The Kdp-ATPase of *Escherichia coli* Mediates an ATP-Dependent, K^+-Independent Electrogenic Partial Reaction†

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ABSTRACT: Charge transport by the K^+ transporting Kdp-ATPase from *Escherichia coli* was investigated using planar lipid membranes to which liposomes reconstituted with the enzyme were adsorbed. To study reactions in the absence of K^+, given some contamination of solutions with K^+, we used a mutant of Kdp whose affinity for K^+ was 6 mM instead of the wild-type whose affinity is 2 μM. Upon rapid release of ATP from caged ATP, a transient current occurred in the absence of K^+. In the presence of K^+, a stationary current was seen. On the basis of their structural similarity, we propose a kinetic model for the Kdp-ATPase analogous to that of the Na^+K^+-ATPase. In this model, the first, K^+-independent step is electrogenic and corresponds to the outward transport of a negative charge. The second, K^+-translocating step is probably also electrogenic and corresponds to transport of positive charge to the intracellular side of the protein.

The Kdp system is a bacterial P-type ATPase of *Escherichia coli* that transports K^+ with high affinity (*1*). The purified enzyme shows characteristic features of this class of ion-motive ATPases: it is inhibited by micromolar concentrations of orthovanadate (*2*) and forms an alkali-labile, acid-stable phosphointermediate as part of the catalytic cycle (*3*). The composition of Kdp, a complex of four membrane-bound subunits (KdpF, 3 kDa; KdpA, 59 kDa; KdpB, 72 kDa; KdpC, 20 kDa) is unique among P-type ATPases (*4*). The four structural genes of Kdp are organized in the *kdpFABC* operon (*5*) whose expression is under the control of the KdpD and KdpE regulatory proteins. These regulatory proteins belong to the class of sensor kinase/response regulator systems (*6, 7*).

The unique composition of the Kdp-ATPase with its four membrane-bound subunits allows for a separation of functional domains. The KdpB subunit shares all conserved regions of the P-type ATPases (*8*−*10*) and is phosphorylated during the reaction cycle (*3*), probably at aspartate 307 in the highly conserved pentapeptide DKTGT, because replacement of this residue by asparagine led to an inactive enzyme (*11*). The analysis of K^+ affinity mutants, generated by random mutagenesis, indicated that K^+ binding is associated with the KdpA subunit (*12*). Topological analysis of KdpA and the locations of the residues changed in the mutant strains suggest that KdpA has 10 membrane-spanning segments and forms two separate and distinct sites where K^+ is bound. These mutations are clustered in regions (*12*) that resemble the H5, M1, and M2 segments of potassium channels (*13*). The essential KdpC subunit is probably required for assembly of the Kdp complex (*1*), but other functions have not been excluded. In contrast, the small, hydrophobic KdpF peptide is not essential in vivo. However, removal of the last 8 residues of KdpF alters the ATPase activity and greatly reduces the stability of the solubilized enzyme (*4*).

The similarity of the enzymatic features of Kdp with those of other P-type ATPases led us to examine the similarities in more detail. Prominent members of this class of ATPases are the Na^+K^+- and H^+K^+-ATPases of eukaryotic cells. Overall transport in the former is electrogenic (*14*) but electroneutral in the latter (*15*). These electrical effects have been explained by electrogenic Na^+ export and electroneutral K^+ uptake in the Na^+K^+-ATPases. In contrast, in the H^+K^+-ATPase electrogenic H^+ export and electrogenic K^+ uptake cancel out. These findings have important implications for the mechanism of transport, specifically as to the number of fixed countercharges cotransported during the transport of cations.

In a preceding publication (*16*) we showed that electrical signals are generated by the wild-type Kdp-ATPase even in the nominal absence of K^+. Given the high affinity of the wild-type Kdp-ATPase for K^+, $K_m = 2$ μM, we could not exclude that the signal was due to K^+ contamination. Therefore, we have studied the purified Kdp-ATPase from the *kdpA42* mutant. In this mutant glutamine 116 in the KdpA subunit is replaced by arginine, resulting in an affinity for K^+ of about 6 mM (*12*). The results show that the Kdp-ATPase mediates a K^+-independent electrogenic partial reaction. On the basis of these results, we propose a model where this electrogenic step is associated with the transport of negative charges in the Kdp complex.

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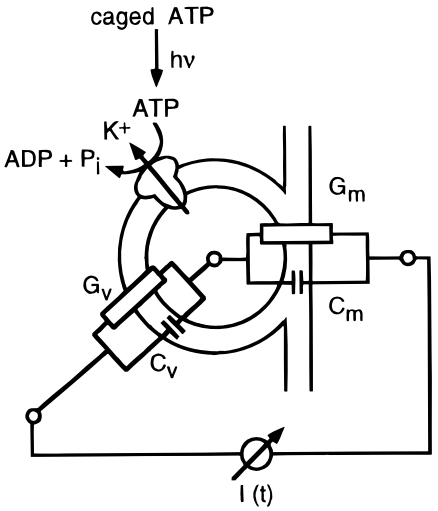

FIGURE 1: Compound membrane: proteoliposomes adsorbed to the planar lipid membrane. The current generated by the integrated Kdp-ATPase is coupled to the measuring system via the conductances G_v, G_m and capacitances C_v, C_m of the proteoliposomes and planar lipid membrane, respectively.

MATERIALS AND METHODS

Bacterial Strains and Growth Conditions. The *E. coli* K12 strain TKR1000 (*kdpA42 Δatp706 nagA trk405 trkD1 thi rha lacZ*) carries mutations in the genes coding for other K^+ uptake systems, Trk, Kup, and in the genes coding for the ATP-synthase (*2*). The strain was transformed with the plasmid pSR5 (*17*) using the $CaCl_2$ method described in ref *18*. Both the chromosomal encoded and the plasmid encoded *kdp*-genes carry the *kdpA42* mutation (according to ref *12*), which is responsible for the reduced affinity to K^+. The resulting transductant TKR1000/pSR5 was grown in minimal medium containing 0.5 mM K^+ as described in ref ref *2*.

Protein Purification and Preparation of the Proteoliposomes. The Kdp-ATPase was purified, and proteoliposomes were prepared and suspended in 15 mM HEPES-Tris, pH 7.5, as previously described (*16*). Protein concentrations were determined by the amido-black method (*19*), and ATPase activity was measured as described by ref *2*.

For experiments where Na^+-free proteoliposomes were required, 500 μL of the solution of protein in 15 mM HEPES-Tris, pH 7.5, 100 mM NaCl, and 0.2% decylmaltoside were dialyzed against 500 mL of the buffer without NaCl for 15 h with one change of the buffer after 12 h. This dialyzed protein suspension was used to prepare proteoliposomes as described by ref *16*. Flame photometry was used to measure the contamination of solutions with Na^+ and K^+.

Bilayer Measurements. The preparation of bilayers, the electrical recording instrumentation, photolysis of caged ATP, and measurements of conductivity were performed as described (*16*). The proteoliposomes are adsorbed to the planar bilayer as shown in Figure 1. For the experiment done at pH 8.4 a slight modification of the procedure was necessary. Under these conditions the adsorption of the proteoliposomes to the planar bilayer is poor. Therefore, at this pH, the experiment was started at pH 7.5 and only after adsorption of the proteoliposomes was the pH adjusted to 8.4 with NaOH.

Caged ATP, P^3-1-(2-nitro)phenylethyladenosine 5′-triphosphate Na^+ salt was purchased from Calbiochem. ACMA, 9-amino-6-chloro-2-methoxy-acridine, was purchased from Sigma. For experiments that required the absence of Na^+ the $(C_2H_5)_3NH^+$ salt of caged ATP was kindly supplied by E. Grell, MPI für Biophysik, Germany.

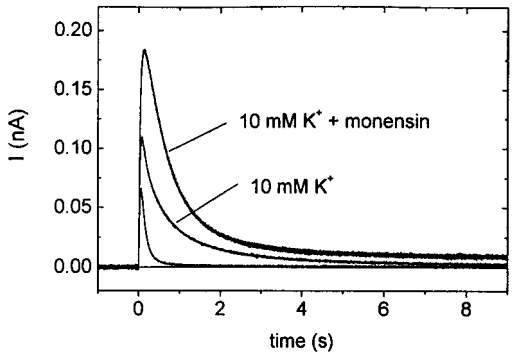

FIGURE 2: Transient currents generated by the A:Q116R Kdp-ATPase after release of ATP from caged ATP at $t = 0$. Buffer: 50 mM Tris-HCl, pH 7.5, 2 mM $MgSO_4$, 1 mM DTT, and 300 μM caged ATP. The three current traces in the figure were recorded before addition of K^+, after addition of 10 mM K^+, and in the presence of 10 mM K^+ and 10 μM monensin as indicated.

RESULTS

The K^+-stimulated ATPase activity of the proteoliposomes prepared with A:Q116R Kdp-ATPase was 20−30 μmol mg^{-1} min^{-1}, somewhat higher than that found for the wild-type used in earlier work. As noted in the earlier work (*16*), ATPase activity was not stimulated by adding valinomycin. This has been tentatively explained by the presence of a large population of unoriented molecules that dominate hydrolysis (*16*). The electrical signals, however, are generated exclusively by inside-out oriented ion pumps, which explains their sensitivity to ionophores (see below). The fact that the electrical measurements represent only the fraction of "correctly" incorporated pumps is an advantage of this technique.

Transient Electrical Currents in the Absence and Presence of K^+. Proteoliposomes were adsorbed to a planar bilayer as described before (*16*). The putative adsorption geometry is shown in Figure 1. Although probably inside-out and right-side-out oriented ion pumps coexist in the liposomal membrane, we only show the inside-out fraction that is activated via caged ATP. Upon irradiation of caged ATP with an excimer laser flash (wavelength 308 nm, duration 10 ns), ATP is generated within 45 ms under the conditions of these experiments (*16*).

In the absence of K^+ a transient current appears rapidly after ATP release (Figure 2), rising with a time constant of about 25 ms and decaying with one of about 100 ms. After addition of K^+ and monensin to facilitate the electroneutral uptake of K^+, the peak current is about three times larger. The current rises with a time constant of about 50 ms and decays with a much larger time constant of about 700 ms. If only K^+ is added the peak current has an intermediate value and the decaying phase is biphasic with relaxation times of 100 and 700 ms, respectively. In all cases an additional very slow time constant of several seconds with a small amplitude is found in the decay. A behavior similar to that observed here in the presence of K^+ was found for the wild-type Kdp-ATPase (*16*).

The stimulation of activity by the uncoupler is explained as showing that the current is limited by access of K^+ to the

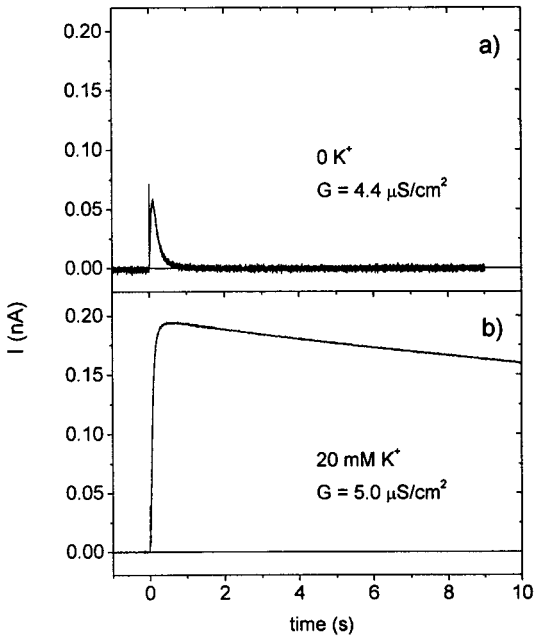

FIGURE 3: Transient current in the absence of K^+ and stationary current in the presence of K^+. The compound membrane was made conductive using the ionophores monensin (10 μM) and 1799 (4 μM). The conductivity is given in the figure. Buffer: 50 mM Tris-HCl, pH 7.5, 2 mM $MgSO_4$, 1 mM DTT, 300 μM caged ATP and KCl as indicated.

inside of the proteoliposomes, representing the external-facing aspect of the Kdp-ATPase, where it is bound for transport. In the absence of monensin, activity is presumably limited by the low concentration of K^+ in the proteoliposomes.

Interpretation of the time constants is complicated by the slow activation of the pump using caged ATP (45 ms). However, qualitative conclusions may be drawn from the different shape of the signal in the presence and absence of K^+. The rise time of the signal (25−50 ms) is apparently determined by the release of ATP. The time constants of the decaying phase measured in the presence of K^+ (700 ms) are too large to be part of the reaction cycle. For comparison, turnover of the enzyme has been estimated to be approximately 40 s^{-1} (*16*). They have to be assigned to the charging of the proteoliposomes and to the build-up of concentration gradients (*16*). The seven times faster decay of the current in the absence of K^+ indicates that no continuous charge transport is taking place under these conditions.

Transient and Stationary Electrical Currents in the Presence of Ionophores. After addition of ionophores, ATP-induced stationary currents can be measured. The combination of the electroneutral Na^+,K^+/H^+ exchanger monensin and the protonophore 1799 acts as a Na^+- and K^+-transport system and makes the planar lipid membrane and the proteoliposomes conductive. This can be described by an increase of G_v and G_m (see Figure 1). We assume that the ionophores induce similar conductivities in the planar membrane and in the proteoliposomes. Therefore, the conductivity of the compound membrane is taken as a measure of the conductivity of the planar membrane and the proteoliposomes.

The rapid transient current that is observed in the absence of K^+ but in the presence of ionophores (Figure 3a) has the same kinetic parameters as seen in the absence of ionophores (compare Figure 2). To ascertain that no K^+ was present in the experiment shown in Figure 3a, we analyzed the electrolyte of this experiment as described in the Materials and Methods section. Contaminating K^+ was only 9 μM in this experiment, far too low to activate the A:Q116R Kdp-ATPase, indicating that this transient current is not due to K^+ transport. In the presence of K^+ there is a rapid appearance of a stationary current which falls slowly over the course of many seconds due to consumption of the released ATP (Figure 3b). The stationary current demonstrates the continuous transport activity of the A:Q116R Kdp-ATPase.

In the absence of ionophores the relationship between the pump current of the enzyme and the measured current is determined by the ratio of the capacitances of the proteoliposome and the planar bilayer (C_m/C_v, see Figure 1). At high ionophore concentration it is determined by the conductances (G_m/G_v). Therefore, if a comparison of currents is made, similar conductance values are preferable.

The current traces in Figure 3, parts a and b, respectively, have been recorded in two different experiments. Note that the conductivity was nearly identical in these two measurements. Constant conductivity in a single experiment before and after addition of K^+ is not possible because the conductivity increases monotonically with time in the presence of the ionophores. Comparable conductivity in Figure 3 excludes the possibility that the absence of a stationary current without K^+ is due to a reduced conductivity of the membranes since monensin will increase conductivity in the presence of K^+ or Na^+.

Transient Currents at Different pH. To determine the role that protons might play, experiments were performed at three different pH values with the results shown in Figure 4. Transient electrical signals were generated by photolytic release of ATP from caged ATP at different times before and after the addition of K^+ and monensin. In the figure, the peak values of the current traces obtained during the experiment are plotted. Each data point corresponds to a current trace obtained at a given time after the photolytic release of ATP.

At each pH the results were similar; there is a current that is independent of K^+, and the magnitude of that current is moderately increased by adding K^+ and monensin. The absolute magnitudes were largest in the experiments at pH 7.5, but quantitative comparisons are not reliable since the amount of proteoliposomes absorbed to the bilayer may differ from one experiment to another. Interpretation of these data is complicated by the fact that the time constant for photolytic ATP release from caged ATP is 5 ms at pH 6.2 and 260 ms at pH 8.4. This was reflected by the slower rise of the signal at basic pH. For instance, the signals in the absence of K^+ rose with a time constant of 120 ms at pH 8.4 as compared to 15 ms at pH 6.2. Also, the temporal development of the peak currents after K^+ and monensin addition was different at different pH which was probably due to a higher leak conductance of the liposomes at basic pH. We will therefore restrict ourselves to a comparison of the peak current in the absence of K^+ and after complete activation using K^+ and monensin.

It is clear from Figure 4 that the peak current obtained without K^+ relative to the current at full activation is largest

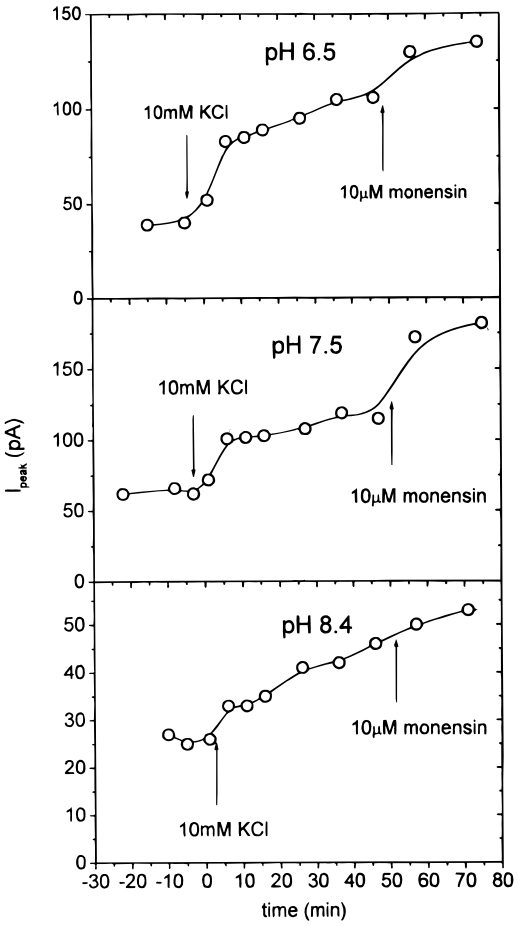

FIGURE 4: Activation of the A:Q116R Kdp-ATPase by K^+ and monensin (see arrows) at three pH values. Each point (○) corresponds to the peak value of a transient current at a time relative to the addition of 10 mM K^+. The solid line connecting the points has been drawn by eye. The buffer was Tris-HCl, 50 mM, at pH 6.5, 7.5, and 8.4, with 2 mM $MgSO_4$, 1mM DTT, and 300 μM caged ATP.

at pH 8.4, that is, at the lowest H^+ concentration. The slow release of ATP from caged ATP at this pH would only act to yield an artificially low amplitude of the current in the absence of K^+. These data suggest that protons do not play a major role in the electrical signal generated by the Kdp-ATPase in the absence of K^+, but certainly do not rule out such a possibility entirely.

K^+ Concentration Dependence of the Electrical Currents. The behavior of the electrical signals in the presence of ionophores at different K^+ concentrations is shown in Figure 5. Here the peak value of a signal (open circles) together with its stationary component (solid squares) are plotted. Peak and stationary currents are normalized to the maximal peak current. The K^+ concentrations given in the figure were corrected for a contamination of about 10 μM. For both curves, half-activation occurred near 1 mM K^+. However, no stationary current is observed at low K^+ concentration while a transient current is present. At high KCl concentrations the transient as well as the stationary current decreases. This is an anion effect that has been previously observed in the wild-type Kdp-ATPase (*16*).

The dependence of the stationary current on K^+ concentration must be interpreted with caution. Since the conductivity induced by the ionophore depends on the presence of K^+ (and partly H^+), conductivity cannot be the same at all K^+

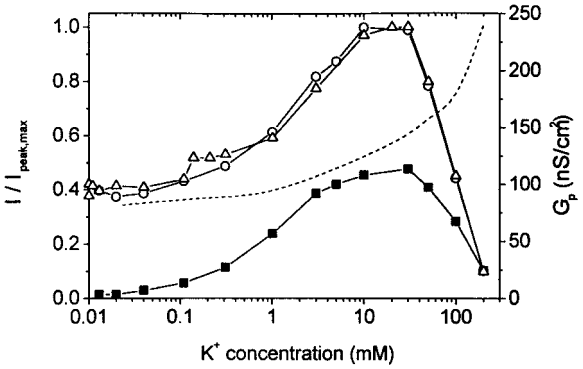

FIGURE 5: K^+ concentration dependence of the normalized stationary currents $I_{stat}/I_{peak,max}$ (■) and peak currents $I_{peak}/I_{peak,max}$ (○). The stationary and peak currents at a given K^+ concentration were determined from the same current trace in the presence of the ionophores monensin and 1799. The dashed line gives the conductivity of the compound membrane (right axis) as measured simultaneously at the different K^+ concentrations. For comparison, also the normalized peak currents in an experiment in the absence of the protonophore 1799 are shown (△). Buffer: 50 mM Tris-HCl, pH 7.5, 2 mM $MgSO_4$, 1 mM DTT, 300 μM caged ATP, and KCl as indicated.

concentrations. The conductivity measured at the different K^+ concentrations is shown by the dashed line in Figure 5. The K^+ dependence of the stationary current may therefore be partially distorted by the conductivity changing with K^+ concentration. However, our claim that no stationary current is measured in the absence of K^+ and that it develops after the addition of K^+ is still valid. This has been shown in Figure 3, where care has been taken to establish the same conductivity level in the absence of K^+ as in its presence. Comparable conductivities could be obtained in Figure 3 because these are separate experiments where the reduced conductivity in the absence of K^+ was compensated for by larger amounts or a longer incubation time of the ionophore. (After addition of the ionophores the conductivity rises slowly within 1 or 2 h. Therefore, the conductivity can be adjusted to the desired level by varying the concentration of ionophores and/or the incubation time.)

The relatively low conductance of the membrane in the experiment shown in Figure 5 also accounts for the fact that at all K^+ concentrations the peak current is always considerably larger than the stationary current. Under these conditions, the system is still partially capacitively coupled. In contrast, the much higher conductivity of the membrane in Figure 3 leads to complete DC coupling and concomitant disappearance of the peak current. To exclude that changing the conductivity of the compound membrane during the K^+ titration influences the peak currents, we also measured the K^+ dependence of the peak currents in the absence of 1799 but in the presence of monensin (Figure 5, open triangles). No difference from the data set registered in the presence of 1799 was found.

The Effect of Na^+. To determine to what extent the results obtained were due to Na^+, we also did experiments in which we took pains to reduce contamination with Na^+ as much as possible. "Na^+-free" proteoliposomes were prepared as described in the methods section. These Na^+-free preparations, which contained 15 μM Na^+, exhibited a transient current in the absence of K^+. However, addition of Na^+ in the absence of K^+ increased the transient current by a factor of 2, with an affinity for Na^+ of 2 mM. This concentration

is similar to that found for activation by K^+ (Figure 5). In contrast to the effect of K^+, no stationary current was obtained with Na^+ alone at concentrations up to 100 mM Na^+ (data not shown).

ATP and Caged ATP Dependence. Caged ATP was found to be a competitive inhibitor of ATP binding in several ATPases (*20*, *21*). In wild-type Kdp-ATPase we determined binding constants of $K_a = 68$ μM for ATP and $K_c = 630$ μM for caged ATP in the presence of 20 mM K^+ (*16*). For A:Q116R Kdp-ATPase similar values were found in the presence of 20 mM K^+: $K_a = 68$ μM, $K_c = 670$ μM. In contrast, no competitive binding of caged ATP was found in the absence of K^+ while ATP binds with $K_a = 160$ μM.

DISCUSSION

Properties of A:Q116R Kdp-ATPase. The mutant *kdpA42* was isolated by random mutagenesis in a selection for Kdp systems with reduced affinity for K^+ (*22*). The corresponding A:Q116R Kdp complex shows an increased K_m for K^+ (6 mM), as monitored by the determination of the K^+-stimulated ATPase activity of the purified complex (*2*, *3*) or in K^+-transport measurements with whole cells (*22*, *12*).[1] However, the narrow ion selectivity of the Kdp system is retained in A:Q116R Kdp-ATPase. Since the A:Q116R Kdp-ATPase shows a comparable phosphorylation reaction and affinity for ATP as the wild-type complex, it was chosen for detailed investigation of the reaction cycle (*3*), thereby avoiding the interfering influence of micromolar K^+ concentration that could activate the wild-type enzyme.

Charge Transport. In many aspects the shape and behavior of the electrical signals of A:Q116R Kdp-ATPase are similar to those of the wild-type enzyme. The A:Q116R Kdp-ATPase is electrogenic: continuous electrogenic charge transport is found as demonstrated by the stationary currents.

Distinct differences between wild-type and A:Q116R Kdp-ATPase exist concerning the K^+ dependence of the enzymatic and electrical activity. From measurements of the K^+-stimulated ATPase activity of the purified A:Q116R Kdp-ATPase (*2*, *3*; S. Dröse & K. Altendorf, unpublished results) and from K^+-transport assays with whole cells (*22*), an apparent affinity for K^+ of 6 mM was reported. This is somewhat larger than the value of 1 mM determined from the electrical experiments, a difference that could be attributed to the nonsaturating ATP concentration used in these measurements. Both the stationary and the transient current are activated by K^+ at the same concentration (see Figure 5). However, even in the absence of K^+, a transient electrical signal is observed.

An Electrical Signal in the Absence of K^+. According to our present knowledge, the Kdp-ATPase is uniquely designed to maintain intracellular K^+ levels in a low K^+ environment. This explains its high K^+ affinity and specificity. It was therefore surprising to find an electrical signal after activation of the pump even in the absence of K^+. This transient current has to be assigned to the Kdp-ATPase since it is inhibited by more than 70% after addition of 1 mM orthovanadate (data not shown). Also an artifact from the photolytic cleavage of caged ATP in the binding site can be ruled out since caged ATP does not bind to A:Q116R Kdp-ATPase in the absence of K^+. There are several possible explanations for an electrical activity of the Kdp-ATPase in the absence of K^+: (1) K^+ contamination of the nominally K^+-free buffer; (2) transport of ions other than K^+, like Na^+ or H^+; and (3) an electrogenic conformational transition, that is, a structural rearrangement of the protein with the concomitant movement of charges fixed on the protein. In the following we will discuss these possibilities and show that it is probably the latter that occurs in A:Q116R Kdp-ATPase.

Buffers with a K^+ concentration of less than 10 μM are difficult to obtain. On the other hand the wild-type Kdp-ATPase has a K^+ affinity in this range (2−10 μM; refs *5*, *3*). To circumvent this problem, we have chosen the A:Q116R complex which exhibits a K_m of 6 mM. In experiments involving a K^+-free electrolyte we measured the solution in the cuvette after the experiment and found K^+ levels of 10 μM or less. A transient transport activity of the A:Q116R Kdp-ATPase due to residual K^+ can therefore be excluded.

To exclude Na^+ transport, we used Na^+-free proteoliposomes in some experiments. When this preparation was used, the Na^+ concentration in the electrolyte was somewhat higher than that of K^+ (15 μM). However, it is highly unlikely that a high-affinity Na^+ binding site exists in the A:Q116R Kdp-ATPase or that Na^+ can replace K^+ at concentrations of 20 μM. In addition, neither a Na^+-activated ATPase activity nor an influence of Na^+ on the phosphorylation level was observed with the purified A:Q116R Kdp-*ATPase* (*3*). Therefore, activation of transient charge movement by Na^+ has to be rejected.

Protons have been shown to substitute for Na^+ and K^+ in Na^+K^+-ATPase under certain conditions (*23*). However, in fluorimetric experiments using the H^+-sensitive dye ACMA, we could not find an indication of H^+ transport (data not shown). Also, the pH dependence of the electrical signal does not seem to indicate an involvement of H^+. The transient signal in the absence of K^+ relative to the signal at full activation in the presence of K^+ and monensin is largest at pH 8.4, that is, at the lowest H^+ concentration (Figure 4).

Current concepts of ionic transport assume that structural rearrangements (conformational transitions) of the protein are involved with the translocation process. The $E_1 \rightarrow E_2$ and $E_1P \rightarrow E_2P$ transitions of the Na^+K^+-ATPase are believed to be such conformational transitions (*24*). Associated with such movement of protein domains, charge movement can easily be envisaged. In particular, it is possible that movement of a charge close to or within a cation binding site is responsible for an experimentally detectable charge translocation. Cation binding sites of the Na^+K^+-ATPase have indeed been proposed to possess negative charges (*25*−*27*).

There is however a principal difference between the charge movement associated with a transport event and that associated with a charge fixed on the protein. Since the transported ion is bound on one side of the protein and released at the other, continuous transport activity will generate a stationary current. On the other hand, if charges fixed on the protein

[1] The reported reduced discrimination between K^+ and Rb^+ of the A:Q116R Kdp-ATPase (K_m: 6 mM for K^+, 50 mM for Rb^+ (*12*)) is probably not correct, because these transport measurements were not corrected for the residual "TrkF" system that does transport Rb^+. Therefore, the comparatively low Rb^+-transport activity of this mutant strain is independent from the Kdp system. The purified A:Q116R Kdp-ATPase shows no Rb^+-dependent ATPase activity (Dröse and Altendorf, unpublished results).

are translocated, the net charge moved has to be zero; no stationary current is observed. Nevertheless a transient charge movement will be measured indicating the electrogenic nature of the conformational transition.

The electrical signal observed in the absence of K^+ shows properties that indicate that the corresponding reaction is an electrogenic conformational transition where charges fixed on the protein are moved. In particular, the absence of a stationary current in the presence of ionophores but in the absence of K^+ is consistent with this assignment. The sign of the signal corresponds to translocation of negative charges to the extracellular side or that of positive charges to the intracellular side of the protein.

Transient versus Stationary Charge Translocation by the Kdp-ATPase. Stationary pump currents can be recorded if the bilayer and the proteoliposomes have been made conductive using the ionophores monensin and 1799. The combination of these two ionophores enables K^+, Na^+, and H^+ to pass through the lipid membrane. Stationary currents indicating a continuous pumping activity of the enzyme could only be obtained with K^+. No stationary electrical activity could be measured in the presence of up to 100 mM NaCl or CsCl (data not shown).

A kinetic model for the Kdp-ATPase has to account for these two key observations, namely a transient current in the absence and a stationary current in the presence of K^+. There are basically two possible explanations for this behavior: (1) a K^+-dependent kinetic bottleneck, that is, an electrogenic reaction followed by a partial reaction that is not possible in the absence of K^+; and (2) an electrogenic conformational transition in the absence of K^+ and an additional K^+ translocation reaction in its presence. In the first case no stationary current is generated in the absence of K^+ because the reaction is stopped by the kinetic bottleneck. In the second case there is no overall charge transport in the absence of K^+ because only charges fixed on the protein are moved.

The behavior of the stationary currents is similar to the hydrolytic activity of the Kdp-ATPase which is approximately 10-fold larger in the presence than in the absence of K^+ (*3*). In contrast, the behavior of the transient electrical signal bears some resemblance with that of the phosphorylation reaction, namely that no monovalent cations are required for this reaction (*3*). Taken together, these findings seem to indicate that the reaction cycle of the Kdp-ATPase contains a partial reaction that is slow in the absence of K^+. A possible candidate for this reaction is the dephosphorylation reaction which was shown to be accelerated by K^+ (*3*). However, more recent measurements indicate only a minor influence of K^+ on the rate constants of the reaction cycle (data not shown). The decision about whether a kinetic bottleneck or the translocation of fixed protein charges or both are responsible for the observed electrical properties has therefore to remain open.

Comparison with Other P-type ATPases and a Kinetic Model. P-type transport ATPases catalyze the transport of a single ion species (H^+-ATPase from fungi) or the countertransport of two different types of ions (Na^+K^+-ATPase, H^+K^+-ATPase from stomach, SERCA Ca^{2+}-ATPase, plasma membrane Ca^{2+}-ATPase). For a review see ref *9*. Details about the transport mechanism are, however, scarce. Therefore, a comparison of the Kdp-ATPase has to be mainly based on facts known about the most thoroughly studied system, the Na^+K^+-ATPase.

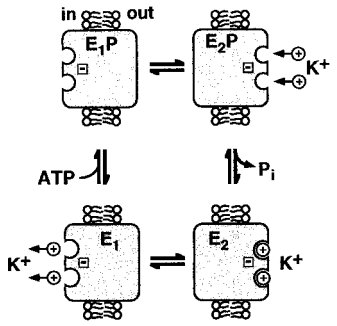

FIGURE 6: Kinetic model for K^+ transport by the Kdp-ATPase. Details are given in the text.

As far as their transport mechanism is known, in all P-type ATPases the formation of the phosphoenzyme E_2P is correlated with the translocation of one or more ions to the extracellular side of the membrane. In a subsequent reaction the enzyme returns to the "ground state" E_1 with (for countertransporting systems) or without concomitant transport of ion(s) to the intracellular side (*9*). The Kdp-ATPase is different from all other P-type ATPases in that it transports ions exclusively to the intracellular compartment. It is therefore tempting to associate K^+ translocation in the Kdp-ATPase with a reaction downstream of E_2P. This is exactly analogous to K^+ transport in the Na^+K^+-ATPase and the H^+K^+-ATPase and leads us to the question whether the Kdp-ATPase is a Na^+K^+-ATPase lacking the Na^+ translocation step.

A kinetic model is shown in Figure 6. The assignment of the K^+ translocation step to the $E_1 \rightarrow E_2$ transition is based on the analogy with the Na^+K^+-ATPase. The assignment to a late event in the reaction cycle is supported by the finding that the turnover in the absence of K^+ is greatly reduced (*3*) while other activities such as phosphorylation and a transient current are still possible. Because Na^+ translocation does not take place in the Kdp-ATPase, the empty binding site must be translocated to the extracellular surface where it is loaded with K^+ ions. Again, the assignment of the translocation step for the empty binding site is made in analogy with the Na^+ translocation step of the Na^+K^+-ATPase.

No experimental information about transport stoichiometry is available at present. We have assumed transport of 2 K^+ ions as in the case of the Na^+K^+- and the H^+K^+-ATPase.

If the binding site carries a negative charge the translocation of the empty binding site would result in a transient current as observed in the Kdp-ATPase in the absence of K^+. The direction of the current expected from this charge translocation is the same as that of K^+ transport into the cell. This model is consistent with the observation of a transient current in the absence of K^+ and that of a stationary current of the same polarity in the presence of K^+.

In this context it is important to note that the expression "translocation of an ion" or "translocation of a binding site" are used from an "electrical" point of view. Rather than considering a translocation from one surface of the protein to the other a change in accessibility of the immobile ion or binding site can be envisaged (alternating-access mechanism, ref *28*). The alternating access mechanism is electrically equivalent to the translocation of the ion, both processes

giving rise to an electrical signal. For simplicity we use the expressions "translocation of an ion" or "translocation of a binding site" for both concepts.

An inherent property of the model shown in Figure 6 is that 2 positive charges are transported per turnover regardless of the number of negative charges in the binding sites. However the relative contribution of the electrogenic steps $E_1P \rightarrow E_2P$ and $E_2 \rightarrow E_1$ is different. We chose one negative charge because the initial current approximately doubles upon addition of K^+ and monensin (Figure 2). This is expected if equal amounts of charge are translocated in the two conformational transitions (and if the second step is not much slower). Because in the first step negative charge is translocated outward and in the second positive charge is transported inward, both reactions give a positive contribution. Note that the outward transport of negative charge and the inward transport of positive charge are both favored by the negative inside membrane potential. The fact that both translocation steps are speeded up by membrane potential could be a strategy to avoid a kinetic bottleneck in one of these steps.

The model of Figure 6 accounts for the experimental finding, namely, a transient electrical signal in the absence and a stationary current in the presence of K^+. Assignments of the electrogenic steps to partial reactions of the reaction cycle are based on analogy with the Na^+K^+-ATPase. Whether this is justified has to await time-resolved phosphorylation and electrical measurements that are in progress. Also, future efforts are aimed at the identification of the negative residues responsible for the transient signal.

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