A K+ Transport ATPase in Escherichia coli*

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Wolfgang Epstein, Virginia Whitelaw, and Joanne Hesse

From the Departments of Biochemistry, and of Biophysics and Theoretical Biology, The University of Chicago, Chicago, Illinois 60637

SUMMARY

A K+ -stimulated ATPase in membranes of Escherichia coli has been identified as an activity of the Kdp system, an ATP-driven K+ transport system. Three characteristics support association of the ATPase with the Kdp system: (i) ATPase and Kdp transport are both repressed by growth in media containing high concentrations of K+; (ii) the ATPase and Kdp system accept only K+ as substrate, neither requires Na+ nor accepts Rb+ as a substrate; (iii) the affinity of the ATPase and that of the Kdp system for K+ is similar and is altered by mutations in the structural genes of the Kdp system. Discovery of an ATPase associated with a bacterial transport system suggests functional similarities with the ATP-driven transport systems of animal cells.

Escherichia coli has four transport systems that can accumulate K+ (1). One of these, the high affinity ($K_m = 2 \mu M$) Kdp system (1), is dependent on ATP (2) and is under the genetic control of the positive regulator coded by the kdpD gene (3). The three structural proteins of the system, coded by the three genes of the kdpABC operon, are inner membrane proteins (4). All previously described ATP-driven transport systems in E. coli have a soluble, periplasmic “binding” protein in addition to membrane protein components (5–8). The unique composition of the Kdp system with only membrane proteins suggested that this system would have ATPase activity in membranes, as is observed for ATP-driven transport systems of animal cells (for reviews see Refs. 9 to 11). We here report a K+ -stimulated membrane ATPase whose properties identify it as due to the Kdp transport system.

MATERIALS AND METHODS

Bacterial Strains and Growth—Strain TK2240 (F- trkD1 trkA405 nagA lacZ rha thi) is wild type for the Kdp system; strains TK2219 (kdp-19) and TK2242 (kdp-42) are UV-induced mutants of strain TK2240 in which the Kdp system has a reduced affinity for K+. In strains such as TK2240 which lack the other two saturable K+ transport systems, TrkA and TrkD, growth is dependent on the Kdp system at K+ concentrations below 10 mM. The only other system present, TrkF, has a very low rate of transport proportional to external K+ concentration (1). When the wild type Kdp system is present, growth rate is independent of K+ concentration to well below 10 $\mu$M. We obtained mutants which had a functional Kdp system but with reduced affinity for K+ by penicillin selection against cells growing rapidly in 30 mM K+ medium, and requiring the cells to grow in medium containing 5 mM K+ medium. For the experiments presented here, homologous kdp diploid derivatives, called TK2219-19 and TK2242-42, carrying an F-109 episome were used. These strains express the Kdp system at a higher level due to a gene dosage effect (1). Strains were grown at 30°C in the phosphate-buffered media previously described (12) containing glucose, 2 g/liter, as carbon and energy source. The Kdp system in strains TK2219-19 and TK2242-42 was derepressed by growth in medium containing 0.35 and 0.2 mM K+, respectively. At these K+ concentrations, the mutants grow at about one-half the rate achieved at K+ concentrations of 5 mM and higher. The Kdp system was repressed by growth in medium containing 115 mM K+.

Preparation of Membranes—All steps were performed at 0 to 4°C. After collection by centrifugation, cells were washed once and suspended at approximately 5 mg dry weight/ml in 30 mM Tris-Cl (pH 8). In Experiment A of Table I, cells were disrupted by passage through a French pressure cell. In other experiments, the cell suspension was made 0.3 M in sucrose, 10 mM in EDTA, 0.1 mg/ml in lysozyme, and sonicated after incubation on ice for 30 min. The sonic lysate was diluted with an equal volume of 30 mM Na,EDTA (pH 7). Unbroken cells were removed by centrifugation at 2000 $\times$ g max for 10 min, and then membranes were pelleted by centrifugation at 110,000 $\times$ g max for 90 min. In most experiments, membranes were stripped of the (Ca++, Mg++)-stimulated ATPase by addition of urea to 6 M before pelleting (13). Membranes were stored at 0–4°C in 50 mM Tris-Cl (pH 7.5).

Assays—ATPase assays contained Tris-Cl (pH 7.5), 50 mM; MgCl2, 1.6 mM; Na,ATP, 1.6 mM; cations as indicated added as chloride salts; and membranes, from 30 to 500 $\mu$g of protein/ml. Reactions were performed at 37°C and initiated by the addition of ATP. The reaction was terminated by dilution with cold 5% perchloric acid. When determined colorimetrically, Pi liberated was determined as described by Chen et al. (14). In the radioactive assay, about 100,000 cpm/ml of [y-32P]ATP was added, and Pi liberated was measured as the increase in radioactivity not absorbed by charcoal. Radiolabeled ATP, kindly provided by Dr. C. Peebles, was repurified from charcoal as needed to reduce contamination with radioactive P3. Assays were performed under conditions that activity was proportional to incubation time and amount of membranes added. Activity is expressed in terms of protein measured by the Folin-phenol method with bovine serum albumin as standard (16).

Net K+ uptake was measured at 30°C as previously described in cells that had been depleted of K+ by treatment with 10 mM dinitrophenol (1). Transport rates are expressed in terms of cell dry weight, obtained from measurements of cell turbidity and a calibration curve.

RESULTS

A transport ATPase which retains normal coupling in membrane fragments should have substrate-dependent ATPase activity. The very high affinity of the Kdp system for K+ makes such a demonstration difficult, since most preparations of membranes and buffers are sufficiently contaminated with K+ that a concentration well above the 2 $\mu$M $K_m$ of the Kdp system is achieved without added K+. We therefore set out to isolate mutants of the Kdp system with a lower affinity for K+. These mutants whose isolation is summarized above will be described in a subsequent publication. We have chosen strains carrying the kdp-19 and kdp-42 mutations to examine membrane ATPase activity. Preliminary mapping indicates that the two mutations are in the kdp gene cluster and very closely linked to a kdpA point mutation, suggesting that they are in the kdpA gene coding for the 47,000-dalton peptide of the Kdp system.

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Kdp system (4). In both strains, good derepression of the Kdp system is achieved by growth in media of suitably low K⁺ concentration, and the system is fully repressed by growth in medium containing 115 mM K⁺.

Membranes prepared from derepressed diploid strain TK2242-42 exhibit ATPase activity that is stimulated by K⁺ (Experiment A in Table I). Much of the activity in these membranes is due to the (Ca²⁺, Mg²⁺)-stimulated ATPase associated with oxidative phosphorylation. Addition of dicyclohexylcarbodiimide, a good inhibitor of this ATPase, reduced activity in the presence of Na⁺ more than in the presence of K⁺. When the (Ca²⁺, Mg²⁺)-stimulated ATPase is removed by treatment with urea, there is a further increase in K⁺-stimulated ATPase which now is insensitive to dicyclohexylcarbodiimide. These results allow us to conclude that these membranes have a K⁺-stimulated ATPase which is not due to the (Ca²⁺, Mg²⁺)-stimulated ATPase, is not inhibited by dicyclohexylcarbodiimide, and appears to be stimulated or unmasked by treatment with urea.

Our identification of this ATPase as an activity of the Kdp system is based on the kinetic analysis in the two mutant strains. Strains TK2242-42 and TK2219-19 have ATPase with markedly different affinity for K⁺. The Kₘ for K⁺ of the former is about 3 mM; for the latter about 0.4 mM (Fig. 1, A and C). K⁺ transport by the Kdp system in each strain exhibits a similar affinity for K⁺, the Kₘ being about 6 mM for the former and 0.3 mM for the latter. The marked similarity in the Kₘ values for these two processes in each strain, and the marked differences between strains due to the particular kdp mutation present, provides genetic proof that both are controlled by the kdp genes. We conclude that this ATPase is an activity of the Kdp system.

We examined two other properties of the ATPase to confirm that it is consistent with an activity of the Kdp system. (i) The Kdp system is repressible by growth in media containing high concentrations of K⁺ (1). As shown in Experiments B and C of Table I, cells grown under repressing conditions have very low ATPase activity which is not stimulated by K⁺. (ii) The Kdp system does not require Na⁺ (3), and is unique in having a very narrow specificity that does not include Rb⁺ or Cs⁺ (16). The ATPase (Table II) has the same properties; it is stimulated only by K⁺. Neither Na⁺ nor Rb⁺ stimulates when added without K⁺, nor do they stimulate or inhibit when added with K⁺. No other cation tested stimulated ATPase. All assays of Table II contained about 4 mM Na⁺ added as Na₂ATP plus contaminating Na⁺ in other solutions. Exper-

### TABLE I

**K⁺ stimulation of membrane ATPase**

Membranes obtained by French press disruption of derepressed strain TK2242-42 (Experiment A) or by sonication of repressed cells of the same strain (Experiments B and C) were pelleted in Tris-Cl buffer (Control) or in Tris-Cl buffer containing 6 M urea (Urea-extracted). Where indicated, membranes were treated with 0.2 mM dicyclohexylcarbodiimide (DCCD) at 37°C for 15 min prior to assay. In Experiments A and B, the colorimetric procedure was used; in Experiment C, the radioactive assay was used. K-stimulated activity is activity in the presence of K⁺ (column b) less that in the presence of Na⁺ (column a).

| Membranes               | DCCD   | Na⁺ = 20 mm (a) | K⁺ = 20 mm (b) | K⁺-stimulated (b-a) | μmol g⁻¹ min⁻¹ |
|-------------------------|--------|-----------------|----------------|---------------------|----------------|
| Experiment A: derepressed cells |        |                 |                |                     |                |
| Control                 |        | 47              | 72             | 25                  |                |
| Control + Urea-extracted|        | 27              | 64             | 37                  |                |
| Urea-extracted          |        | 17              | 91             | 74                  |                |
| Experiment B: repressed cells |        |                 |                |                     |                |
| Urea-extracted          |        | 2.1             | 2.2            | 0.1                 |                |
| Experiment C: repressed cells |        |                 |                |                     |                |
| Urea-extracted          |        | 1               | 1              | 0                   |                |

![Fig. 1. Dependence on K⁺ of membrane ATPase and K⁺ transport in two mutant strains. Strains TK2242-42 (Panels A and B) and TK2219-19 (Panels C and D) grown under derepressing conditions were used to prepare urea-extracted membranes obtained by sonication for radioactive ATPase assays (A, C), or in separate experiments to measure the initial rate of net K⁺ uptake in K⁺-depleted cells (B, D). For the ATPase measurements, the insets show the reciprocal of the difference between ATPase activity at a given K⁺ concentration and that in the absence of K⁺ versus the reciprocal of the K⁺ concentration. ATPase is expressed per g of membrane protein; transport rates are per g of cell dry weight.](http://www.jbc.org)
mements with Tris$_2$ATP which contained about 1 mM contaminating Na$^+$ failed to show any stimulation by or dependence on Na$^+$. If the ATPase has a Na$^+$ requirement, it must be fully satisfied by 1 mM Na$^+$.

The Kdp ATPase is insensitive to millimolar concentrations of ouabain, the classical inhibitor of the mammalian (Na$^+$, K$^+$)-stimulated ATPase. Only slight inhibition (12%) resulted from treatment with 1 mM N-ethylmaleimide, a good inhibitor of several transport ATPases (17-19). The divalent cation specificity of the ATPase is shown in Table III. There is only negligible activity in the absence of added divalent cation. Mn$^{2+}$ and Ca$^{2+}$ serve as well as Mg$^{2+}$, while Zn$^{2+}$ is only 30% as active. Ca$^{2+}$ does not support activity, and in the presence of Mg$^{2+}$ is a good inhibitor. The nucleotide specificity is narrow too. The membranes had negligible triphosphatase activity with GTP and UTP, very low activity with ITP and ATP. ATP, however, is a good substrate at the rate of 150 pmol/min, well over an order of magnitude higher than the rates are about 150 μmol/min, well over an order of magnitude higher. Incorporation of the ATPase during membrane isolation could account for low rates of ATPase activity. We have not yet tested widely different ways of obtaining membranes, nor optimum conditions for stabilization of the ATPase. Storage in the refrigerator leads to slow loss of activity, accounting for lower activity in the experiments of Fig. 1A and Table III.

Low ATPase activity could be due to an inhibitor of ATPase which might regulate the activity in vivo. There is good evidence for a regulatory subunit of the (Ca$^{2+}$, Mg$^{2+}$)-stimulated ATPase of mitochondria which inhibits activity in the direction of ATP hydrolysis (20). The presence of an inhibitor is supported by the stimulation of ATPase after urea treatment (Table I). Every Kdp mutant with altered affinity for K$^+$ that we examined showed higher K$^+$-stimulated ATPase after urea treatment. Urea treatment removes the F1 component of the (Ca$^{2+}$, Mg$^{2+}$)-stimulated ATPase, which is the reason we used urea. Whether the F1 component or some other protein is an inhibitor in vitro, and the role of this inhibitor in vivo, remain to be determined.

Rates of transport would be higher than those of the ATPase if the stoichiometry of moles of K$^+$ transported per mole of ATP hydrolyzed is greater than 1. A stoichiometry of 2 could be obtained if K$^+$ is transported electrogenically. The Kdp system can create a K$^+$ concentration gradient of 4 x 10$^{11}$ (1), equivalent to 9400 cal/mol. In electrogenic transport, the membrane potential of −140 mV (21) would contribute 3300 cal/mol, leaving 6100 cal/mol to come from ATP. This value would allow a stoichiometry of 2, allowing for some uncertainties in these figures and in the AG for ATP hydrolysis in vivo. If K$^+$ transport is electroneutral, as an exchange for H$^+$ or by symport with an anion, the most likely stoichiometry is 1. In such transport, no energy is provided by the membrane potential and it is not likely that movement of the coupled ion will contribute significant amounts of energy, so that about 9400 cal/mol must be provided by ATP. These considerations of the stoichiometry of transport indicate that the expected ATPase activity is at least one-half that of Kdp transport.

The identification of a membrane ATPase associated with K$^+$ transport indicates that bacteria have transport systems that are at least superficially similar to the ATP-driven systems of animal cells. Further studies will permit us to determine if the parallel with animal cell transport ATPases extends to specific details, such as formation of a phosphoenzyme intermediate. Regardless of details, identification of the ATPase will greatly facilitate studies of the mechanism of energy coupling to this system, and purification of the system in a functional state.

### DISCUSSION

The genetic evidence shown in Fig. 1 of the effect of kdp mutations on the affinity for K$^+$ of the ATPase indicate that this activity is due to the Kdp system. Two other unusual characteristics of the Kdp system, repressibility by growth in high K$^+$ and a very narrow cation specificity, are shared by the ATPase. The only property of the ATPase that does not fit perfectly with those of the Kdp system is the relatively low energy coupling to this system, and purification of the system in a functional state.

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