BBA 65862

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

XXIII. A Mg^{2+}-ATPase IN *ESCHERICHIA COLI*, ACTIVATED BY MONOVALENT CATIONS

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(Received November 1st, 1968)

SUMMARY

1. The properties of a Mg^{2+}-activated ATPase (ATP phosphohydrolase, EC 3.6.1.4) with optimum pH 8.7 in *Escherichia coli*, strain K-12, are described.

2. Addition of KCl increases the specific activity over the entire pH range.

3. Various monovalent cations, added as chlorides, have a different activating effect.

4. Of all phosphates tested, ADP is the best substrate next to ATP, and of all bivalent cations tested, Mg^{2+} is the best cofactor for this phosphatase activity.

5. The optimal temperature for the Mg^{2+}-ATPase activity is 45°, both in the presence and in the absence of KCl.

6. Disintegration of the bacteria by sonication leads to a loss of the activating effect of monovalent cations on the Mg^{2+}-ATPase activity.

7. In the light of these properties of the Mg^{2+}-ATPase, the occurrence of a (Na^+-K^+)-ATPase system in *E. coli* was reinvestigated and confirmed.

INTRODUCTION

The active transport of Na^+ and K^+ across the cell membrane is qualitatively and quantitatively related to the (Na^+-K^+)-activated Mg^{2+}-dependent ATPase system (ATP phosphohydrolase, EC 3.6.1.4)[1-3]. Besides Mg^{2+}, this enzyme system requires Na^+ and K^+ ions together for activity, while it can be inhibited by cardiac glycosides like ouabain. The occurrence of the (Na^+-K^+)-ATPase system is ubiquitous and it has been detected in many tissues[4]. It is always found accompanied by a Mg^{2+}-dependent ATPase activity, which is not stimulated by Na^+ and K^+ ions together and is not inhibited by ouabain.

Recently HAFKENSCHEID AND BONTING[5] described the occurrence of a (Na^+-K^+)-ATPase activity, in *Escherichia coli*. In studies of the properties of this enzyme, the presence was confirmed of a Mg^{2+}-dependent ATPase, which can be activated by one monovalent cation and which has previously been described by GÜNTHER AND DORN[6] and by BRAGG AND HOU[7].

This necessitated a careful study of the properties of this enzyme in order to reevaluate our previous conclusion about the occurrence of a (Na^+-K^+)-ATPase system in *E. coli*. Our results are reported in this paper.

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, harvested and lyophilized as described previously[5]. All homogenates were prepared by homogenization of 4.0 mg of the freeze-dried bacteria in 1 ml of twice-distilled water.

The assay of the Mg^{2+}-ATPase was performed in 0.5 ml of a medium containing 200 mM Tris–HCl (pH 8.7), 2 mM $MgCl_2$ and 2 mM ATP. The incubation was carried out for 1 h at 37°, after which the reaction was stopped by adding 2.25 ml of 10% trichloroacetic acid, the precipitate was removed by centrifugation and the inorganic phosphate released was measured as described by BONTING, SIMON AND HAWKINS[4].

The Mg^{2+}-activation curve was obtained by varying the Mg^{2+} concentration from 0 to 6 mM while maintaining the ATP concentration at 2 mM. The pH-activity curves were obtained by using Tris–histidine buffers (final concentration of each compound: 100 mM) in a pH range from 6.0 to 9.5. The pH of each resulting medium was determined and used in plotting the assay results. The influence of the various monovalent cations was measured by adding the corresponding chloride (up to 140 mM final concentration) to the incubation medium. The substrate specificity was investigated by replacing ATP with each of the different nucleotides (2 mM). The specificity of the bivalent cations was measured by replacing Mg^{2+} with other bivalent cations in the same concentration (2 mM). The effect of temperature on activity was determined after incubation for 1 h at the indicated temperatures. Disintegration of the bacteria was achieved by sonicating for 3 min. The homogenate was centrifuged at 100 000 × *g* for 1 h at 4°. The supernatant was separated from the precipitate, which was taken up in the required amount of water. The protein content was measured according to the method of LOWRY *et al.*[8].

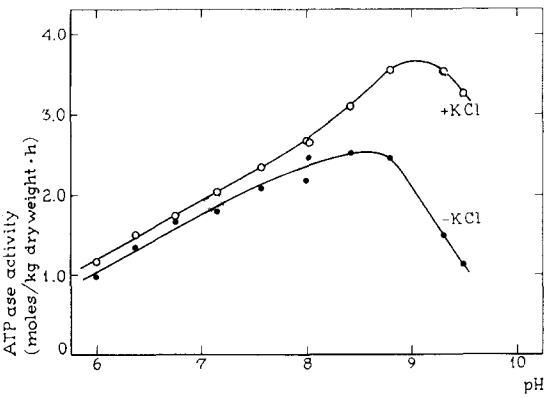
Assay media for (Na^+-K^+)-ATPase were those described previously[5] with the following differences: urea pretreatment was omitted, the Mg^{2+} concentration was raised from 1 to 2 mM, and 3 new media (F, G and H) were added. Composition of the media in mmoles/l final concentration was therefore as follows: Medium A: Tris–HCl (pH 7.5), 94; ATP, 2; Mg^{2+}, 2; Na^+, 60; K^+, 5; EDTA, 0.1. Medium B: the same as A, but with no K^+. Medium C: Tris–HCl (pH 7.5), 98; ATP Trissalt, 2; Mg^{2+}, 2; K^+, 5; EDTA, 0.1. Medium D: the same as A, but with 10^{-4} M ouabain present. Medium E: the same as B, but with 10^{-4} M ouabain present. Medium F: the same as C, but with 10^{-4} M ouabain present. Medium G: Tris–HCl (pH 7.5), 100; ATP, 2; EDTA, 0.1. Medium H: the same as G, but with 10^{-4} M ouabain present.

RESULTS

The enzymic reaction of the Mg^{2+}-ATPase was linearly dependent on the amount of freeze-dried bacteria added under the conditions used. There was also a linear

relationship between substrate hydrolyzed and time of incubation up to at least 1 h. Maximal substrate utilization never exceeded 40%.

The pH-activity curve is reproduced in Fig. 1. In the absence of added KCl the Mg^{2+}-ATPase had a maximum at pH 8.7. This is in agreement with the values obtained by different authors for the Mg^{2+}-ATPase in various microorganisms. In intact cells of *E. coli* a pH optimum of 8.5 was detected by GÜNTHER AND DORN[6], while ABRAMS[9] could show a pH optimum of 8.0 for a soluble ATPase obtained from



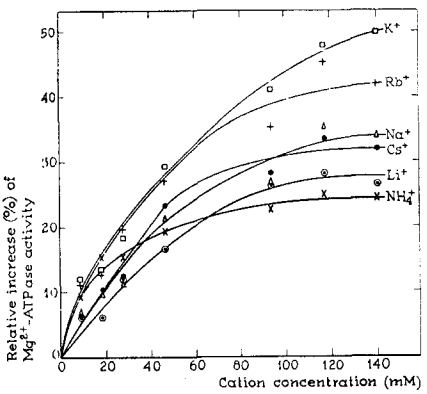


Fig. 1. Effect of pH on Mg^{2+}-ATPase activity of *E. coli* both in the presence and absence of KCl (140 mM final concentration).

Fig. 2. Effect of various monovalent cations on Mg^{2+}-ATPase activity of *E. coli*.

Streptococcus faecalis. Also in *Vibrio parahaemolyticus*[10] a pH optimum of 8.5 was observed in the presence of 2 mM Mg^{2+}. In contrast, no pH optimum was detected by WEIBULL, GREENAWALT AND LÖW[11] for ghosts of *Bacillus megaterium* or by GÜNTHER AND DORN[6] for lysed protoplasts of *E. coli*. Addition of 140 mM KCl increased the specific activity over the entire pH range measured, while a slight shift of the pH optimum from pH 8.7 to 9.1 was noticed (Fig. 1).

Fig. 2 shows that all monovalent cations, added as chlorides, enhanced the Mg^{2+}-ATPase activity, although there is a clear difference between the various cations: K^+ and Rb^+ caused the highest increase, while Li^+ and NH_4^+ caused a less pronounced effect. Choline chloride, added in the same concentrations to the incubation medium, did not cause any activation at all. The results obtained here are in contrast to those of GÜNTHER AND DORN[6], who report an optimal activating effect on the Mg^{2+}-ATPase of intact cells or lysed protoplasts of *E. coli* by 30 mM Na^+ in the absence of added K^+, while K^+ did not have any activating effect up to 100 mM. ABRAMS[9] demonstrated that Na^+ and K^+ in concentrations of 50 mM or higher increased the activity. He could not observe any difference between Na^+ and K^+ in a soluble Mg^{2+}-ATPase of *S. faecalis*, while NEUJAHR, HANSSON AND FERM[12] could hardly detect any stimulating effect of 100 mM Na^+ on the enzyme in the same microorganism. In the cell membranes of halophilic *Vibrio parahaemolyticus*[10] there was a stimulation by high concentrations of Na^+ (1.2–1.6 M) and K^+ (3.0 M) at pH 8.5

TABLE I

EFFECT OF VARIOUS ANIONS ON THE Mg^{2+}-ATPase ACTIVITY OF *E. coli*

Activity in moles ATP hydrolyzed per kg dry wt. per h. Composition of the incubation medium in mmoles per l (final concentration): Tris–HCl (pH 8.7), 200; Mg^{2+}, 2; ATP, 2; EDTA, 0.1.

Anion added (140 mM final conc.)	*Activity*	
	Na^+ salt	*K^+ salt*
no salt added	2.38	2.38
F^-	0.67	1.07
Cl^-	3.25	3.94
Br^-	3.27	3.98
I^-	2.75	3.28
NO_3^-	2.07	2.32
HCO_3^-	2.85	3.46
SO_4^{2-}	2.96	3.81
CH_3COO^-	5.62	6.15

in the presence of 2 mM Mg^{2+}. GREENAWALT, WEIBULL AND LÖW[13] found a stimulating effect of K^+ and Na^+ (0.05 M) on the Mg^{2+}-ATPase activity of the soluble fraction obtained from lysed protoplasts of *B. megaterium*. KCl was 2 to 3 times more effective than NaCl in producing this increased activity. Higher concentrations (up to 1.0 M) of these salts were inhibitory to the Mg^{2+}-ATPase of the soluble protoplasm and to the ghosts of this microorganism[13]. It appears, therefore, that monovalent cations can have quite different effects on the Mg^{2+}-ATPase activities of various bacteria.

Table I shows the influence of various K^+ and Na^+ salts on the Mg^{2+}-ATPase of *E. coli*. In every case the K^+ salt gave a higher activity than the corresponding Na^+ salt, in agreement with the greater stimulating effect of K^+ ions to Na^+ ions already presented in Fig. 2. There is a close parallelism between the values for the K^+ salts and those for Na^+ salts. In both series the acetates caused the highest enhancement of the Mg^{2+}-ATPase activity, while the nitrates and especially the

TABLE II

SUBSTRATE SPECIFICITY OF NUCLEOTIDE PHOSPHATASE ACTIVITIES OF *E. coli* BOTH IN PRESENCE AND ABSENCE OF KCl

Activity in moles substrate hydrolyzed per kg dry wt. per h. Composition of the incubation medium in mmoles per l (final concentration): Tris–HCl (pH 8.7), 200; Mg^{2+}, 2; substrate, 2; EDTA, 0.1.

Substrate	*Activity*	
	– KCl	*+ KCl (140 mM)*
ATP	2.41	3.80
ADP	0.89	1.44
AMP	0.07	0.08
GTP	1.75	1.88
CTP	1.07	1.24
UTP	0.76	0.95
ITP	1.57	1.82
TTP	0.51	0.68

fluorides were inhibitory. This shows that, besides the activation by monovalent cations, anions also have an effect on the activity of the Mg^{2+}-ATPase of *E. coli*.

The substrate specificity of this phosphatase in the presence or absence of 140 mM KCl is represented in Table II. Without the addition of KCl, ATP was the best substrate for this phosphatase activity of *E. coli*. Of the various triphosphates tested, the purine nucleotides ATP, GTP and ITP gave a higher phosphatase activity than the pyrimidine nucleotides, like CTP, UTP and TTP. When KCl (140 mM) was added to the incubation medium, an enhancement was observed with all substrates, but the greatest increase was obtained with ATP and ADP. OLIVER AND PEEL[14] have demonstrated an adenylate kinase (ATP:AMP phosphotransferase, EC 2.7.4.3) in *E. coli*, suggesting that the activity for ADP could be due to conversion of ADP to ATP and AMP and subsequent hydrolysis of the ATP thus formed. We have indeed been able to demonstrate the formation of both AMP and ATP after incubation of *E. coli* with ADP by means of column chromatography on Dowex 1-X2 (formate)[15]. The substrate specificity described here is higher than that obtained by GÜNTHER AND DORN[6], who demonstrated nearly equal activities for ATP and ADP for lysed protoplasts of *E. coli*.

The Mg^{2+}-activation curves with and without added KCl are given in Fig. 3. Without added KCl a maximum was detected at 2 mM Mg^{2+}, *i.e.*, at a Mg^{2+}:ATP ratio of 1. The same result was obtained by ROTTEM AND RAZIN[16] for mycoplasma-membranes, while NEUJAHR, HANSSON AND FERM[12] detected a Mg^{2+}:ATP ratio close to 1:2 in membranes of *S. faecalis*. Addition of 140 mM KCl shifted the optimal ratio of Mg^{2+}:ATP to 0.75. At higher Mg^{2+} concentrations the Mg^{2+}-ATPase activity decreased at a constant concentration of 2 mM ATP.

Mg^{2+} added as chloride, sulfate or nitrate did not show any difference in ATPase activity either with or without added KCl. The results of replacement of Mg^{2+} by various bivalent cations is presented in Table III. The highest activity was obtained

TABLE III

EFFECT OF VARIOUS BIVALENT CATIONS ON THE ATPase ACTIVITY OF *E. coli* BOTH IN THE PRESENCE AND ABSENCE OF KCl

Activity in moles ATP hydrolyzed per kg dry wt. per h. Composition of the incubation medium in mmoles per l (final concentration): Tris–HCl (pH 8.7), 200; bivalent cation, 2; ATP, 2; EDTA, 0.1.

Cation added (2 mM final conc.)	*Activity*	
	– KCl	*+ KCl (140 mM)*
—	0.49	0.54
Mg^{2+}	2.57	3.72
Mn^{2+}	1.97	2.75
Ca^{2+}	1.36	1.03
Cu^{2+}	0.80	0.67
Sr^{2+}	0.40	0.58
Ba^{2+}	0.58	0.71
Fe^{2+}	1.78	1.43
Co^{2+}	1.16	1.18
Ni^{2+}	0.61	0.74
Zn^{2+}	0.81	0.50

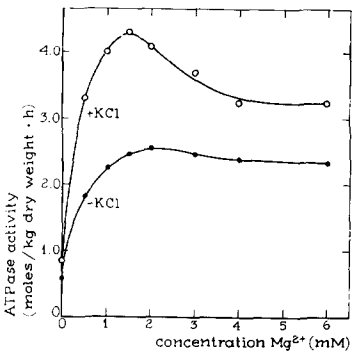


Fig. 3. Effect of Mg^{2+} concentration on Mg^{2+}-ATPase activity of *E. coli* both in the presence and absence of KCl (140 mM final concentration).

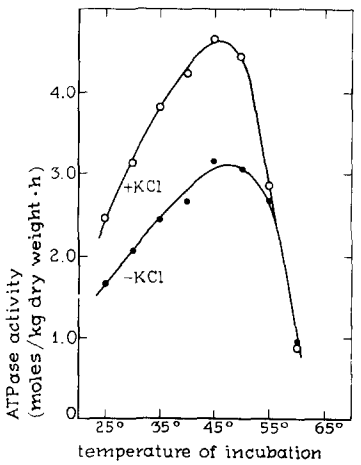


Fig. 4. Effect of temperature on Mg^{2+}-ATPase activity of *E. coli* both in the presence and absence of KCl (140 mM final concentration).

with Mg^{2+} as cofactor, while Fe^{2+} and especially Mn^{2+} gave considerable activity. Addition of 140 mM KCl increased only the Mg^{2+}- and Mn^{2+}-stimulated ATPase activity. Hence it can be concluded that Mg^{2+} is the best cofactor for this ATPase activity.

The effect of temperature on the Mg^{2+}-ATPase activity is shown in Fig. 4. The maximum activity was reached at 45° both in the presence and in the absence of 140 mM KCl. At higher temperatures the Mg^{2+}-ATPase activity with KCl fell more rapidly until at 60° KCl had no activating effect.

Table IV shows that after sonication of the bacteria for 3 min, the Mg^{2+}-ATPase activity was enhanced. It is clear that this activity is localized in the supernatant fraction obtained after centrifugation at 100 000 × *g* and not in the sediment. The activation by KCl was only detectable in the intact cells of *E. coli*. After sonication the enhancement had disappeared.

TABLE IV

Mg^{2+}-ATPase ACTIVITY OF DISINTEGRATED CELLS OF *E. coli* BOTH IN THE PRESENCE AND ABSENCE OF KCl

Activity in moles ATP hydrolyzed per kg protein per h. Composition of the incubation medium in mmoles per l (final concentration): Tris–HCl (pH 8.7), 200; Mg, 2; ATP, 2; EDTA, 0.1.

	Activity	
	− KCl	*+ KCl (140 mM)*
untreated bacteria	2.90	4.52
disintegrated bacteria	3.48	2.81
100 000 × *g* supernatant	6.75	4.96
sediment	1.91	1.65

In view of the small amount of (Na^+-K^+)-ATPase activity previously detected by us[5] in the presence of a large amount of this Mg^{2+}-ATPase activity, the question arises as to whether the former activity really represents (Na^+-K^+)-ATPase activity, especially in view of the single cation activation of the Mg^{2+}-ATPase activity. In order to settle this question the activities in the media A–H (Table V) were deter-

TABLE V

RELATIVE ATPase ACTIVITIES OF *E. coli* IN VARIOUS SUBSTRATE MEDIA

Composition of the incubation media: see text. Activities are expressed with ± S.E., and in parentheses, number of determinations. Differences between medium A and each of the other media are significant ($P < 0.001$). Differences between medium G and H and each of the other media are significant ($P < 0.001$). Differences between medium B and E, C and F, G and H are not significant ($P > 0.1$). Differences between media B, C, D, E and F are not significant ($P > 0.1$).

Code	*Incubation media*				*ATPase activities* %	
	Mg^{2+}	Na^+	K^+	*Ouabain* (10^{-4} *M*)		
A	+	+	+	−	100	(21)
B	+	+	−	−	91.5 ± 0.9	(21)
C	+	−	+	−	92.6 ± 0.8	(8)
D	+	+	+	+	93.1 ± 1.0	(21)
E	+	+	−	+	91.9 ± 0.5	(21)
F	+	−	+	+	92.0 ± 0.5	(8)
G	+	−	−	−	83.8 ± 0.7	(21)
H	+	−	−	+	83.8 ± 0.7	(21)

mined at pH 7.5. Setting the total ATPase activity in medium A at 100%, the lowest activities (83.8%) are found in media G (no Na^+, no K^+, no ouabain) and H (no Na^+, no K^+, plus ouabain, 10^{-4}M). This activity thus represents the non-stimulated Mg^{2+}-ATPase, which is not affected by ouabain. Addition of Na^+ (60 mM) alone gives some stimulation (medium B, 91.5%), which is not affected by ouabain (medium E, 91.9%). Similar behavior is found upon addition of K^+ (5 mM) alone (medium C, 92.6%; with ouabain: medium F, 92.0%). Addition of the second cation gives further stimulation (medium A, 100%), which is abolished by adding 10^{-4} M ouabain (medium D, 93.1%). This stimulation of the second cation could not be due to an additive effect of one cation upon the other, because the inhibition by ouabain would not then be observed. Hence we may conclude that this latter stimulation represents the true ouabain-sensitive (Na^+-K^+)-ATPase activity. Since there are no significant differences in the activities in media B, C, D, E and F, each of these media is suitable for the determination of the activity of this enzyme system. The best measure for its activity is the difference in the activity in medium A and the average activity in media B, C, D, E and F. In *E. coli* this amounts to 7.8% of the total ATPase activity or 0.24 mole ATP hydrolyzed per kg dry wt. per h at 37° and pH 7.5.

Removal of the anion chloride from medium A is not responsible for the difference in activities between medium A and media B, C, D, E and F, since the same results were obtained by using the bromides or sulfates instead of the chlorides. Therefore an effect of the anion in this relatively low concentration can be excluded.

This is in agreement with the observations of OPIT, POTTER AND CHARNOCK[17], who found no influence of Cl^-, Br^- and SO_4^{2-} on the (Na^+-K^+)-ATPase of some organs of the guinea pig.

DISCUSSION

In this paper the properties of a Mg^{2+}-ATPase of *E. coli* are described. This ATPase can be activated by a single monovalent cation, although anions also have some effect on the enzymic activity. The widely varying results reported for pH optimum, substrate specificity and activation by a single monovalent cation for the Mg^{2+}-ATPase in different microorganisms must be due to factors like the bacterial strain, the growth medium and the method of isolation of the membrane-bound Mg^{2+}-ATPase from the cells. The last factor is illustrated by the loss of cation activation of the Mg^{2+}-ATPase of *E. coli* upon ultrasonic treatment.

The presence of a single cation-activated Mg^{2+}-ATPase in *E. coli* raised the question of whether the (Na^+-K^+)-ATPase activity previously reported by us[5] was real. Since the Mg^{2+}-ATPase activity in the presence of Mg^{2+} alone can be activated by monovalent cations, it would seem to be hazardous to calculate the activity of the (Na^+-K^+)-ATPase from the difference in activity between media A (complete) and B (no K^+) or between media A and C (no Na^+). Careful consideration of the data in Table V, however, leads us to the conclusion that the loss in activity in media B, C, D, E and F, compared to the activity in medium A, represents the true ouabain-sensitive (Na^+-K^+)-ATPase activity. The absolute activity of 0.24 mole ATP hydrolyzed per kg dry wt. per h thus found is low relative to the Mg^{2+}-ATPase activity in *E. coli* (2.54 mole/kg dry wt./h). However, it is not very low compared to many other tissues, but the low relative activity of (Na^+-K^+)-ATPase (7.8%) and the activation of the Mg^{2+}-ATPase by a single monovalent cation complicate its determination.

It can thus be concluded that *E. coli*, strain K-12, contains a Mg^{2+}-ATPase which can be activated by a single monovalent cation and is not inhibited by ouabain, as well as the (Na^+-K^+)-ATPase system which is activated by Na^+ and K^+ together and inhibited by ouabain.

ACKNOWLEDGEMENTS

The skillful technical assistance of Miss. C. TH. M. BEUNEN is gratefully acknowledged. The authors wish to thank Mr. E. LOMMEN for determining AMP, ADP and ATP after incubation of *E. coli* with ADP.

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