles) but not in right-side-out ones (Kaback vesicles), indicating that this ATPase is only accessible from the cytoplasmic side of the membrane (table 1).

Table 1
K^+-stimulated ATPase activities of membranes derived from repressed and derepressed cells from *E. coli* DG 20/1

Membranes *E. coli* DG 20/1 (*uncA*)		ATPase (μmol . g^{-1} . min^{-1})
Repressed cells		
Ribi-press vesicles	($-K^+$)	4.6
Ribi-press vesicles	($+K^+$)	5.0
Kaback vesicles	($+K^+$)	3.8
Derepressed cells		
Ribi-press vesicles	($-K^+$)	10.4
Ribi-press vesicles	($+K^+$)	66.8
Ribi-press vesicles	(+DCCD, $+K^+$)	66.0
Kaback vesicles	($+K^+$)	14.4
Kaback vesicles	(Sonication, $+K^+$)	63.1

The preparation of the membranes (everted vesicles) and the measurement of the ATPase activities are in section 2.3 and 2.4. If added to the reaction mixture, K^+ was 50 μM. Treatment with *N,N'*-dicyclohexylcarbodiimide (DCCD) was performed by incubating membranes (4 mg/ml) for 30 min at room temperature in 50 mM Tris–SO_4 (pH 7.8), containing 0.02 mM DCCD. Right-side-out vesicles (Kaback vesicles) were prepared as in [13]. As indicated, these vesicles were sonicated for 5 min at 4°C in 50 mM Tris–SO_4 (pH 7.8)

Further support for the notion that the ATPase is consistent with an activity of the Kdp system comes from studies using monovalent cations. It was established for intact cells that the Kdp system does not require Na^+ for its function [4] and that Rb^+ cannot subsitute for K^+. The ATPase detected in membranes of derepressed cells has similar properties. The ATPase activity is not stimulated further by Na^+ in the presence or absence of K^+. It should be mentioned that similar results were obtained when K^+ or Na^+ were added to different sides of the cytoplasmic membrane. Furthermore neither, Rb^+, Cs^+ nor Li^+ have a stimulatory effect (data not shown).

Since sodium ATP was normally used for the enzyme assay resulting in Na^+ at ~4 mM, experiments were also performed using Tris–ATP. These experiments also failed to show any significant stimulation by or dependence on Na^+ (data not shown).

Interesting results were also obtained testing the divalent cation specificity of the ATPase. In the absence of divalent cations the ATPase activity is very low (table 2). Mn^{2+} and Co^{2+} can stimulate ATPase activity to the same extent as Mg^{2+}. It is noteworthy to mention that Ca^{2+} had no effect on the ATPase activity in the absence of Mg^{2+}. In the presence of Mg^{2+}, however, Ca^{2+} was somewhat inhibitory; Zn^{2+}, Cu^{2+} or Fe^{2+} showed, of it all, only slight stimulatory effects.

The nucleotide specificity is narrow, too. Only ATP serves as a good substrate; with all the others (UTP, GTP, ITP) negligible activity was observed in the presence of K^+. The pH optimum of the K^+-stimulated ATPase was found to be ~7.8. Therefore, the assays were performed at this pH. Finally, it should be mentioned, that ouabain, the inhibitor of the mammalian Na^+, K^+-ATPase has no effect on the K^+-stimulated ATPase of *E. coli* (data not shown).

Table 2
Divalent cation specificity of the Kdp ATPase

Divalent cation (1.5 mM)	ATPase (μmol . g^{-1} . min^{-1})
None	2
Mg^{2+}	53
Mn^{2+}	51
Co^{2+}	55
Zn^{2+}	8
Ca^{2+}	6
Ca^{2+}, Mg^{2+}	36

The preparation of membranes from derepressed cells, and the measurement of the ATPase activities are in section 2.3 and 2.4. K^+ was present at 50 μM

4. Discussion

It was demonstrated [2] that the Kdp transport system for K^+ is repressed by growth in high K^+ and [11] that it exhibits a very narrow cation specificity. These characteristics are shared by the ATPase described here. Our data, as well as results in [12], have so far established that K^+ stimulates the Kdp ATPase activity whereas Na^+ has no effect.

Evidence is still lacking as to whether K^+ is transported electrogenically by the Kdp ATPase or whether the transport is an electroneutral process, as an exchange for H^+ or by symport with an anion. Furthermore, it would also be interesting to know how many moles of K^+ are transported per mole of ATP hydrolyzed. These and other questions are presently under investigation.

Having identified a K^+-transport ATPase in bacteria it is tempting to speculate about the role of the 3 membrane bound subunits of the system [3]. Based on the preliminary findings in [12] that a change in K_m for K^+ is probably due to a mutation in the *kdpA* gene, it seems reasonable to assume that the KdpA protein(s) might be responsible for the translocation of K^+ through the cytoplasmic membrane. Experimental evidence from our and also from Epstein's laboratory (personal communication) revealed that the KdpB protein becomes phosphorylated during ATP hydrolysis. Whether the remaining KdpC protein is the link between the 'energy input' part (KdpB protein) and the transport part (KdpA protein) of the system, or whether it has other functions, remains to be seen. Regardless of details, this view makes studies to the mechanism of energy coupling to this system very interesting, and the purification of the K^+-ATPase in a functional state and the reconstitution in an artificial system would be rewarding.

Acknowledgements

The authors are grateful to Professor Dr A Trebst for financial support. We thank Professor Epstein for providing us with a preprint of his work prior to publication. This work was supported in part by the Deutsche Forschungsgemeinschaft (Al 118/4) and by the Fonds der Chemischen Industrie.

References

[1] Harold, F. M. and Altendorf, K. (1974) in: Current Topics in Membranes and Transport (Bronner, F. and Kleinzeller, A. eds) pp. 1–50, Academic Press, New York.
[2] Rhoads, D. B., Waters, F. B. and Epstein, W. (1976) J. Gen. Physiol. 67, 325–341.
[3] Laimins, L. A., Rhoads, D. B., Altendorf, K. and Epstein, W. (1978) Proc. Natl. Acad. Sci. USA 75, 3216–3219.
[4] Rhoads, D. B., Laimins, L. and Epstein, W. (1978) J. Bacteriol. 135, 445–452.
[5] Rhoads, D. B. and Epstein, W. (1972) J. Biol. Chem. 252, 1394–1401.
[6] Sachs, G., Spenney, J. G. and Lewin, M. (1978) Physiol. Rev. 58, 106–173.
[7] Schairer, H. U., Friedl, P., Schmid, B. J. and Vogel, G. (1976) Eur. J. Biochem. 66, 257–268.
[8] Epstein, W. and Davies, M. (1970) J. Bacteriol. 101, 836–843.
[9] Chen, P. S., jr, Toribara, T. Y. and Warner, H. (1956) Anal. Chem. 28, 1756–1758.
[10] Hartree, E. F. (1972) Anal. Biochem. 48, 422–427.
[11] Rhoads, D. B. and Epstein, W. (1977) Biochim. Biophys. Acta 469, 45–51.
[12] Epstein, W., Whitelaw, V. and Hesse, J. (1979) J. Biol. Chem. in press.
[13] Kaback, H. R. (1971) Methods Enzymol. 22, 99–120.