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The Bacterial Kdp K<latex>$^+$</latex>-ATPase and Its Relation to Other Transport ATPases, Such as the Na<latex>$^+$</latex>/K<latex>$^+$</latex>- and Ca<latex>$^{2+}$</latex>-ATPases in Higher Organisms [and Discussion]

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The bacterial Kdp K^+-ATPase and its relation to other transport ATPases, such as the Na^+/K^+- and Ca^{2+}-ATPases in higher organisms

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The Kdp system is a three-subunit member of the E_1–E_2 family of transport ATPases. There is sequence homology of the 72 kDa KdpB protein, the largest subunit of Kdp, with the other members of this family. The predicted structure of the 21 kDa KdpC subunit resembles that of the β subunit of the Na^+,K^+-ATPase, suggesting that these subunits may have a similar function. The 59 kDa KdpA subunit has no known homologue; it is very hydrophobic and is predicted to cross the membrane 10–12 times. Genetic studies implicate this subunit in the binding of K^+. As the binding site must be close to the beginning of the transmembrane channel, we suggest that KdpA also forms most or all of the latter. KdpA may have evolved from a K^+/H^+ antiporter that was recruited by the KdpB precursor to achieve the high affinity and specificity for K^+, and the activation of transport by low turgor pressure characteristic of Kdp.

Turgor pressure controls the expression of Kdp. This action is dependent on the 70 kDa KdpD and 23 kDa KdpE proteins. We are in the process of sequencing these genes. KdpE is homologous to the smaller protein of other members of a family of pairs of regulatory proteins implicated in control of a variety of bacterial processes such as porin synthesis, phosphate regulon expression, nitrogen metabolism, chemotaxis and nodule formation.

Introduction

The K^+-ATPase of *Escherichia coli*, commonly referred to as Kdp, is a member of the E_1–E_2 class of transport ATPases (Skou 1975; Inesi 1985; for extensive coverage see symposium volumes edited by Carafoli & Scarpa (1982) and by Skou *et al.* (1988)). These are also called P-type ATPases (Pederson & Carafoli 1987) to distinguish them from ATPases that do not form a phosphorylated intermediate. This class of enzyme is widely distributed in nature; representatives from all kingdoms except the archaebacteria have been described. The E_1–E_2 label came from the biochemical studies, primarily of the Na^+/K^+- and the Ca^{2+}-ATPases, indicating that the acyl-phosphorylated intermediate characteristic of these enzymes exists in two different functional states. In the E_1 state the intermediate is of 'high energy' and can form ATP from ADP, whereas in the E_2 state the intermediate appears to be of 'low energy' and can be formed from inorganic phosphate under suitable conditions. The forms do not differ in the covalent attachment of the phosphate and represent alternate conformations of the enzyme. Transitions from E_1 to E_2 and vice versa are associated with conformational changes that lead to occlusion or release of the transported ion, indicating that these transitions are key steps in transport.

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Almost all of these ATPases have a single type of protein subunit of relative molecular mass (M_r) in the range of 60000 to 140000, and all share sequence homology. The key structural features of this large subunit are represented in figure 1. Four membrane-spanning segments in the N-terminal half of the protein form two loops joined by small extramembranous regions (*a*) and (*b*). The N-terminus and the large extramembranous regions between and beyond the loops are cytoplasmic. The C-terminal region is more variable, with at least two membrane-spanning segments and sometimes a sizeable cytoplasmic C-terminal domain. Three regions of highly conserved sequence are found in all of these ATPases. One of the regions of homology includes the acylphosphorylated aspartate residue (P). The region with greatest conservation is that labelled (3). As is to be expected, systems with the same cation substrate or that are in the same kingdom, or both, are more homologous than less related enzymes.

There are two enzymes of this family that have more than one type of subunit: the Na^+/K^+-ATPase has two subunits, α and β and Kdp has three subunits, KdpA, KdpB, and KdpC. There is no reason to believe that the basic mechanism of transport in multi-subunit ATPases differs markedly from that in single subunit enzymes. This is clearest in the comparison of data for the two subunit Na^+,K^+-ATPase and the single subunit Ca^{2+}-ATPase (Skou 1957; Inesi 1985). Although biochemical studies of Kdp are not extensive, all of the evidence (Epstein 1985; Siebers & Altendorf 1988, 1989) suggests that it undergoes transitions like those of the other ATPases.

Enzymes very similar to Kdp are widely distributed in eubacteria. Sequences with good homology to all *kdp* genes have been detected in a number of closely-related Gram-negative bacteria, and there is homology to the gene for the largest Kdp subunit in many distantly related Gram-negative species (Walderhaug *et al.* 1989). Similar enzymes occur in Gram-positive bacteria. There is a single subunit K^+-ATPase in *Streptococcus faecalis* (Solioz *et al.* 1987) and what seems to be a close relative of Kdp in *Bacillus acidocaldarius*. This thermoacidophile has a K^+-ATPase whose three subunits are similar in size to those of Kdp and whose largest subunit reacts with antibody to the largest Kdp subunit (Hafer *et al.* 1989).

Comparisons of Kdp with other ATPases

In this article we will try to draw conclusions from a comparison of Kdp with other ATPases, and summarize recent findings. We assume that the function(s) of each of the three types of subunit of Kdp is done by parts of the single, large subunit of most members of this family of ATPases.

1. *KdpC may be an analogue of the* β *subunit of the* Na^+-K^+-*ATPase*

There is some similarity of the predicted structures of KdpC (Siebers 1988) and of the β subunit (Kawakami *et al.* 1985; Ovchinnikov *et al.* 1986; Brown *et al.* 1987); each has only a single predicted membrane spanning region that is close to the N-terminus. The extent of homology between the two is weak at best. It is known that the large extramembranous C-terminal part of the β subunit is exposed to the outside of the cell because it has the three sites of glycosylation. Because eubacteria do not glycosylate proteins, no such information is available for KdpC. The KdpC protein as assembled in the Kdp complex is resistant to proteases from both sides of the membrane (Epstein 1985; Siebers 1988), suggesting that only small parts are exposed in the intact Kdp complex. None the less, these two subunits may have a similar function that is yet to be determined.

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2. *KdpA binds* K^+ *and may form the transmembrane channel*

The large KdpA subunit must be present in most if not all of the close bacterial relatives of Kdp because Southern blotting with a probe for its gene showed it to be present in many bacteria (Walderhaug *et al.* 1989). However, it has no counterpart in other members of this class of transport ATPases. A search of the National Biomedical Research Foundation and EMBO protein sequence databases with the FastP program revealed no proteins that had more than small, probably random, regions of homology. KdpA is the most hydrophobic subunit of Kdp, and hydrophobicity plots suggest that it has up to 12 membrane-spanning regions (Epstein 1985; Siebers 1988). The structure of this subunit should resemble that of membrane porters, such as the chemiosmotic porters for sugars discussed in the articles by P. Henderson, R. Kaback and G. LeBlanc in this symposium.

Genetic studies implicate KdpA as the site of K^+ binding. Most of the mutants in which the normal high affinity of the system for K^+, $K_m = 2\ \mu M$, is reduced by a factor of 100 or more (Epstein *et al.* 1978) alter the KdpA subunit. Mapping shows that these mutations are clustered in a few regions distributed over much of the length of KdpA (Dorus *et al.* 1985). Recent sequencing of the mutations confirms this clustering (J. M. Daniel *et al.*, in preparation).

Because the K^+ binding site must be near the beginning of the path for transmembrane movement of K^+, it is only a small step to postulate that KdpA performs both binding and transmembrane movement. This suggests models of Kdp like that drawn in figure 2*b*. Here the main membrane component is KdpA, drawn as a dimer in view of genetic evidence (intracistronic complementation between some *kdpA* mutations (Epstein 1985)) that Kdp is oligomeric. The larger KdpB subunit is shown apposed to the cytoplasmic face of KdpA, where it could transduce the energy from ATP hydrolysis to produce the conformational changes needed for transport. The dashed line indicates the channel for cation movement through the membrane.

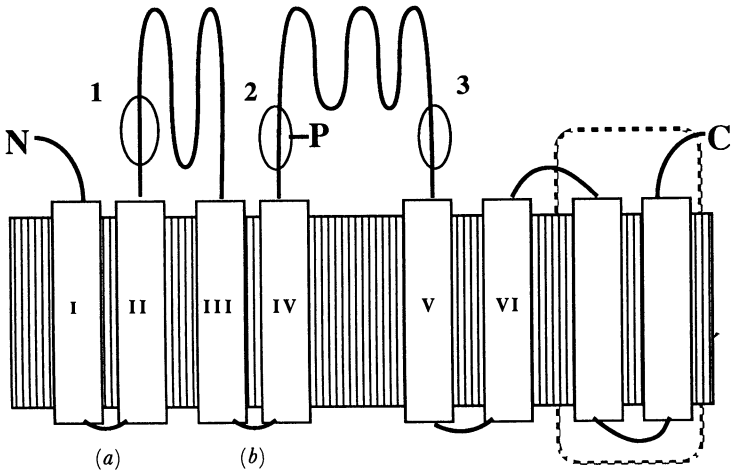

FIGURE 1. A schematic representation of the conserved structural features of the large subunit of the E_1–E_2 ATPases. The first six membrane spanning regions are identified by roman numerals. Ovals with arabic numbers identify the approximate location of the regions of highest sequence conservation, and (P) indicates the site of the phosphorylated aspartate residue. The small connecting regions that are believed to be exposed externally in other ATPases but not in Kdp are labelled (*a*) and (*b*); their sequences are shown in table 1. A dashed line surrounds the C-terminal and un-numbered membrane-spanning regions to indicate variability in different enzymes. Where the extramembraneous C-terminal region is short it may be external rather than internal as shown.

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TABLE 1. HYDROPHILIC CENTRES OF MEMBRANE LOOPS OF ATPASES

enzyme	first residue shown[a]	sequence[b]	reference
first loop			
Na/K	(105)	FLAYGIQAATEEEPQNDNLYLGVV	Shull *et al.* (1985)
Ca	(73)	FVLAWF EEGEETITAFVEPV	MacLennan *et al.* (1985)
H/K	(71)	LIAFAIQASEGDLTTDDNLYLALA	Shull & Lingrel (1986)
H (yeast)	(133)	ILAAGL SDWVDFGV	Serrano *et al.* (1986)
H (mould)	(133)	VLAAGL EDWVDFGV	Addison (1986); Hager *et al.* (1986)
S. fecalis	(23)	VYSFIA NLISPHTHVMDFFWELA	Solioz *et al.* (1987)
KdpB	(54)	ASGAMP GNALFS	Hesse *et al.* (1984)
Second loop			
Na/K	(301)	ILSLIL EYTWLEAVIFLI	
Ca	(274)	INIGHF NDPVHGGSWIRGAI	
H/K	(269)	AMCIGY TFLRAMVFFM	
H (yeast)	(308)	LLLVWTACFYRTNGIVRILRYTLGIT	
H (mould)	(305)	LLIVWVSSFYRSNPIVQILEFTLAIT	
S. fecalis	(215)	FLANLP DALERMVTVFI	
KdpB	(245)	WGGNAV SVTVLV	

[a] Numbering of residues is from the N-terminus.

[b] Residues in the middle (not underlined) are presumed to be extracellular; these correspond to the regions labelled (*a*) and (*b*) in figure 1 for the first and second loops, respectively. The six flanking, underlined residues represent the adjacent sequences that are presumed to be in the membrane. Gaps are used to align the residues at the right presumed to be in the membrane.

The ATPases that do not have a KdpA-like subunit must bind and transport their cation substrates by a functionally analogous region of their one (or two) subunits. Transmembrane movement must be mediated by several of the membrane-spanning regions of the large subunit, rather than by a separate subunit as in Kdp. The loop formed by membrane segments I and II, and that formed by segments III and IV (see figure 1) appear to be involved in ion transport in other ATPases but not in Kdp. If these segments are to mediate ion binding or transport, or both, at least part of each ought to extend to the outside of the membrane. In the other ATPases the middle of each loop has a short hydrophilic region (figure 1*a*, *b*) that would be expected to project beyond the bilayer (table 1). These loops in KdpB, in contrast, lack any clearly defined hydrophilic region and may not extend to the external face of the membrane. If KdpA binds (and transports) the cation substrate, there is no apparent reason for either of the loops in KdpB to have access to the outside. The action of the energy-coupling domain of the large subunit on its transporting domain is converted, in Kdp, to the action of one subunit on a separate polypeptide. Models in which energy transduction is dependent on covalent bonds between energy-transducing and cation binding–transport regions cannot hold for Kdp.

3. *Suggested origin of KdpA*

We suggest that Kdp evolved from a single subunit ATPase, perhaps one similar to the K^+-ATPase of *Streptococcus faecalis*, by acquiring a separate subunit to mediate cation binding and transport. It could have commandeered an existing porter for K^+, such as a K^+/H^+ antiporter. It appears that such antiporters are ubiquitous in bacteria (Walderhaug *et al.* 1987). A separate large subunit, whose sole functions are cation binding and transport, could more readily evolve to achieve high affinity and high specificity for K^+ characteristic of Kdp than could a single subunit ATPase. The KdpB precursor, a single subunit ATPase (figure 2*a*), would have

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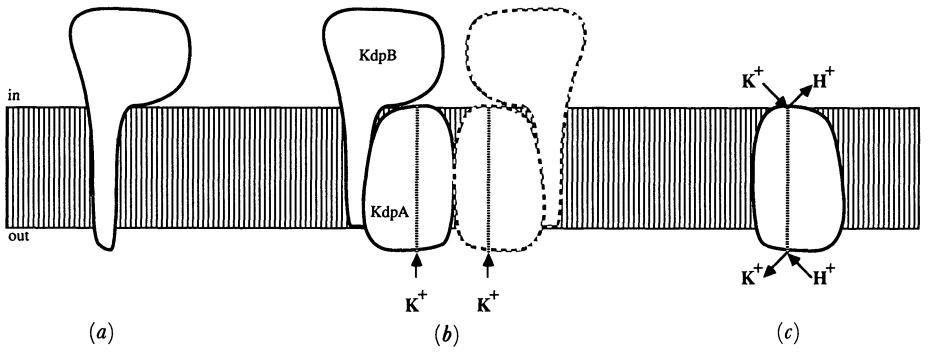

FIGURE 2. (*a*) A diagrammatic representation of an ATPase with only a single type of subunit. (*b*) A diagrammatic representation of the presumed structure of Kdp, in which only the two largest subunits are drawn. A second copy of each subunit is drawn to indicate the active structure is an oligomer, perhaps a dimer. (*c*) Representation of a chemiosmotic porter, here drawn as a K^+/H^+ antiporter. Dashed lines indicate the paths for cation movement. It is suggested that Kdp evolved from an ATPase like that in (*a*) by acquiring an additional subunit like (*c*) that replaced the ion binding and transport functions of some of the membrane-spanning parts; the latter still serve to anchor and assemble the large subunit but do not participate in ion movement.

obtained the KdpA precursor from a porter (figure 2*c*) that displaced some of the membrane-spanning regions of the KdpB precursor. The large cytoplasmic domain that mediates energy transduction became positioned above the KdpA subunit to form the Kdp complex (figure 2*b*). The membrane-spanning regions of KdpB, retaining little, if any, role in cation movement, now serve to assemble this subunit with the other subunits and into the membrane.

REGULATION OF Kdp

Intracellular K^+ is one of the major internal osmotic solutes in bacteria. In species like *E. coli* it appears to be the primary solute whose concentration is most closely and most directly controlled by the osmotic pressure difference across the membrane, the turgor pressure (Epstein 1985, 1986). This control is exerted at two levels as illustrated in figure 3: at the level of activity, turgor appears to affect most K^+ transport systems; at the level of gene expression turgor appears to control only Kdp.

1. *Control of transport activity*

The most immediate controls are those on transport activity. At the optimal turgor pressure, uptake and efflux are at low levels and balanced so that internal K^+ remains unchanged. Cell growth, by increasing cell volume, will dilute internal solutes to reduce turgor whereupon there is an increase in K^+ uptake that restores turgor toward the optimal. This type of control is manifested by several K^+ uptake systems, not only Kdp, in *E. coli* as well as by K^+ uptake systems in other organisms (Walderhaug *et al.* 1987). The requirements of osmoregulation predict that the net K^+ movements are always controlled by turgor to avoid unphysiological levels of turgor pressure. Under most conditions of growth, Kdp is not expressed, so control of the activity of constitutive systems for K^+ uptake is of key importance in osmoregulation. However, Kdp is controlled in the same way if it is present (Rhoads & Epstein 1978). An increase in turgor, produced by dilution of the medium or by major intracellular accumulation

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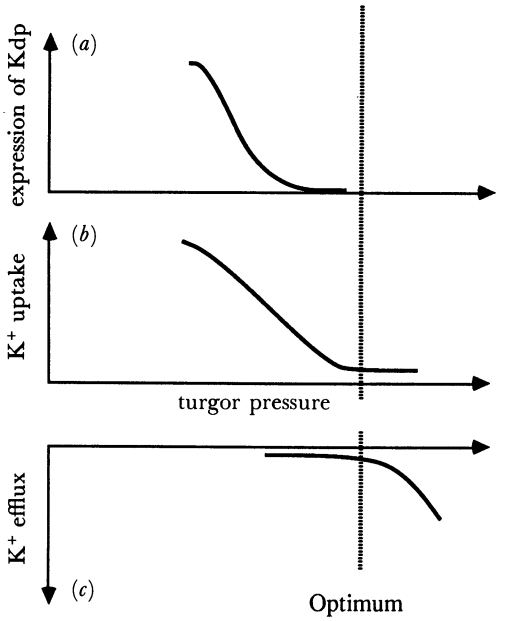

FIGURE 3. The types of control that turgor pressure exerts on K^+ transport. (*a*) The dependence of Kdp expression on turgor. At the optimum turgor and at moderate reductions, Kdp remains repressed but when turgor drops significantly there is progressive expression of Kdp. The behaviour of uptake systems (*b*) is similar except that a much smaller reduction of turgor is required to stimulate uptake. It is presumed that this relation accounts for control of cell K^+ under most conditions; only when existing uptake systems cannot maintain turgor close to normal levels is Kdp expressed. (*c*) The behaviour of efflux systems. These are activated by slight increases in turgor. The diagram shows that both influx and efflux remain at low levels at and near optimum turgor; this behaviour accounts for the presence of considerable rates of K^+/K^+ exchange under steady-state conditions.

of other solutes, stimulates K^+ efflux (Meury *et al.* 1985; Bakker *et al.* 1987) by what we assume are constitutive systems designed for this purpose. Analysis of mutants indicates that efflux is not mediated by any known uptake system. The shapes of the curves for uptake and efflux in figure 3 are somewhat arbitrary as few quantitative determinations of the dependence of the rate of transport on the change in turgor have been done (Meury & Kepes 1981).

The way that low turgor pressure activates Kdp is not known. There must be a component of Kdp, or another protein (see Martirosov 1979) that mediates control by turgor. A good candidate for this role is KdpA. The transport channel could be gated by turgor pressure. Such control presents another rationale for a separate subunit; the added flexibility of limited function in a large protein would make it easier to evolve this type of control.

2. *Control of Kdp expression*

Kdp is generally expressed only when growth is limited by the availability of K^+. Analysis of this phenomenon showed that neither external nor internal K^+ concentrations can be the direct signal, but that a change in turgor is most likely the signal to express Kdp (Laimins *et al.* 1981). The genetics of the system are clear: Kdp is encoded by the *kdpABC* operon whose expression is dependent on the expression of the adjacent regulatory *kdpDE* operon. Mutations in either of the two genes of the *kdpDE* operon block expression of Kdp, so both appear to encode positive regulators (Polarek *et al.* 1988). A clue to their role is emerging from the sequence analysis of the regulatory operon underway at present. We have compared the

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TABLE 2. HOMOLOGY OF KdpE TO PhoB AND OmpR

(Number is the first amino acid of the sequence shown, beginning at the N-terminus. The PhoB sequence is from Makino *et al.* (1986); OmpR from Wurtzel *et al.* (1982).)

KdpE	VIVLSARSEESDKIAALDAGADDYLSKPFGIGELQA
PhoB (79)	VVMLTARGEEEDRVRGLETGADDYITLPFSPKELVA
OmpR (79)	IIMVTAKGEEVDRIVGLEIGADDYIPKPFNPRELLA

sequence of KdpE, the smaller of the two proteins, with the smaller of several other pairs of related regulatory proteins (table 2). There is sequence homology with regions of the OmpR protein involved in control of porin synthesis and the PhoB protein that controls phosphate regulon expression. Other members of this group include proteins of chemotaxis, nitrogen metabolism, and nodule formation (Stock *et al.* 1985; Kofoid & Parkinson 1988). In each of these pairs of proteins, it is presumed that the larger one is a sensor of environmental information, whereas the smaller effects the response. The locations of the Kdp regulatory proteins, KdpD in the membrane and KdpE in the cytoplasm (J. W. Polarek *et al.*, in preparation) are consistent with a model where the turgor pressure regulated action of KdpD alters KdpE to turn on transcription of the *kdpABC* operon. The finding that the smaller proteins regulating chemotaxis and that nitrogen metabolism are phosphorylated (Ninfa *et al.* 1988) suggests that phosphorylation of KdpE may be the key step in turning-on expression of Kdp.

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Discussion

N. M. Green (*National Institute for Medical Research, London, U.K.*). The reaction cycle of the P-type ATPases consists of two halves. In the first, ATP brings about a cation dependent phosphorylation of the pump, the cation being pumped out of the cytoplasm. In the second step the phosphoenzyme is hydrolysed. A second cation is often required for this and, if so, it

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behaves as a counterion and is pumped into the cell. In Kdp B the potasium is the counterion and there seems to be no evidence of a cation requirement for phosphorylation by ATP. Is there any evidence that protons could be involved in this step?

W. Epstein. We do not know whether Kdp transports protons out as well as moving K^+ in. This question cannot be readily answered from studies in intact cells, and will require either studies of Kdp in membrane vesicles or after reconstitution into liposomes. It is certainly possible that Kdp exchanges protons for K^+. It is unlikely that another ion, such as Na^+ is transported out, because no monovalent cation requirement for formation of the phosphorylated intermediate has been detected.

J. Broome-Smith (*University of Sussex, Brighton, U.K.*). The catalytic α subunit of the mammalian Na^+-K^+-ATPase shares significant homology with the kdpB protein. Intriguingly, the β subunit has only limited homology with the kdpC protein, yet, from their amino acid sequences similar transmembrane topologies are predicted for both proteins. No known function has yet been attributed to either of these proteins, and given a possible commonality of structure, could it be that they are both involved in assembly/oligomerization of the membrane protein components into a functional membrane complex?

W. Epstein. An assembly role for KdpC is very plausible, but no information on this point is available as yet. An examination of whether the KdpA and KdpB subunits are associated when KdpC is not present should address this question.

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