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PROPERTIES OF A SOLUBLE Ca^{2+}- AND Mg^{2+}-ACTIVATED ATPase RELEASED FROM *ESCHERICHIA COLI* MEMBRANES

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SUMMARY

1. Spheroplasts of *Escherichia coli* NRC 482 were prepared by lysozyme digestion alone without the addition of EDTA. Subsequent osmotic lysis in 0.2 mM $MgCl_2$ gave less than 0.1 % survival of viable cells.

2. The spheroplast membranes from the lysate were put through a washing procedure similar to that used by Abrams[1]. During the 5th washing a Ca^{2+}- and Mg^{2+}-activated ATPase (ATP phosphohydrolase, EC 3.6.1.4) was released.

3. On gel filtration through Sepharose 6B the enzyme eluted as a sharp peak corresponding to a molecular weight of 365 000–390 000.

4. The ATPase activity was dependent on the addition of a divalent metal ion. With Ca^{2+} and Mg^{2+} the pH optimum was at 9.5 and the optimum ratio of ion to substrate was less than 1. The Ca^{2+}- and Mg^{2+}-dependent activities appeared to be catalysed by the same enzyme.

5. In the presence of Ca^{2+} the enzyme was specific for ATP but with Mg^{2+} there was some hydrolysis of ADP.

6. There was no stimulation of ATPase activity by Na^+ or K^+ or by a mixture of the two ions.

7. The enzyme was cold labile at 0° but the addition of glycerol (20 % v/v) overcame this instability.

8. The Arrhenius plot for the ATPase reaction was linear over the range 10–36° and from this an activation energy of 20.7 kcal/mole was determined.

INTRODUCTION

Many workers have reported the presence of a Mg^{2+}-activated ATPase activity associated with the membranes of both Gram-positive[2–4] and Gram-negative bacteria[5,6]. In some Gram-positive strains the enzyme has been released from the membranes by washing with Mg^{2+}-free buffer[1,7]. Mg^{2+} may play an important role in binding the ATPase to the membrane. Thus successive washings are done to deplete the magnesium level of the membrane until there comes a point at which the ATPase is specifically released in a soluble form. This procedure has been used in the isolation of ATPases from Gram-positive cells in a homogeneous state[8].

Gram-negative cells are more resistant to lysozyme digestion[9]. In *Escherichia coli* the formation of lysozyme spheroplasts usually requires pretreatment of the cells with a chelating agent prior to the addition of lysozyme. If, in this organism, the ATPase is bound to the membrane by Mg^{2+} then the use of chelating agents is undesirable if the membrane-bound enzyme is to be isolated initially.

With *E. coli* NRC 482 we have avoided the use of chelating agents in the preparation of spheroplast membranes. We have subsequently solubilized from these membranes by repeated washings a Ca^{2+}- and Mg^{2+}-activated ATPase which has properties similar to the membrane-bound enzyme. The partial purification and properties of the soluble enzyme are described in this paper.

MATERIALS AND METHODS

Reagents

All chemicals were of reagent-grade purity. Crystalline bovine serum albumin, yeast hexokinase, yeast alcohol dehydrogenase, yeast glucose 6-phosphate dehydrogenase, and equine hemoglobin were obtained from Calbiochem. Porcine thyroglobulin and equine apoferritin were products of Mann Research Laboratories. Glucose 6-phosphate was obtained from Boehringer, Sepharose 6B from Pharmacia, and deoxyribonuclease from Nutritional Biochemicals Corporation. Egg-white lysozyme was obtained from both Worthington Biochemical Corporation and Calbiochem. Coenzymes were from Calbiochem with the exception of the Tris salt of ATP which came from Sigma Chemical Company. ADP was freed from traces of ATP by chromatography on DEAE-cellulose with an NH_4HCO_3 gradient.

Assay procedures

Protein concentrations were determined by the method of Lowry *et al.*[10] with crystalline bovine serum albumin as a standard. ATPase (1–5 μg protein) was routinely assayed in 0.5 ml of a medium made 100 mM in Tris–HCl (pH 9.0), 5 mM in ATP (disodium salt) and, either $MgCl_2$ or $CaCl_2$ (concentration given in legends). The reaction was stopped by the addition of 0.25 ml of 10 % (v/v) trichloroacetic acid after 30 or 60 min of incubation at 37°. If there was noticeable turbidity at this point then the solutions were centrifuged at 15000 $\times$ g for 10 min. Aliquots of the trichloroacetic acid-treated assay medium were assayed for inorganic phosphate as described by Ames[11]. The amount of enzyme assayed was adjusted such that no more than 10 % of the substrate was hydrolyzed by the end of the incubation. Corrections were made for endogenous inorganic phosphate. One unit of enzyme activity is defined as the amount of enzyme which liberates 1 μmole of phosphate per min.

NADH-menadione reductase was assayed as described by Bragg and Hou[12], and catalase as described by Beers and Sizer[13]. Methods used to assay for glucose 6-phosphate dehydrogenase, hexokinase and alcohol dehydrogenase were taken from Bergmeyer's manual[14].

Variations in the ATPase assay

When the effects of monovalent cations on the ATPase were tested the Tris salt of ATP was used instead of the disodium salt. When the optimum ion to sub-

strate ratio was investigated the concentration of substrate was held at 5 mM while the Mg^{2+} and Ca^{2+} concentrations were varied from 0 to 10 mM. For this experiment the enzyme was dialysed against 5 mM Tris–HCl (pH 7.4) to remove the 0.2 mM EDTA which was present in the enzyme fractions from the Sepharose 6B column and which might have altered the effective divalent ion concentrations.

For studies on the pH optimum of the enzyme the Tris–HCl buffer (pH 9.0) was replaced by 0.1 M Tris–acetate buffers of pH values ranging from 4.5 to 9.0. Solutions of pH 9.5 and 10.0 were obtained by using 0.1 M glycine adjusted to the required pH with NaOH.

For studies on the substrate specificity of the enzyme, ATP was replaced by other substrates at the same concentration of 5 mM.

Cold lability study on the soluble enzyme

Four enzyme solutions were prepared. Two were kept in 20 % glycerol by the addition of 1 vol. of glycerol to 4 vol. of the pooled enzyme-containing fractions from Sepharose 6B. In the other two preparations distilled water replaced the glycerol to act as a control. One control tube and one with added glycerol were kept at 0°, and the other two were kept between 22–24°. At time intervals samples were removed from all four tubes and assayed for Mg^{2+}- and Ca^{2+}-activated ATPase activities.

ADP inhibition study

The standard assay mixture contained added ADP (Na salt) ranging from 0 to 8 mM. No corrections were made for the presence of small amounts of ADP (about 2 %) in the ATP substrate.

Organism and growth conditions

E. coli (strain 482 of the culture collection of the National Research Council of Canada) was grown with vigorous aeration at 37° on minimal salts medium[15] in 3-l cultures. The cells were grown from a 10 % (v/v) inoculum to the late logarithmic stage of growth (about 4 h) before harvesting at 2° by centrifuging the culture for 10 min at 6000 × *g*. The cells were resuspended in 0.9 % NaCl (1 g wet wt. per 20 ml) at 0° and the suspension centrifuged at 6000 × *g* under the same conditions to give washed cells.

Preparation and lysis of spheroplasts

The washed cells were suspended at 22° to a concentration of 25 mg/ml in 20 % sucrose solution made 30 mM in Tris–HCl (pH 8.0). Lysozyme (5 mg/g of cells) was then added as a solution in a few drops of 0.05 % NaCl and the suspension was stirred gently. After 30 min at 22° the suspension was diluted 10-fold into 0.2 mM $MgCl_2$ which contained a small amount of deoxyribonuclease.

The resulting lysate was stirred rapidly and the decrease in turbidity was followed at 420 nm. After 20–40 min when the absorbance at 420 nm had reached a constant, minimum value the lysate was centrifuged at 15000 × *g* for 30 min at 18° to recover the spheroplast membranes as an opalescent, reddish-brown pellet.

Investigation of the extent of lysis

During osmotic lysis of the spheroplasts the lysate was sampled at timed intervals and immediately filtered at 22° through a Millipore apparatus containing a filter of pore size 0.65 μm and a fibreglass prefilter disc. The filtrates were kept at 0°. Part of the lysate (30 ml) was disrupted for 10 min in an ice-cooled 20-kHz Bronwill sonic oscillator. The filtrates and the sonicate (not filtered) were assayed for two soluble enzymes, catalase and NADH–menadione reductase. The level of each enzyme in the filtrate was compared to the value for the sonicate and was used as an approximate indicator of lysis.

After 40 min a sample of the lysate was removed for determination of the number of viable organisms. The viability counts were done by the pour plate method[16]. Dilutions were made with 25 % Ringer's solution. A sample of the cell suspension in 20 % sucrose made 30 mM in Tris–HCl (pH 8.0) was, prior to the addition of lysozyme, treated in the same fashion for comparison.

Release of ATPase from membranes

The spheroplast membranes (170 mg protein) from 10 g of cells were suspended in 250 ml of 10 mM Tris–HCl buffer (pH 7.4) containing 0.5 mM $MgCl_2$ using a glass homogeniser with a teflon plunger. They were then sedimented at 23700 × *g* for 30 min and the resulting supernatant was set aside. This same procedure was followed through 5 more washings. The resuspension media were 2 M LiCl in 50 mM Tris–HCl (pH 7.4) for the second and third washings, 50 mM Tris–HCl (pH 7.4) for the fourth, and 1 mM Tris–HCl (pH 7.4) for the fifth and sixth washings. The membrane pellet which remained after the sixth washing was resuspended in 250 ml of 1 mM Tris–HCl (pH 7.4). All these and previous manipulations were performed at 0–4°. However, subsequent steps and storage of samples were done at 22–24°.

Gel filtration of ATPase

The supernatant from the fifth washing of the membranes was concentrated to 1.5–1.9 ml by ultrafiltration using a PM 10 ultrafilter in model 402 and 8MC cells (Amicon Corporation).

In order to mark the total volume (V_t) of the column 0.1 ml of 0.2 M phosphate buffer (pH 7.1) was added to the sample and its volume was then made up to 2 ml with water. The sample was applied to a column of Sepharose 6B (2.5 cm × 38 cm). A buffer containing 0.2 mM disodium EDTA in 50 mM Tris–HCl (pH 7.4) was used for equilibration and elution of the column. 1.7-ml fractions were collected at a flow rate of 30 ml/h. The region of peak ATPase activity was pooled as shown in Fig. 2 and this fraction was used as the soluble ATPase in the subsequent experiments. Calibration of the column was carried out at room temperature, also, with the eluting medium as previously described. Yeast hexokinase (mol. wt. 102000), alcohol dehydrogenase (mol. wt. 150000), and glucose 6-phosphate dehydrogenase (mol. wt. 257000), equine apoferritin (mol. wt. 480000), equine hemoglobin (mol. wt. 64500), and porcine thyroglobulin (mol. wt. 670000) were used as molecular weight markers. The elution positions of the last three proteins were measured by their absorbance (apoferritin and thyroglobulin at 230 nm and hemoglobin at 415 nm) while the remaining proteins were estimated by their enzyme activities.

RESULTS

Fragility of the cell envelope of E. coli NRC 482

In contrast to wild type *E. coli* B (ATCC 11303), *E. coli* NRC 482 was completely converted to osmotically sensitive spheroplasts with lysozyme (5 mg/g wet wt. of cells) in the absence of EDTA. Furthermore lysis could be carried out in the presence of 0.2 mM $MgCl_2$ without affecting the extent of lysis. That the fall in turbidity during lysis measured the extent of cell breakage is shown in Fig. 1 where the release of the cytoplasmic enzymes catalase and NADH–menadione reductase paralleled the fall in absorbance of the system. When the turbidity was at a constant, minimum value cell survival was only 0.04 % of that measured before lysozyme digestion. The rate and extent of lysis were both decreased if the $MgCl_2$ concentration in the lysing medium was above 0.2 mM. When $MgCl_2$ was omitted complete lysis was rapid.

Release of ATPase from the spheroplast membranes

As has been observed with membranes from Gram-positive cells ATPase activity can be released by washing the membranes with Mg^{2+}-free buffers. There was a specific release of Ca^{2+}- and Mg^{2+}-activated ATPase during the 5th washing step (Table I). The 3-fold increase in specific activity and the percentage re-

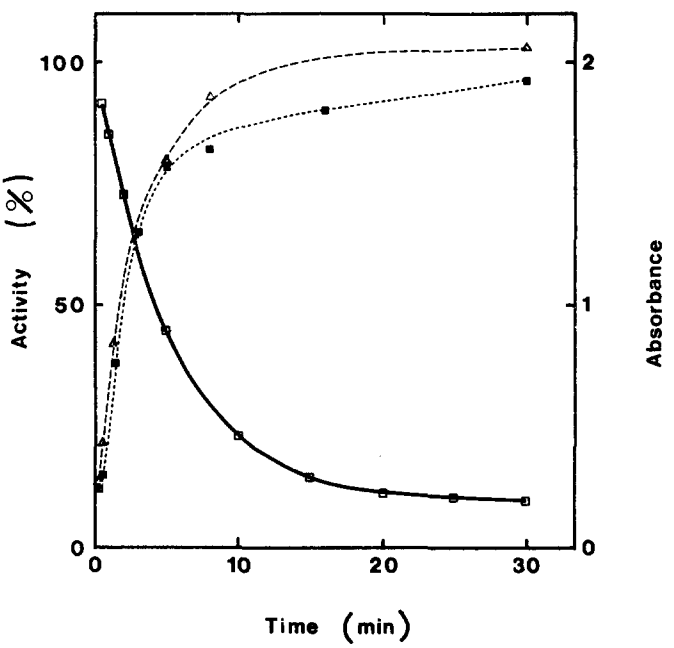

Fig. 1. Decrease in absorbance and release of soluble enzymes during lysis of *E. coli* spheroplasts. The preparation and lysis of spheroplasts is described in Materials and Methods. Time was measured from the moment of dilution in 0.2 mM $MgCl_2$. Absorbance (solid line) was measured at 420 nm. Activity of catalase (open points on broken line) and NADH-menadione reductase (solid points on broken line) are both expressed relative to the activity of the sonicated lysate (specific activity at 22°: catalase, 156; menadione reductase, 4.76). The protein concentration of the whole lysate was 0.248 mg protein/ml. After filtration 89 % of the protein occurred in the filtrate.

lease of enzyme (20 %) were somewhat lower than the values obtained for the release of ATPase from Gram-positive cells[1,7]. Most of the enzyme (66 %) remained bound to the membranes and could not be released by the washing procedure. It had similar properties to that which was released.

TABLE I

DISTRIBUTION OF ATPase DURING WASHING OF *E. coli* SPHEROPLAST MEMBRANES

The experimental procedure is described in Materials and Methods. All resuspensions were done to a volume of 250 ml at pH 7.4. ATPase was measured in the presence of 5 mM $CaCl_2$. These results represent the average of two experiments starting with spheroplast membranes (170 mg protein) from 10 g of freshly grown *E. coli* NRC 482.

Washing step	*Fraction*	*Total protein (mg)*	*Total activity (units)*	*Specific activity* $\times 10^{-2}$ *(units/mg protein)*
	Initial membrane suspension in 10 mM Tris–HCl–0.5 mM $MgCl_2$	170	17.8	10.5
1	Supernatant from the initial membrane suspension	7.8	1.51	19.4
2	Supernatant from second membrane suspension in 2 M LiCl–50 mM Tris–HCl	9.4	0.04	0.4
3	Supernatant from third membrane suspension in 2 M LiCl–50 mM Tris–HCl	6.6	0.02	0.3
4	Supernatant from fourth membrane suspension in 50 mM Tris–HCl	6.4	0.14	2.2
5	Supernatant from fifth membrane suspension in 1 mM Tris–HCl	11.7	3.36	28.7
6	Supernatant from sixth membrane suspension in 1 mM Tris–HCl	7.7	1.12	14.1
	Final membrane suspension in 1 mM Tris–HCl	121	11.8	9.8
	Recovery	170.6	18.0	

The ATPase activities of supernatants from washing steps 2 and 3 increased by up to 100 % on removal of LiCl by dialysis. Li^+ is a mild inhibitor of the ATPase but washing with LiCl removes contaminating lysozyme[1].

Gel filtration of soluble ATPase

The Ca^{2+}- and Mg^{2+}-activated ATPase activity eluted as a single peak from Sepharose 6B (Fig. 2). The small amount of ATPase activity in the turbid, void fractions may be membrane bound. The fractions covered by the bar in Fig. 2 were pooled and used for further experiments. When the column was run at room temperature there was 100 % recovery of ATPase activity. This step gave a 20-fold purification of the enzyme and showed that the ATPase was indeed soluble. Specific activities of up to 10.6 units/mg of protein were obtained with this fraction in the presence of 2 mM $MgCl_2$. The purification procedure is summarized in Table II.

Two separate experiments with independently calibrated columns gave molecular weight values of 390000 and 365000 for the ATPase provided that the enzyme is a spherical molecule.

Influence of pH on soluble ATPase activity

The variation of Ca^{2+}- and Mg^{2+}-activated ATPase activity was similar to that of the membrane-bound enzyme[6] with optimal activity between pH 8.5 and 9.5 in the presence of 5 mM divalent cation.

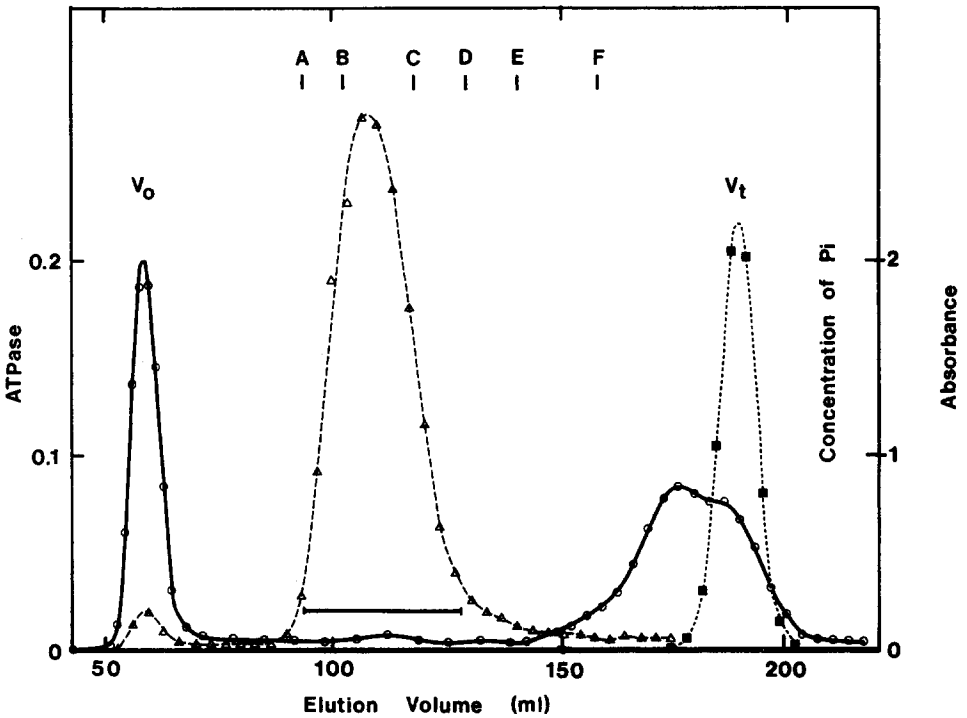

Fig. 2. Elution pattern from Sepharose 6B column of the supernatant from 5th washing of *E. coli* spheroplast membranes. Chromatography was done as described in Materials and Methods. Absorbance (○—○) was measured at 280 nm. Ca^{2+}-activated ATPase activity measured in the presence of 5 mM Ca^{2+} (△---△) is expressed as units/ml fraction, and concentration of phosphate (■---■) as μmoles/ml fraction. Markers A–F indicate elution positions of thyroglobulin, apoferritin, glucose 6-phosphate dehydrogenase, alcohol dehydrogenase, hexokinase and hemoglobin, respectively. Fractions covered by the bar constitute the pooled enzyme used in later experiments.

TABLE II

PARTIAL PURIFICATION OF SOLUBLE ATPase OF *E. coli*

ATPase activity was measured in the presence of 5 mM $CaCl_2$. These results are the average of two experiments starting with spheroplast membranes (170 mg protein) from 10 g of freshly grown *E. coli* NRC 482.

Fraction	*Total ATPase activity (units)*	*Total protein (mg)*	*Specific activity (units/mg protein)*	*Purification*	*Recovery (%)*
Initial membrane suspension	17.8	170	0.105	1	100
Supernatant from fifth washing step	3.36	11.7	0.287	2.75	19
Pooled fractions from Sepharose 6B	3.82	0.66	5.80	55	21

Optimum divalent cation: substrate ratios for soluble ATPase activity

In the absence of divalent metal ions there was essentially no ATPase activity. The optimum ion to substrate ratio for Mg^{2+}-activated ATPase activity was 0.4:1 while for Ca^{2+}-activated ATPase activity it was 0.66:1. As with the membrane-bound enzyme[6] higher ratios of metal ion to ATP result in inhibition.

Substrate specificity of soluble ATPase

There was no detectable hydrolysis of AMP with either of the divalent ions: thus, it is unlikely that 5'-nucleotidase and non-specific phosphatases were present in the preparation. Hydrolysis of ADP occurred only in the presence of Mg^{2+} to the extent of 19 % of the value for ATP hydrolysis (Table III). Since hydrolysis of ADP did not occur with Ca^{2+} this effect may have been due to a contaminating activity like polynucleotide phosphorylase or adenylate kinase in conjunction with the ATPase.

Of the other substrates tested dATP was by far the most active especially with Mg^{2+}.

The purine nucleoside triphosphates, ITP and GTP, were more readily attacked than the pyrimidine nucleoside triphosphates UTP and CTP. With each of these substrates, as with the adenosine derivatives, Ca^{2+} gave a higher degree of specificity than Mg^{2+}.

Effect of monovalent cations on soluble ATPase

At a concentration of 50 mM, Li^+, Na^+, K^+, Cs^+, and Na^+ *plus* K^+ inhibited the enzyme between 10 and 30 %. With these ions there was no differential effect between the Ca^{2+}-activated and the Mg^{2+}-activated ATPase activities. However, NH_4^+ gave more inhibition with Ca^{2+} (55 %) than with Mg^{2+} (26 %). We could not detect any $(Na^+ + K^+)$-activated ATPase similar to that described by Hafkenscheid and Bonting[17,18] for lyophilized cells tested in the presence of urea.

Inhibition of soluble ATPase by ADP

ADP inhibited the Ca^{2+}-activated ATPase. In the presence of 5 mM Ca^{2+}, 0.8, 2, 4 and 8 mM ADP gave 17, 36, 63 and 86 % inhibition, respectively. The effect of ADP on the Mg^{2+}-activated ATPase was not investigated since ADP was attacked in the presence of Mg^{2+} (Table III).

Cold lability of soluble ATPase

The soluble ATPase was very labile when stored at 0°. The half-life of its activity was 4 h at this temperature compared to a half-life of 6 days for the membrane-bound enzyme. The soluble enzyme was much more stable at 22–24° and less than 40 % of the activity was lost after 80 h. In the presence of 20 % glycerol there was no apparent loss of ATPase activity under these conditions. Glycerol (20 %) also stabilized the enzyme kept at 0°. There was no significant difference in the behaviour of the Ca^{2+}- and Mg^{2+}-activated enzymes.

Activation energy of reaction catalysed by soluble ATPase

The soluble enzyme was heat labile and lost 50 % of its activity in a 7-min preincubation at 55°.

Sweetman and Griffiths[19] observed that in the reaction catalysed by a membrane-bound ATPase of *E. coli* there was a sharp break at 18.8° in the Arrhenius plot. This transition temperature is thought to indicate the point at which the lipid of the membrane undergoes a phase change[20]. If the solubilized enzyme contained any lipid it might well show such a break in the Arrhenius plot, although this might also be due to a conformational change in the enzyme protein. The effect of temperature on the ATPase reaction was examined over the range of 4–36° and as can be seen in Fig. 3 all the points fell close to a straight line with

TABLE III

SUBSTRATE SPECIFICITY OF SOLUBLE ATPase OF *E. coli*

The enzyme tested was the pooled enzyme-containing fractions from Sepharose 6B. Enzyme activity is expressed as a percentage relative to the amount of hydrolysis of ATP. Specific activities of the ATPase were 4.57 units/mg protein in the presence of Mg^{2+} and 3.19 units/mg protein with Ca^{2+}. The concentrations of $MgCl_2$ and $CaCl_2$ were 2 mM and 5 mM, respectively. Substrate concentration was 5 mM in each assay. Each assay contained 2.2 μg protein. These results represent the average of two experiments.

Substrate	*Activity in the presence of Ca^{2+}* (%)	*Activity in the presence of Mg^{2+}* (%)
ATP	100	100
dATP	55	131
GTP	22	62
ITP	26	41
UTP	9	27
CTP	1	19
ADP	0	19
AMP	0	0

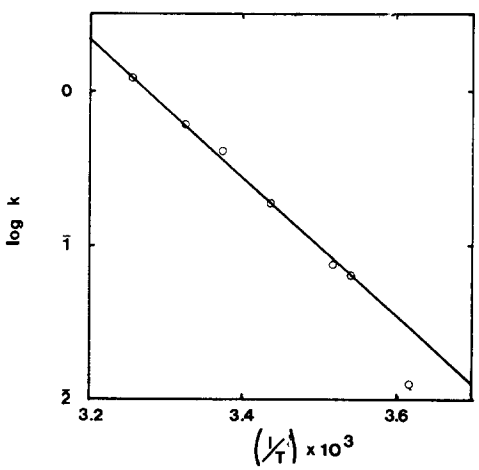

Fig. 3. Arrhenius plot for hydrolysis of ATP by soluble Ca^{2+}-activated ATPase of *E. coli*. The enzyme tested was the pooled enzyme-containing fractions from Sepharose 6B. Each assay mixture contained 4.4 μg protein and 5 mM Ca^{2+}. k, rate of hydrolysis of ATP (μmoles/h); T, incubation temperature (°K). The incubation time was extended beyond 60 min for assays done at low temperature so that the extent of hydrolysis of ATP was approximately the same at each temperature.

no indication of a transition point. The anomalous activity at 4.3° may have been due to the cold lability of the enzyme. The activation energy for the reaction was 20.7 kcal/mole.

DISCUSSION

Mg^{2+}- and Ca^{2+}-activated ATPase was released by use of the washing procedure described in Materials and Methods from spheroplast membranes of *E. coli* NRC 482 prepared in the presence of 0.2 mM $MgCl_2$. $MgCl_2$ was included to optimize binding of the ATPase to the membrane. Subsequent washing with 2 M LiCl was used to remove other proteins and did not cause release of ATPase from the membrane. ATPase could then be liberated by lowering the concentration of salt in the absence of $MgCl_2$. This result is similar to that found with the Gram-positive organisms *Streptococcus faecalis*[1] and *Micrococcus lysodeikticus*[7]. Only a third of the enzyme was released from the *E. coli* spheroplast membranes by the washing technique. Since the membrane-bound enzyme has similar properties to the soluble enzyme this may be due to the remainder of the ATPase being less accessible to washing. However, subsequent experiments have shown that at least 65 % of the Ca^{2+}-activated ATPase can be released from the membranes by dialysis against 0.5 mM EDTA at pH 9.0. This result is in accordance with that obtained by Neujahr[21] for membranes of *Lactobacillus fermenti*. We have not used this latter method in the present report since it yields a less-pure preparation.

The soluble ATPase of *E. coli* NRC 482 has many properties in common with the enzymes of the Gram-positive bacteria besides cellular location on the cell membrane and mode of release. All the enzymes are activated by Mg^{2+}, and most of them by Ca^{2+} and other divalent cations. The pH optimum is usually in the alkaline region although for *Bacillus megaterium*[22] it varies widely with the ion to substrate ratio. Where data is available as in *Bacillus sterothermophilus*[23] and *M. lysodeikticus*[24], the optimum ion to substrate ratio is less than unity in agreement with our findings. All the enzymes give little or no hydrolysis of AMP and ADP. With regard to nucleoside triphosphate substrates our enzyme follows the general observation that the comparative rates of hydrolysis are ATP > GTP > UTP > CTP. As with the *E. coli* enzyme ADP is found to be an inhibitor of the ATPase reaction wherever this has been examined. The soluble ATPases of *B. sterothermophilus*[23] and *S. faecalis*[25] are large molecules with molecular weights of 280000 and 385000 respectively compared to 365000–390000 for the *E. coli* enzyme. The enzyme from *M. lysodeikticus*[24] has a sedimentation value of 14–15 S and so is also in this range.

Both Ca^{2+}- and Mg^{2+}-activated ATPases of *E. coli* NRC 482 show similar properties. This is good evidence for the two activities being catalysed by the same enzyme. For example both activities were released from the membrane at the same stage and co-chromatographed on Sepharose 6B, and had the same pH optimum. Their optimum ion to substrate ratios, their substrate specificity, the effects of monovalent cations on the two activities, and their cold lability properties were also very similar. Furthermore the activities were not additive.

The properties of our soluble enzyme are also in accord with those of the membrane-bound ATPase of *E. coli* B as described by Evans[6]. Evans[26] has also

described an ATPase solubilized by detergents from the spheroplast membranes of *E. coli* B. Since he did not report on a number of the properties of the solubilized enzyme which he previously described for the membrane-bound enzyme, it is difficult to compare the two solubilized enzymes from *E. coli*. However, there is a marked difference in the molecular weights of the two preparations. The *E. coli* B enzyme was found to have a molecular weight of 100000 by gel filtration. The smaller size of this enzyme compared to that of *E. coli* NRC 482 is most likely due to it having been treated with sodium lauryl sulphate. The detergent may have caused disaggregation of a trimer or tetramer form of the enzyme, or it may have stripped off other associated proteins which are not needed for catalytic activity and which might be present in our enzyme. It does not seem likely that removal of lipid accounts for the difference in molecular weight since the Arrhenius plot of our enzyme reaction gives no indication that lipid is required for activity. Moreover Munoz *et al.*[24] concluded that lipid does not play a major role in the enzymatic properties of the soluble ATPase of *M. lysodeikticus* and no lipid content was reported for the enzymes of *B. sterothermophilus*[23] or *S. faecalis*[25] for which amino acid compositions have been determined.

The soluble ATPase of *E. coli* NRC 482 is cold labile whereas that of the membrane is relatively stable. This behaviour has been observed with the ATPases of *B. megaterium*[4,22] and *S. faecalis*[1] as well as *E. coli* B and is often used to compare these enzymes with the mitochondrial ATPase. Another difference between the soluble and the membrane-bound enzyme is that the activity of the latter shows a transition temperature[19] which can be attributed to changes in the orientation of the lipid in the membrane[20].

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