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STUDIES ON (Na^+-K^+)-ACTIVATED ATPase

XIX. OCCURRENCE AND PROPERTIES OF A (Na^+-K^+)-ACTIVATED ATPase IN *ESCHERICHIA COLI*

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SUMMARY

1. An ouabain-sensitive (Na^+-K^+)-ATPase (ATP phosphohydrolase, EC 3.6.1.3) has been demonstrated in *Escherichia coli*, strain K-12.

2. Pretreatment of the freeze-dried bacteria with urea lowers the Mg^{2+}-ATPase activity, while it has no effect on the (Na^+-K^+)-ATPase activity.

3. The Mg^{2+}-activation curve gives an optimal Mg^{2+}:ATP ratio of 1:1.

4. In the presence of 58 mM Na^+ there is a maximum activation of the (Na^+-K^+)-ATPase at 30–40 mM K^+.

5. The pH optimum for the Mg^{2+}-ATPase is 8.9 and for the (Na^+-K^+)-ATPase 7.7.

6. There is a high specificity of the (Na^+-K^+)-dependent activity for ATP and ADP.

7. The results obtained for *E. coli* are discussed in relation to the corresponding enzyme system in mammalian tissues.

INTRODUCTION

In 1957 SKOU[1] described the occurrence of an ATPase (ATP phosphohydrolase, EC 3.6.1.3) in crab nerve, which was highly stimulated in the presence of Na^+ and K^+ together. POST *et al.*[2], as well as DUNHAM AND GLYNN[3], demonstrated that the (Na^+-K^+)-ATPase activity in ghosts of erythrocytes is closely related to the active transport of Na^+ and K^+ across the membrane, *e.g.* both are inhibited by cardiac glycosides like ouabain. BONTING, SIMON AND HAWKINS[4] have found this (Na^+-K^+)-ATPase in several tissues of the cat, being undetectable only in non-cellular tissues. They determined the presence of the enzyme in all tissues in which glycoside-sensitive cation transport had been shown[5]. A quantitative correlation of the enzyme activity with the cation transport was demonstrated in six tissues[6]. It was impossible to detect in *Ulva lactuca* either any (Na^+-K^+)-ATPase activity or an ouabain-sensitive cation transport, so that the correlation between these two systems is so far unbroken[7].

SCHULTZ AND SOLOMON[8] have studied the cation transport in *Escherichia coli*. From their results they concluded that there must be two separate processes of K^+ uptake, a K^+–H^+ exchange and a K^+–Na^+ exchange[9]. The first process is rapid and accounts for approx. 60% of the total net K^+ uptake. The second process may be ascribed to a 1 for 1 exchange of extracellular K^+ for intracellular Na^+. SOLOMON[10] detected in the membrane of *E. coli* an ATPase activity which was inhibited by omission of K^+ or by addition of ouabain.

Recently GÜNTHER AND DORN[11] described the presence of an ATPase in *E. coli* which was only stimulated about 20% by Na^+, while K^+ and ouabain did not have any effect.

KLEBER AND AURICH[12] postulated in *Pseudomonas aeruginosa* the occurrence of a (Na^+-K^+)-activated ouabain-sensitive ATPase, which would be involved in the transport of carnitine.

The present paper deals with the occurrence and properties of a (Na^+-K^+)-activated ATPase in *E. coli*.

MATERIALS AND METHODS

Escherichia coli, strain K-12, was kindly supplied by Professor T. O. WIKÉN, Laboratory of Microbiology, Institute of Technology at Delft, The Netherlands. The organism was cultivated on the 5 mM K^+ medium[8], with a little modification, containing in mM: NaH_2PO_4, 22; Na_2HPO_4, 40; $(NH_4)_2SO_4$, 8; sodium citrate, 5; $MgSO_4$, 0.4; KCl, 5; glucose, 60. The salt solution and glucose solution were autoclaved separately and then mixed aseptically. A volume of 500 ml of this medium was inoculated with 15 ml of a culture in the logarithmic phase and incubated for 24 h at 37° without aeration. The cells in the late stationary phase were harvested by centrifugation at 4°, washed 2 times with distilled water to remove the growth medium, lyophilized at −20° and stored at −25°.

Assay media for (Na^+-K^+)-ATPase were those described previously[7], except that all volumes were multiplied by 50. Medium A (complete) gave total Mg^{2+}-ATPase activity; Media B (no K^+), C (no Na^+), D (Medium A *plus* 10^{-2} or 10^{-4} M ouabain) and E (no K^+; 10^{-4} M ouabain) gave Mg^{2+}-ATPase activity. Activity in Medium A *minus* the average activity in Media B, C, D and E gave the ouabain-sensitive (Na^+-K^+)-ATPase activity. Incubation was carried out at pH 7.6 for 1 h. For convenience, in most experiments, the ATPase activity was determined only in Media A and E.

The dependence of the ATPase activities on pretreatment with urea was measured by homogenisation of the freeze-dried bacteria in solutions of different urea concentration. The Mg^{2+}-activation curve was obtained by varying the Mg^{2+} concentration in Media A and E from 0 to 6 mM while maintaining the ATP concentration at 2 mM. The K^+-activation curve was obtained by adding KCl (0–40 mM) to Medium B. The pH–activity curves were obtained by preparing Media A and E with Tris–histidine buffers (final concentration of each compound: 50 mM) in a pH range from 6.2 to 9.4. The pH of each resulting medium was measured and used in plotting the assay results.

The substrate specificity was investigated by adding one each of the different nucleotides (2 mM) instead of ATP to Media A and E. The specificity of cation

activation was tested by adding one each of the different cations (5 mM) instead of KCl to Medium A. All cations were added as chlorides.

For routine assays 6 mg of the freeze-dried bacteria were homogenized in 1 ml of twice-distilled water or 12 mg in 1 ml of a solution of 1.5 M urea. The final tissue concentration was about 0.4 and 0.8 mg, respectively, per ml incubation medium and the final urea concentration was 0.09 M. All homogenates were stored for 1 h at 0°. The urea had no effect on color development in the phosphate determination.

ATP, ADP and AMP were purchased from C. F. Boehringer & Soehne GmbH., Mannheim and GTP, UTP, CTP, TTP and ITP from Sigma Chemical Co.

RESULTS

Attempts to demonstrate (Na^+-K^+)-ATPase activity by removing the cell wall of *E. coli* by ultrasonic treatment, by the lysozyme–EDTA method of REPASKE[13] or by making spheroplasts with penicillin were without result, since only Mg^{2+}-ATPase was detectable.

The relative ATPase activities in the various substrate media with and without pretreatment with 1.5 M urea are shown in Table I. These data indicate the presence

TABLE I

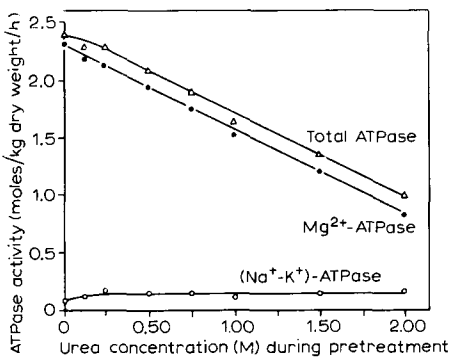
RELATIVE ATPase ACTIVITIES OF *E. coli* WITHOUT PRETREATMENT OR PRETREATED WITH 1.5 M UREA IN VARIOUS SUBSTRATE MEDIA

The final tissue concentrations were 0.4 and 0.8 mg per ml incubation medium, respectively. Activities are expressed with ±S.E. and, in parentheses, number of determinations. All percentages are significantly different from 100% at $P = 0.05$ level.

Medium	*ATPase activity (%)*	
	− Urea	*+ Urea*
A (complete)	100 (12)	100 (11)
B (no K^+)	95.2 ± 1.3 (10)	88.8 ± 1.0 (8)
C (no Na^+)	92.0 ± 3.2 (12)	88.1 ± 3.2 (9)
D (10^{-4} M ouabain)	96.8 ± 1.0 (10)	
D (10^{-2} M ouabain)		96.0 ± 0.7 (9)
E (no K^+; 10^{-4} M ouabain)	95.6 ± 1.9 (9)	87.6 ± 1.0 (8)
Average in Media B, C, D, E	95.2 ± 0.6	90.1 ± 2.0

of a (Na^+-K^+)-ATPase activity. Pretreatment of the freeze-dried bacteria with urea decreased the Mg^{2+}-ATPase activity. After this pretreatment there was about 12% inhibition of the total ATPase by omission of K^+ or Na^+. The inhibition in Medium D (10^{-2} M or 10^{-4} M ouabain) was less than in the other media (3.2–4.0%). SOLOMON[10] found an inhibition of 20% by omission of K^+. GÜNTHER AND DORN[11] demonstrated an increase of the Mg^{2+}-ATPase activity of about 20% by adding Na^+, but they could not find any effect of K^+ either on the intact bacteria or on the lysed protoplasts of *E. coli*.

Fig. 1 shows that pretreatment of the freeze-dried material with urea solutions of different molarities led to a decrease in the total and the Mg^{2+}-ATPase activity,



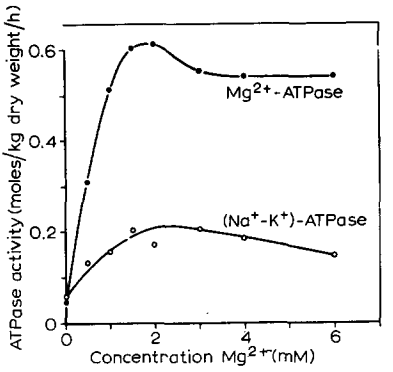
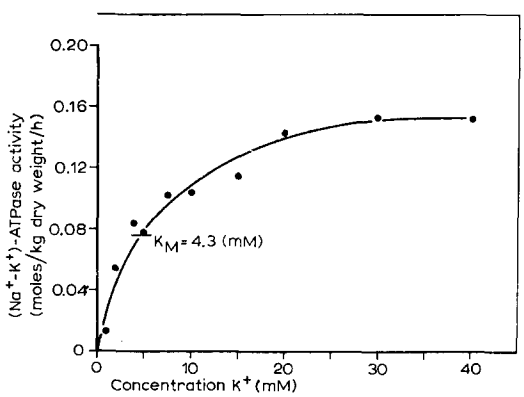


Fig. 1. Effect of pretreatment with urea on total, Mg^{2+}- and (Na^+-K^+)-ATPase of *E. coli*. The final tissue concentration was 0.4 to 0.8 mg/ml incubation medium.

Fig. 2. Effect of Mg^{2+} concentration on Mg^{2+}-ATPase and (Na^+-K^+)-ATPase activities in *E. coli*. Freeze-dried bacteria were pretreated with 1.5 M urea.

while the (Na^+-K^+)-ATPase remained unaffected. This treatment made it easier to measure the (Na^+-K^+)-ATPase, because the percentage of this ATPase with respect to the total ATPase was greater. Urea directly added in the same concentration to the incubation media had no effect. These findings are in agreement with those of SKOU AND HILBERG[14] for ox brain.

Fig. 2 shows the Mg^{2+}-activation curve for the Mg^{2+}-ATPase and the (Na^+-K^+)-ATPase. It can be seen that for both activities the optimal Mg^{2+}:ATP ratio was 1:1. There was some ATPase activity without adding Mg^{2+}, possibly because a significant amount of Mg^{2+} was present in *E. coli*. GÜNTHER AND DORN[11] found an optimal Mg^{2+}:ATP ratio of 3:5 for Mg^{2+}-ATPase in intact cells of *E. coli*, while NEUJAHR, HANSSON AND FERM[15] showed a ratio close to 1:2 for the enzyme in membranes of *Streptococcus faecalis*.



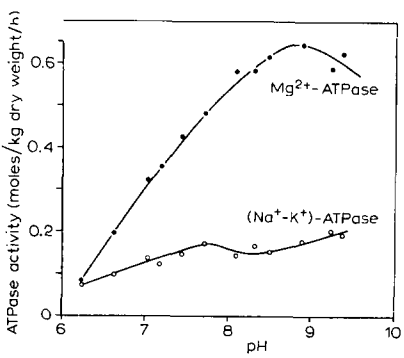


Fig. 3. Effect of K^+ concentration on Mg^{2+}-ATPase and (Na^+-K^+)-ATPase activities in *E. coli*. Freeze-dried bacteria were pretreated with 1.5 M urea.

Fig. 4. Effect of pH on Mg^{2+}-ATPase and (Na^+-K^+)-ATPase activities in *E. coli*. Freeze-dried bacteria were pretreated with 1.5 M urea.

TABLE II

SUBSTRATE SPECIFICITY OF NUCLEOTIDE PHOSPHATASE ACTIVITIES IN *E. coli*
Freeze-dried bacteria were pretreated with 1.5 M urea.

Substrate	*Activity (moles substrate ATP hydrolyzed/kg dry wt. per h)*		
	Total	*Mg^{2+}-activated*	*(Na^+-K^+)-activated*
ATP	0.94	0.76	0.18
GTP	0.47	0.51	0
UTP	0.30	0.27	0.03
CTP	0.30	0.28	0.02
TTP	0.21	0.20	0.01
ITP	0.48	0.43	0.05
ADP	0.56	0.44	0.12
AMP	0.10	0.08	0.02

The K^+-activation curve, measured in the presence of 58 mM Na^+ is shown in Fig. 3. There was a maximum activity at 30–40 mM, while the K_m value was 4.3 mM. This is different from the results of BONTING *et al.* for vertebrate tissues; they showed a maximum activity at 5 mM K^+ and a K_m value of 0.7 to 1.5 mM in the rectal gland of the elasmobranchs[16] and in the salt gland of the herring gull[17]. GÜNTHER AND DORN[11] could not detect any influence of K^+ on the ATPase activity in *E. coli*, while NEUJAHR, HANSSON AND FERM[15] found a maximum of activity at 10 mM K^+ in the presence of 100 mM Na^+ in membranes of *S. faecalis*.

Fig. 4 shows the pH–activity curves for the Mg^{2+}-ATPase and the (Na^+-K^+)-ATPase. The Mg^{2+}-ATPase has a maximum at pH 8.9 in agreement with the value for the rectal gland of the elasmobranchs[16]. The (Na^+-K^+)-ATPase shows a very indistinct optimum at pH 7.7, compared with optima at pH 6.3 to 7.0 for the rectal gland of the elasmobranchs[16], at pH 7.2 for the salt gland of the herring gull[17] and at pH 7.3 for rabbit lens epithelium[18]. GÜNTHER AND DORN[11] observed an optimum

TABLE III

EFFECT OF DIFFERENT CATIONS ON ATPase ACTIVITIES OF *E. coli* IN THE PRESENCE OF 58 mM Na^+
Freeze-dried bacteria were pretreated with 1.5 M urea.

Cation added (5 mM of the chloride)	*Activity (moles ATP hydrolyzed/kg dry wt. per h)*		
	Total ATPase	*Mg^{2+}-ATPase*	*Cation-activated ATPase*
Li^+	0.45	0.43	0.02
Na^+	0.44	0.43	0.01
K^+	0.57	0.42	0.15
Rb^+	0.54	0.42	0.12
Cs^+	0.45	0.42	0.03
NH_4^+	0.60	0.45	0.15

at pH 8.5 for the intact bacteria and for the lysed protoplasts of *E. coli* no optimum until 9.2, while WEIBULL, GREENAWALT AND LÖW[19] could not detect a pH optimum for ghosts of *Bacillus megaterium*.

The substrate specificity is shown in Table II. Only ATP and ADP gave a reasonable (Na^+-K^+)-phosphatase activity, while with the other triphosphates and AMP little or no activity was observed. There existed considerable Mg^{2+}-phosphatase activity for each of the substrates tested. Thus ATP and ADP are specific substrates for the (Na^+-K^+)-phosphatase and not for the Mg^{2+}-phosphatase. GÜNTHER AND DORN[11] found for ATP and ADP nearly equal activity, while AMP gave about half the activity for ATP.

Table III shows the effect of different cations on the $(Na^+ - K^+)$-ATPase activity in the presence of 58 mM Na^+. Besides K^+, only Rb^+ and NH_4^+ showed activation, while Li^+, Na^+ and Cs^+ did not give any activation. It was already shown that Rb^+ can take the place of K^+ in the activation of $(Na^+ - K^+)$-ATPase of crab nerve by SKOU[20] and in lens epithelium by BONTING, CARAVAGGIO AND HAWKINS[18].

DISCUSSION

A Mg^{2+}-ATPase, as described here, has been demonstrated in several species of bacteria. GÜNTHER AND DORN[11] could show a Mg^{2+}-ATPase in intact bacteria as well as in lysed protoplasts of *E. coli*. WEIBULL, GREENAWALT AND LÖW[19] found a Mg^{2+}-ATPase activity in *B. megaterium*, while ABRAMS, MCNAMARA AND JOHNSON[21] demonstrated the same activity in membranes of *S. faecalis*. Also in mycoplasma membranes a Mg^{2+}-ATPase activity was demonstrated[22].

Varying types of cation activation of this Mg^{2+}-ATPase were also obtained by different authors for various microorganisms. In *E. coli* there was a Na^+ activation in the absence of K^+ or in the presence of 100 mM K^+, but there was no K^+ activation[11]. In the cell membranes of halophilic *Vibrio parahaemolyticus* there was a stimulation by Na^+ (1.2–1.6 M) and K^+ (1.0–3.0 M) in the presence of 2 mM Mg^{2+} and 1 mM ATP at pH 8.5 (ref. 23). In mycoplasma membranes there was no stimulation by Na^+, K^+ or NH_4^+, tested alone or in various combinations[22], while in the soluble fraction of *B. megaterium* an activation by Na^+ or K^+ at pH 7.2 was demonstrated[24].

In the present study *E. coli*, strain K-12, was found to contain an ATPase which required Mg^{2+} in a Mg^{2+}:ATP ratio of 1:1. This Mg^{2+}-ATPase activity could be decreased by pretreatment with urea without affecting the (Na^+-K^+)-ATPase. SKOU AND HILBERG[14] previously concluded that the vertebrate Mg^{2+}-ATPase is more sensitive to pretreatment with urea than the (Na^+-K^+)-ATPase, a conclusion which was here confirmed for *E. coli*.

Since the relative (Na^+-K^+)-ATPase activity was quite low and this activity has to be determined by a differential assay, it is understandable that difficulties were encountered in studying the properties of the enzyme. Therefore a pretreatment of the enzyme preparation with concentrated urea solution was performed. BAKKEREN AND BONTING[25] found evidence that this pretreatment, at least in the case of rat liver, does not change the properties of the (Na^+-K^+)-ATPase.

The Mg^{2+}-ATPase could be activated by cations. In the presence of 58 mM Na^+ there was maximal activation of K^+ at 30–40 mM, which is different from results

obtained by GÜNTHER AND DORN[11]. On the other hand activation by Na^+ in the presence of 5 mM K^+ could not be obtained, while GÜNTHER AND DORN[11] showed in intact bacteria an activation by 30 mM Na^+ in the absence of K^+. In our experiments the activation of the Mg^{2+}-ATPase by one cation (Na^+ or K^+) was always measured in the presence of the other cation (K^+ or Na^+).

It was also possible to obtain activation by one cation alone. At pH 7.6 either Na^+ or K^+ alone could increase the Mg^{2+}-ATPase activity[26]. The question arises therefore, whether there is a true (Na^+-K^+)-ATPase present in *E. coli*. By definition[5] this enzyme activity is activated by Na^+ and K^+ together and inhibited by digitalis glycosides. Since there is a certain degree of activation of Mg^{2+}-ATPase by Na^+ and K^+ alone, the safest measure of (Na^+-K^+)-ATPase in *E. coli* would seem to be the inhibition of the total ATPase by ouabain. After addition of 10^{-4} to 10^{-2} M ouabain to Medium A there was a small but significant inhibition (3.2–4%) of the total ATPase, indicating that there is some (Na^+-K^+)-ATPase present in *E. coli*. The inhibition of 30% of the total ATPase by $5 \cdot 10^{-3}$ M ouabain reported by SOLOMON[10] could not be reproduced subsequently (W. EPSTEIN AND A. K. SOLOMON, private communication). Our best estimate for the absolute activity of this (Na^+-K^+)-ATPase in *E. coli* was 0.21 mole ATP hydrolyzed per kg dry weight per h. From results obtained by EPSTEIN AND SCHULTZ[27] it is possible to calculate that the total K^+ flux under steady-state conditions is 0.85 to 1.70 moles/kg dry weight per h of which 40% or 0.34–0.68 mole/kg dry weight per h was due to K^+–Na^+ exchange. This gives a ratio of K^+–Na^+ flux to ATP hydrolyzed by ATPase of 1.6–3.2 which is within the range of values obtained by BONTING[28].

Only ATP and ADP gave an increased phosphatase activity in the presence of Na^+ and K^+. OLIVER AND PEEL[29] have demonstrated an active adenylate kinase (ATP:AMP phosphotransferase, EC 2.7.4.3) in *E. coli*, suggesting that the high activity with ADP as substrate could represent an ATPase activity. This substrate specificity also suggests that we are dealing with a true (Na^+-K^+)-ATPase.

The pH optima for the two ATPases agreed with those for other tissues[16,17]. For the (Na^+-K^+)-ATPase there was an indistinct optimum at pH 7.7, while at alkaline pH the activity slowly increased. This increase may represent an ATPase activated by a single cation. GÜNTHER AND DORN[11] obtained a steady increase in activity at increasing pH in the lysed protoplasts of *E. coli* in the presence of Mg^{2+}, Na^+ and K^+ without an optimum in the measured range (up to pH 9.2).

It can be concluded that *E. coli* does contain a (Na^+-K^+)-ATPase, although its relative activity is very low compared with the corresponding enzyme system in most vertebrate tissues. Attempts are now in progress to investigate whether this (Na^+-K^+)-ATPase in *E. coli* is related to the active transport of Na^+ and K^+ across the cell membrane.

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