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The K^+-translocating KdpFABC complex from *Escherichia coli*: A P-type ATPase with unique features

Jörg-Christian Greie · Karlheinz Altendorf

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Abstract The prokaryotic KdpFABC complex from the enterobacterium *Escherichia coli* represents a unique type of P-type ATPase composed of four different subunits, in which a catalytically active P-type ATPase has evolutionary recruited a potassium channel module in order to facilitate ATP-driven potassium transport into the bacterial cell against steep concentration gradients. This unusual composition entails special features with respect to other P-type ATPases, for example the spatial separation of the sites of ATP hydrolysis and substrate transport on two different polypeptides within this multisubunit enzyme complex, which, in turn, leads to an interesting coupling mechanism. As all other P-type ATPases, also the KdpFABC complex cycles between the so-called E1 and E2 states during catalysis, each of which comprises different structural properties together with different binding affinities for both ATP and the transport substrate. Distinct configurations of this transport cycle have recently been visualized in the working enzyme. All typical features of P-type ATPases are attributed to the KdpB subunit, which also comprises strong structural homologies to other P-type ATPase family members. However, the translocation of the transport substrate, potassium, is mediated by the KdpA subunit, which comprises structural as well as functional homologies to MPM-type potassium channels like KcsA from *Streptomyces lividans*. Subunit KdpC has long been thought to exhibit an FXYD protein-like function in the regulation of KdpFABC activity. However, our latest results are in favor of the notion that KdpC might act as a catalytical chaperone, which cooperatively interacts with the nucleotide to be hydrolyzed and, thus, increases the rather untypical weak nucleotide binding affinity of the KdpB nucleotide binding domain.

Keywords Kdp · P-type ATPase · Potassium · Potassium channel · FRET · ALEX-FRET

J.-C. Greie (✉) · K. Altendorf
Universität Osnabrück, Fachbereich Biologie/Chemie,
Arbeitsgruppe Mikrobiologie,
Barbarastraße 11,
49069 Osnabrück, Germany
e-mail: greie@biologie.uni-osnabrueck.de

Introduction

P-type ATPases generally resemble a superfamily, which represents primary ion pumps transporting cations across lipid bilayers. By use of the Gibbs free energy of ATP hydrolysis, they can establish and maintain large electrochemical gradients across these membranes. A characteristic intermediate step in the catalysis of P-type ATPases, from which the name of this class of enzymes is derived, is the formation of a transient phosphointermediate, in which a conserved aspartic acid residue gets reversibly autophosphorylated. Substrate transport generally occurs via sequential alterations in both the affinity and accessibility of at least two ion binding sites exposed to either side of the membrane, which are driven by large conformational rearrangements of the enzyme. This widely accepted reaction cycle can be described by the so-called E1/E2 catalytic cycle (Møller et al. 1996). By far the most information about the structure and mechanism of P-type ATPases has to date been derived from eukaryotic family members like the sarcoplasmic reticulum Ca^{2+}-ATPase (SERCA), the Na^+, K^+-ATPase of animal cells, and the H^+, K^+-ATPase of the gastric mucosa. In contrast, much less information is available for the prokaryotic counterparts, like the CopA copper-transporting ATPase. Another long-time overlooked member of the P-type ATPase family

is the KdpFABC K^+-translocating ATPase of *Escherichia coli*, which features outstanding characteristics that clearly point to a unique status of at least this bacterial P-type ATPase with respect to its eukaryotic relatives and justified an allocation to the type IA subgroup within the P-type ATPase family (Haupt et al. 2006).

As in case of all other P-type ATPases, also the KdpFABC complex is supposed to undergo a characteristic E1/E2 catalytic cycle (Fig. 1). Most recently, by use of a new time-resolved single-molecule FRET technique applying two alternating excimer lasers (ALEX-FRET), we were able to show distinct E1 and E2 conformational states of reconstituted KdpFABC in the absence of K^+ or in the presence of *ortho*-vanadate, respectively, as well as fluctuating FRET signals in the working KdpFABC complex (T. Heitkamp, N. Zarrabi, M. Börsch, and J.-C. Greie, to be published). However, only one of the four subunits of the complex, namely the 72 kDa KdpB, is catalytic and comprises all typical signature motifs of P-type ATPases, but is not responsible for substrate translocation (Bramkamp and Altendorf 2004). Thus, ATP hydrolysis in KdpB is energetically coupled to K^+ transport mediated by another subunit, namely the 59 kDa KdpA polypeptide (Altendorf et al. 1998), which comprises strong sequence homologies to KcsA-type potassium channels (Durell et al. 2000). The unique status of this P-type ATPase is further outlined by the abundance of two additional smaller subunits, KdpF (3 kDa) and KdpC (20 kDa). Whereas the former is thought to represent a molecular glue being of structural importance for the integrity of the enzyme complex (Gaßel and Altendorf 2001), the latter is most likely involved in the reaction cycle as a catalytical chaperone (Ahnert et al. 2006).

The physiological relevance of the KDP system

Besides protons, which are mainly employed for pH maintenance and bioenergetic purposes, potassium is the

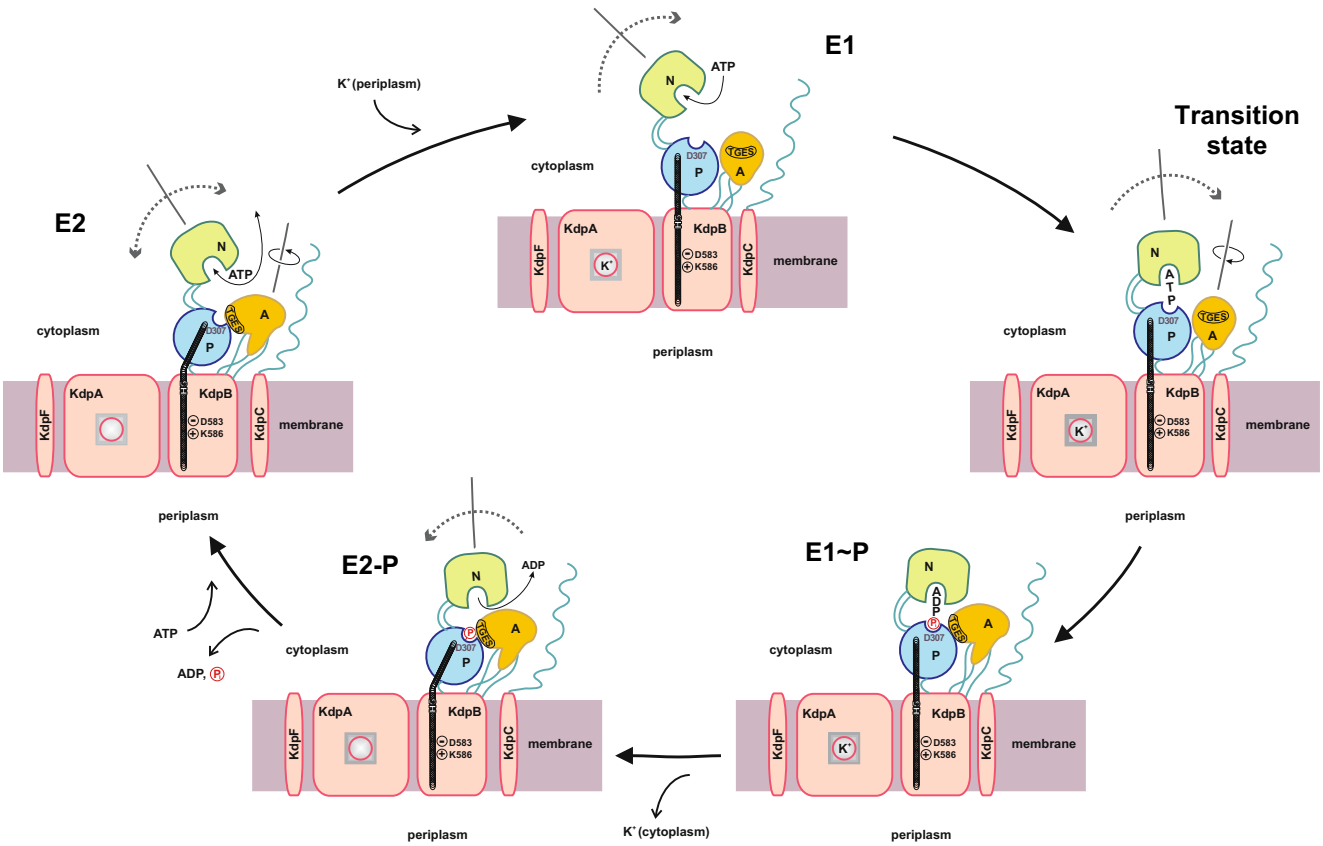

Fig. 1 Proposed reaction cycle for the *E. coli* KdpFABC complex. Assuming a resting position of KdpFABC in the E2 state, the binding of K^+ promotes high-affinity ATP binding to the N-domain of KdpB. Concomittantly, the complex turns to the E1 state, and the potassium ion enters the binding site in the center of the selectivity filter of KdpA and gets occluded in the transition state of the enzyme upon phosphorylation of the conserved aspartic acid residue (D307) in the P-domain of KdpB. The subsequent E1/E2 transition is accompanied by major conformational changes in KdpB, which alters the orientation of the conserved dipole formed by D583 and K586 in the transmembrane domain of KdpB. This renders the position of the potassium ion in the center of the selectivity filter of KdpA energetically unfavorable and, as a consequence, pushes the ion toward the cytoplasmic side of the membrane, where it is finally released. Finally, dephosphorylation of D307 by the interaction with the TGES motif of the KdpB A-domain regenerates the E2 state

main and most abundant monovalent cation in the *E. coli* cell. Although K^+ is also involved in pH homeostasis by use of K^+/H^+ and Na^+/H^+ antiport processes, it serves fundamental tasks in the activation of enzymes, acting as co-factor for example in ribosomes, and it is involved in the maintenance of turgor even in case of an osmotic upshock of the cell, thereby acting as a first-response osmoprotectant. Besides the house-keeping, constitutively expressed and $\Delta\Psi$-driven K^+ uptake systems Kup and Trk, which exhibit a high transport speed but a rather low affinity for the substrate, *E. coli* has employed the high-affinity ($K_d \approx$ 2 μM) and ATP-driven K^+-scavenging KdpFABC complex, the synthesis of which is induced if the external potassium concentration drops below 100 μM. Regulation of the transport complex synthesis is governed by a two-component sensory systems, in which KdpD represents the membrane-bound sensor kinase, and the soluble KdpE polypeptide constitutes the cognate response regulator. Whereas the nature of the stimulus is still controversially discussed, a rather detailed view on the signal transduction pathway has already emerged (reviewed in Jung and Altendorf 2002). Upon stimulus perception, dimeric KdpD undergoes autophosphorylation by ATP at a conserved histidine residue within a C-terminal hydrophilic extension of the polypeptide. This phosphoryl group is subsequently transferred to the response regulator KdpE at a conserved aspartate residue. KdpE-P then dimerizes and triggers the transcription of the *kdpFABC* operon as a classical helix-turn-helix transcription factor. On the other hand if there is no stimulus, non-phosphorylated KdpD is able to de-phosphorylate KdpE~P in order to terminate KdpFABC synthesis and, thereby, ATP-driven K^+ uptake.

With this battery of different K^+ transport systems *E. coli* effectively meets the vital prerequisite of a steady potassium supply even under conditions of low driving force and/or extreme K^+ limitation.

A unique division of labor within a P-type ATPase

As already mentioned, only the KdpB subunit accounts for all the key motifs of P-type ATPases. Although structural information is so far only available for the isolated N-Domain (Haupt et al. 2006), the KdpB polypeptide can easily be modeled based on the SERCA coordinates in both the E1 and E2 states (Fig. 2). At first glance, KdpB closely resembles a common P-type ATPase with corresponding N-, P-, A-, and transmembrane domains. However, especially the nucleotide binding mode clearly differs from that of other P-type ATPases, for example with respect to type IB ATPases a completely different set of residues is involved in ATP binding. In addition, other P-type ATPases, especially the eukaryotic ones, contain larger insertions

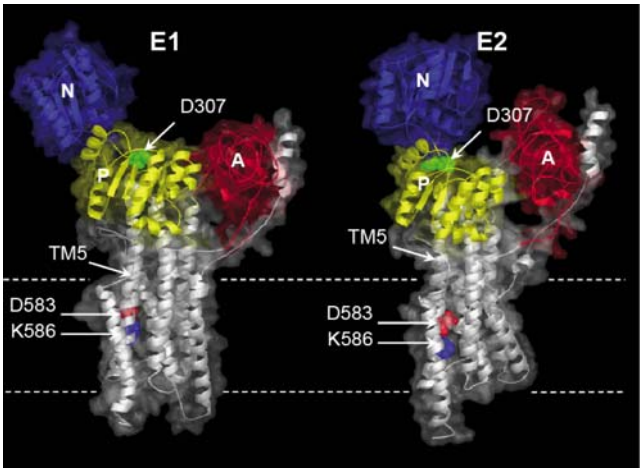

Fig. 2 SERCA-derived model of KdpB in E1 and E2 state. The N-, P-, and A-domains are depicted in *blue*, *yellow*, and *red*, respectively. The site of phosphorylation (D307) as well as the conserved dipole residues D583 and K586 within transmembrane helix 5 (TM5) are indicated

within the N-domain, so that KdpB constitutes a minimal core structure. A comparative interpretation of the various structures so far available and of the different nucleotide binding modes has already extensively been carried out recently (Haupt et al. 2006, Bramkamp et al. 2007), so this will not be a subject of this review. However, also the transmembrane portion of KdpB is comparatively small and consists of only seven transmembrane helices. Whereas in all other P-type ATPases the transmembrane portion of the catalytic subunit harbors the ion binding site(s), KdpB is not transporting any ions via its transmembrane domain. Instead of a conserved ion binding site, two residues can clearly be highlighted, namely D583 and K586, which are located in the middle of the transmembrane section on helix 5 and which are conserved among all KdpB polypeptides found so far. Since the elimination of either of these residues leads to a more or less severe uncoupling of ATP hydrolysis and K^+ transport (Bramkamp and Altendorf 2005), these amino acids are held responsible for the functional coupling between ATP hydrolysis in KdpB and potassium translocation in KdpA (also compare legend to Fig. 1).

The recruitment of a potassium channel by a P-type ATPase—or vice versa

The fact that KdpB comprises the unique number of only seven transmembrane helices together with the lack of any ion-translocating properties closely reflects an evolutionary reduction of structural elements upon the acquisition of another substrate-translocating polypeptide. Whether a potassium channel has made use of ATP fueling by

hijacking a P-type ATPase or if a P-type ATPase has recruited a potassium channel during evolution in order to gain higher turnover numbers cannot be decided from the data so far available. However, it is clear from our present results that within the KdpFABC complex, P-type ATPases and potassium channels merged in order to build a unique type of transporter, which displays characteristics of both of the two worlds.

No corresponding counterpart or domain element of KdpA can be found in other P-type ATPases nor does KdpA comprise any significant homologies to transmembrane domains of other P-type ATPases, so that it can be regarded as a unique component of the KdpFABC complex. Initial emphasis of KdpA mimicking a potassium channel of the KcsA type came from comparative computational sequence analysis of potentially related proteins by Durell, Guy, and Bakker (Durell et al. 2000), who concluded that KdpA is evolutionary derived from a homotetrameric MPM-type (*m*embrane/*P*-loop/*m*embrane) K^+ channel by gene duplication and fusion events. In contrast to the *S. lividans* KcsA channel, which features a homotetrameric array with each monomer comprising a single MPM motif (Doyle et al. 1998), KdpA was shown to possess four of these putative MPM motifs within a single polypeptide, flanked by two additional transmembrane helices one on each side (Fig. 3a). Although this hypothesis was primarily based on sequence similarity patterns, it is now in good accord with already existing experimental data relying on both the analysis of mutants affecting K^+ selectivity and the determination of transmembrane topology (Buurman et al. 1995; Durell et al. 2000) as well as it served as an appropriate template for further characterization of potential selectivity filter regions (Van der Laan et al. 2002; Bertrand et al. 2004). Mutations affecting ion binding and selectivity of the transport complex cluster in four defined hydrophilic loop regions, each of which being separated from its next neighbor by two transmembrane helices. The eight central helices containing the four putative MPM motifs can easily be modeled pairwise together with the connecting loop regions against the coordinates of KcsA, thereby clearly demonstrating that the clusters in fact form corresponding selectivity filter regions within the assembled K^+ conduit (Fig. 3b).

Since sequence analysis and mutagenesis as well as molecular homology modeling clearly favor the notion that KdpA displays K^+ channel-like characteristics, recent studies aimed at the electrophysiological characterization of distinct channeling properties of the polypeptide. For these analyses, KdpA was isolated from purified KdpFABC complexes and reconstituted into planar lipid bilayers (D. Becker, T. A. Götze, R. Wagner, K. Altendorf, and J.-C. Greie, unpublished data). The results obtained demonstrated

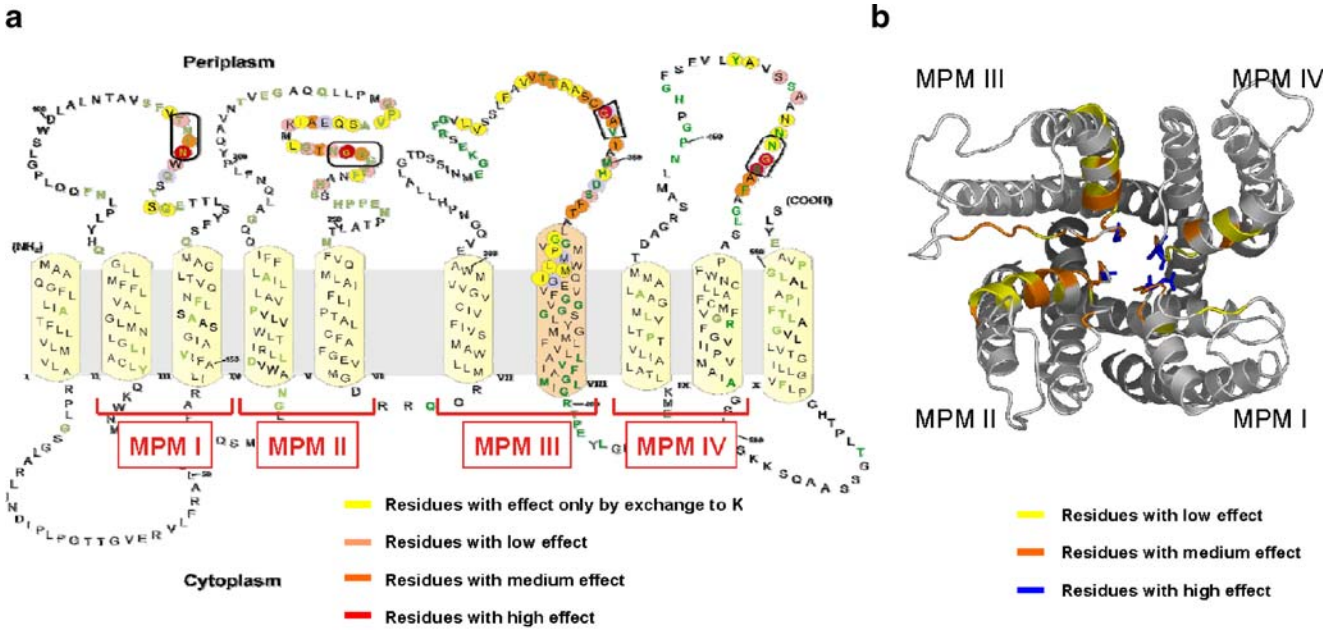

Fig. 3 **a** Topology model of the KdpA polypeptide. Based on topology analysis, KdpA folds into 10 transmembrane helices, the central eight of which constitute four consecutive MPM-type potassium channel domains (MPM I–IV). Mutations affecting KdpFABC ion selectivity cluster in four potential selectivity filter regions and are depicted in different colors as indicated. The potential core residues of the selectivity filter are boxed in *black*. **b** MPM-type K+ channel model of KdpA. The four MPM motifs of KdpA were modeled pairwise together with the connecting loop regions based on the coordinates of the *S. lividans* KcsA K^+ channel [1K4C] (Zhou et al. 2001) and arranged in a corresponding tetrameric assembly, with the color code ranging from *yellow* to *blue* with increasing effect on ion selectivity upon mutagenesis

that the isolated KdpA exhibited a K^+-selective ($K^+/Na^+ \leq$ 18:1 in 1 M KCl or NaCl) rectifying (rectification ratio > 6:1 in 250 mM KCl) conduit. Since the conductance revealed to be rather high (~ 500 pS in 250 mM KCl), it should clearly be noted that despite the high homology of KdpA and KcsA, K^+ gating in KdpA should differ from the KcsA mechanics. First, at least KdpB is involved in K^+ translocation by coupling ATP hydrolysis to the site of substrate transport as described above. Second, there are the two additional transmembrane helices in KdpA flanking the four MPM domains, which could either mediate subunit interactions or could also be directly involved in ion translocation. In addition, in strong contrast to KcsA, KdpA has to function as a unidirectional valve in order to prevent passive K^+ backflow. As a consequence, the K^+ conduit has to be tightly sealed in the direction of K^+ efflux, since KdpFABC is able to accumulate potassium inside the cell against a gradient of more than a 10,000-fold.

A catalytical chaperone as yet another unique feature of the Kdp FABC complex

Although it has been known for some time that the KdpC subunit is an essential part of KdpFABC (Gaßel and Altendorf 2001), its specific structure and function has not yet been appropriately elucidated. KdpC contains just one N-terminal transmembrane helix, with the remainder of the polypeptide facing the cytoplasm. Although this hydrophilic portion of KdpC is the only larger hydrophilic domain of the KdpFABC complex beside the P-, N-, and A-domains of the catalytic KdpB subunit, its distinct function is still unknown. A possible stabilizing role like the β subunit of Na^+, K^+- and H^+, K^+-ATPase in Eukarya has been discussed in the past (Axelsen and Palmgreen 1998), but this is rather unlikely for the sake of the opposing orientation with respect to the membrane. On the other hand, KdpC has been regarded as a member of the FXYD protein family like phospholamban or calmodulin in case of the Ca^{2+}-ATPase (Kimura and Inui 2002) or the γ subunit of the Na^+, K^+-ATPase (Therien et al. 1999), which are known to exert a regulatory function on enzyme activity via interactions with the large cytoplasmic loop regions of the pump. As another example, the single-subunit H^+-ATPase from *Neurospora crassa* contains an intrinsic hydrophilic regulatory domain at the C terminus of the polypeptide, which is proposed to interact with the catalytic N- and P-domains depending on the (de)phosphorylation by a specific kinase (Kühlbrandt et al. 2002). However, in contrast to KdpC, all of these so-called FXYD proteins are not essential for enzymatic activity, thereby pointing out that KdpC exerts a different, probably more unique regulatory function.

Recently, the isolated hydrophilic portion of KdpC ($KdpC_{sol}$) was shown to specifically bind one ATP molecule to a well-defined binding site within its hydrophilic portion facing the catalytic domains of KdpB (Ahnert et al. 2006), which argues in favor of a regulatory function of KdpC either via interaction with the nucleotide binding pocket of KdpB or via the direct binding of ATP. The ATP-labeling could neither be competed with GTP or CTP, nor with ADP or AMP, which, in turn, leaves the ribose moiety of the nucleotide left for the interaction, which also explains the rather low nucleotide affinity observed in the labeling studies. Hence, to be of physiological relevance, this specific ATP binding to KdpC needs an interaction partner in order to increase at least binding affinity if not also nucleotide selectivity, which renders a cooperative nucleotide binding mechanism together with KdpB likely for the following reason: The isolated N-domain of KdpB (KdpBN) exhibits an extraordinarily high binding constant for ATP of about 1.4 mM (Haupt et al. 2006). In contrast, other well-characterized nucleotide binding domains display much lower K_d values for ATP, which are in the range of 10–100 μM in case of the Ca^{2+}-ATPase (Abu-Abed et al. 2002) or 70 μM for the Wilson disease protein (Dmitriev et al. 2006). With respect to the latter, another special feature of KdpBN comes into play: Whereas in KdpBN there are no interactions between the ribose moiety of the nucleotide and the protein (Haupt et al. 2006), in its Wilson's disease counterpart the ribose is well-coordinated within a distinct and rather complex ribose binding pocket (Dmitriev et al. 2006), which readily explains the low ATP affinity of KdpBN.

As already mentioned, in the ATP binding studies with KdpC, alterations in neither the phosphate moiety (i.e. no competition between ATP, ADP, and AMP), nor the base (ATP vs. GTP and CTP) had any influence on the overall nucleotide binding rate, which would leave the ribose moiety of the nucleotide to interact with KdpC. The distinct ATP binding site has most recently been assigned to a stretch of C-terminal residues with a QXXRVAXARXL consensus motif, which is conserved in all KdpC sequences known so far (F. Ahnert, K. Altendorf, and J.-C. Greie, unpublished data). In addition, it is present in over 160 different proteins, which at least interact with ATP, and, most strikingly, in the ABC cassettes of the corresponding family of ABC transporters. In these ABC cassettes, this consensus is right next to the conserved LSGGQ signature motif of ABC transporters, with the Q residues overlapping. The mechanistic role of this essentially conserved glutamine residue can clearly be derived from the crystal structure of the homodimeric *Methanococcus jannaschii* MJ0796 ABC motor domain (Smith et al. 2002). In this structure, the Q side chain residue forms double H-bonds with the two hydroxyl groups of the ribose from the ATP molecule, thereby clamping the ribose moiety in a nucleotide sandwich between the two protein monomers.

In KdpBN, the nucleotide has to be properly oriented in order to enter the binding site in accord with a balanced equilibrium of nucleotide uptake and release (Haupt et al. 2006). Whereas the base moiety of the ATP is deeply embedded in the nucleotide binding pocket, the lack of additional coordination sites targeting the ribose moiety renders an additional regulatory process likely. In this scenario, KdpC would act as a catalytical chaperone, which orients and/or locks the nucleotide into the low-affinity catalytic binding site of KdpB via cooperative ribose interactions. Recently, we titrated [^{15}N]-labeled KdpBN with $KdpC_{sol}$, and corresponding backbone chemical shifts were analyzed by NMR (D. Becker, M. Haupt, H. Kessler, K. Altendorf, and J.-C. Greie, unpublished data). Strikingly, the residues affected by the presence of $KdpC_{sol}$ were almost completely identical to those known to form the entrance of the nucleotide binding pocket. Thus, the formation of a cooperative ternary complex between the N-domain of KdpB, KdpC, and the ATP molecule seems to be another unique feature in the mechanism of this P-type ATPase.

Conclusions

The KdpFABC complex comprises all of the characteristic P-type ATPase properties but it also exhibits some unique features with respect to its subunit composition and mode of action, which are based on the spatial separation of ATP hydrolysis and substrate transport on two different subunits. Whereas the catalytic cycling of the KdpB subunit and most of its structure clearly belong to the P-type ATPase family, the recruitment of the KdpA subunit as the site of potassium translocation mechanistically merges P-type ATPases and K^+ channels. The cooperative nucleotide binding mode by KdpC, which represents a catalytical chaperone with properties of ABC transporters, blends characteristics of yet another kind of transporter into this complex machinery.

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