Binding of a Single Rb\(^+\) Increases Na\(^+\)/K\(^+\)-ATPase, Activating Dephosphorylation without Stoichiometric Occlusion*

Sergio B. Kaufman, Rodolfo M. González-Lebrero, Rolando C. Rossi\(^1\), and Patricio J. Garrahan\(^1\)

From the Instituto de Química y Físicoquímica Biológicas y Departamento de Química Biológica, Facultad de Farmacia y Bioquímica, Universidad de Buenos Aires, Junín 956, C1113AAD Buenos Aires, Argentina

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We used partially purified Na\(^+\)/K\(^+\)-ATPase from pig kidney to study dephosphorylation, occlusion, and ATPase activity in the same enzyme preparation and in media of identical composition containing 10 \(\mu\)M ATP and different concentrations of Rb\(^+\), used as a K\(^+\) congener. The experiments were performed using a rapid-mixing apparatus with a time resolution of 3.5 ms. The main findings were as follows. (i) At sufficiently low Rb\(^+\) concentration the initial rate of dephosphorylation was higher than that of occlusion, (ii) as [Rb\(^+\)] tended to zero the slope of the time course of occlusion but not that of the time course of dephosphorylation approached zero and, (iii) as Rb\(^+\) concentration increased, ATPase activity first increased and, after passing through a maximum, tended to a value that was lower than that observed in media without Rb\(^+\). None of these results is compatible with the currently held idea that binding of a single Rb\(^+\) to the \(E_2P\) conformer of the ATPase does not modify the rate of dephosphorylation and strongly suggest that a single Rb\(^+\) does promote dephosphorylation through a mechanism that is not stoichiometrically coupled to Rb\(^+\) occlusion. If this mechanism is included in the currently accepted scheme for ATP hydrolysis by the Na\(^+\)/K\(^+\)-ATPase, a reasonable prediction of the experimental results is obtained.

During its physiological operation, the plasma membrane Na\(^+\)/K\(^+\)-ATPase couples the hydrolysis of ATP to the transport of three intracellular sodium ions in exchange for two extracellular potassium ions. According to the currently accepted scheme of ATP hydrolysis, this takes place in a series of steps that include at least (Fig. 1) (i) the (Na\(^+\) + Mg\(^2+\))-dependent phosphorylation by ATP of the \(E_1\) conformer of the enzyme to form the \(E_1P\) phosphoenzyme (phosphorylation requires the binding of three Na\(^+\) ions that are taken up from the cytosol and trapped in \(E_1P\)), (ii) the \(E_1P \rightarrow E_1P\) transition of the phosphoenzyme (this is accompanied by the release of Na\(^+\) to the extracellular medium), (iii) the activation by extracellular K\(^+\) of the dephosphorylation of \(E_1P\) (it is generally accepted that this requires the binding of two K\(^+\) ions that are taken up from the extracellular medium and trapped in \(E_2\)), and finally (iv), the return of \(E_2\) to \(E_1\) with the release K\(^+\) into the cytosol. The binding of ATP to a noncatalytic site in \(E_2\) whose \(K_m\) (≈200 \(\mu\)M) is at least 10\(^4\) times higher than the \(K_m\) of the active site of the ATPase (≈0.2 \(\mu\)M) leads to a 100-fold increase in the rate of the \(E_2(K_2) \rightarrow E_1 + 2K^+\) reaction. Hence, as the concentration of ATP tends to zero the release of K\(^+\) becomes the slowest step of the Na\(^+\)/K\(^+\)-ATPase cycle (see Refs. 1 and 2). The Na\(^+\) and K\(^+\) taken up by the enzyme in steps (i) and (iii) are in a state in which they exchange slowly with the incubation media. This is usually called "occlusion" and is considered to be the expression of an intermediate step in the movement of Na\(^+\) and K\(^+\) across the ATPase from one surface of the membrane to the other. No direct structural information with enough resolution is yet available to identify the occlusion domains in the enzyme.

Phosphorylation is absolutely dependent on Na\(^+\), whereas for activation of dephosphorylation K\(^+\) can be replaced with varying degrees of effectiveness by Rb\(^+\), Cs\(^+\), Li\(^+\), NH\(_4^+\), or Tl\(^+\). At least Rb\(^+\) (3) or Tl\(^+\) (4) also replaces K\(^+\) for occlusion. Dephosphorylation persists in the absence of K\(^+\), albeit at a rate that is 100 \(\times\) slower than in the presence of K\(^+\). This gives rise to an ATPase activity, called the Na\(^+\)-ATPase, which requires Na\(^+\) and Mg\(^2+\) and is coupled to the transport of Na\(^+\). Na\(^+\)-ATPase is switched off as K\(^+\) or its congeners drive dephosphorylation toward the pathway that leads to occlusion.

The model in Fig. 1 has three branching points at the \(E_2P\) states that lead to three pathways. The velocity of dephosphorylation in the absence of products \(v_{\text{dephos}}\) will be the sum of the contributions of the three pathways, i.e.

\[
v_{\text{dephos}} = k_{40}[E_2P] + k_{41}[E_2PK] + k_{42}[E_2PK_2] \quad \text{(Eq. 1)}
\]

The relative abundance of each state of \(E_2P\) will depend on the concentration of K\(^+\). Assuming rapid equilibrium for the addition of K\(^+\), this dependence will be

\[
[E_2P] = \frac{E_2P_0}{1 + \frac{[K^+]^2}{K_{K1}} + \frac{[K^+]^2}{K_{K2}}} \quad \text{(Eq. 2)}
\]

\[
[E_2PK] = \frac{E_2PK_0}{1 + \frac{[K^+]^2}{K_{K1}} + \frac{[K^+]^2}{K_{K2}}} \quad \text{(Eq. 3)}
\]

\[
[E_2PK_2] = \frac{E_2PK_2}{1 + \frac{[K^+]^2}{K_{K1}} + \frac{[K^+]^2}{K_{K2}}} \quad \text{(Eq. 4)}
\]

where \(K_{K1}\) and \(K_{K2}\) are equilibrium dissociation constants and \(E_2P_0 = [E_2P] + [E_2PK] + [E_2PK_2]\). \(E_2P_0\) will be equal to the concentration of \(E_2P\) in the absence of K\(^+\) when all the enzyme is catalyzing Na\(^+\)-ATPase activity.

Equations 2–4 illustrate the more general fact that, as [K\(^+\)] goes from zero to infinity, the following will occur. (i) \([E_2P]\) will decrease continuously toward zero (Equation 2), and the same will happen with Na\(^+\)-ATPase activity. (ii) \([E_2PK]\) will start at zero, pass through a maximum,
through a maximum, tend to a lower value as \([\text{Rb}^+]\) reaches saturation. This is so because it is known that under these conditions the \(E_2(K_3) \rightarrow E_1 + 2K^+\) step is slower than any other.

We further analyzed the consequences of our hypothesis by simulating the behavior of the model in Fig. 1 to which we added the pathway enclosed within the shaded area. \(E_1\) was included in the reaction scheme to evaluate whether the dephosphorylation of \(E_2PK\) leads to occlusion. To perform this, we fitted analytical solutions of the model to the experimental data of the effects of \([\text{Rb}^+]\) on the steady-state levels of ATPase activity, \(E_P\), and occluded \(\text{Rb}^+\), measured in media of identical composition and temperature as those used to measure the rates of dephosphorylation and occlusion. The values of the kinetic parameters thus obtained were used to predict the effects of \([\text{Rb}^+]\) on the above-mentioned rates.

Results reported here constitute the first detailed study of the time course of \([\text{Rb}^+]\) occlusion through the physiological route. This allowed us also for the first time to correlate the kinetics of occlusion with that of dephosphorylation. A preliminary report of some of the results presented in this work has been published (6).

**EXPERIMENTAL PROCEDURES**

\(\text{Na}^+\)/\(\text{K}^+\)-ATPase was partially purified from pig kidney outer medulla according to Jensen et al. (7) and kindly provided by the Department of Biophysics, University of Århus, Denmark. All the results shown in this paper were obtained from experiments performed at 25 °C with enzyme suspended in reaction media containing 150 mM NaCl, 10 \(\mu\)M ATP, 0.7 mM MgCl\(_2\), 0.2 mM EDTA, and 25 mM imidazole-HCl (pH 7.4 at 25 °C) and the concentrations of \([\text{Rb}^+]\) indicated in the figures.

The time courses of dephosphorylation and of occlusion were measured in experiments carried out using a rapid-mixing apparatus, SFM4/Q, developed by Bio-Logic (France). The experiments were started adding the desired concentrations of \([\text{Rb}^+]\) to the incubation medium in which the enzyme (final concentration 40–50 \(\mu\)g/mL) performed steady-state \(\text{Na}^+\)/\(\text{K}^+\)-ATPase activity. The time course of dephosphorylation was measured by monitoring the transition of the \(^32\text{P}\)-labeled phosphoenzyme to a new steady state after the addition of enough of \([\text{Rb}^+]\) to attain the concentrations indicated in the legend to the figures. Reactions were stopped by chemical quenching with trichloroacetic acid (final concentration 10 g/100 mL) as described by Schwarzbaum et al. (8). The time course of occlusion was measured using \(^{86}\text{Rb}^+\). Because acid denaturation by chemical quenching releases occluded \([\text{Rb}^+]\), occlusion was measured after stopping the reaction by rapid cooling-and-washing following the procedure developed in our laboratory (9). This procedure, which preserves the structural integrity of the enzyme, is able to stop deocclusion reactions that proceed with rate constants of up to 25 s\(^{-1}\) without significant loss of \([\text{Rb}^+]\) (9).

The initial rates of \([\text{Rb}^+]\)-dependent dephosphorylation and of \([\text{Rb}^+]\) occlusion were calculated from (i) the solution at \(t = 0\) of the first derivative of the functions fitted to the complete time courses (Fig. 2), (ii) the slope of the straight lines fitted to data of occluded \([\text{Rb}^+]\) and of phosphoenzyme concentrations measured at the time of addition of \([\text{Rb}^+]\) (\(t = 0\)) and after a 93-ms-long incubation. This alternative (“single-time measurements”), which was particularly convenient to increase the precision of the comparison between the initial rates of occlusion and dephosphorylation, allowed a higher density of data at the lower \([\text{Rb}^+]\) concentrations (Fig. 3, B–E), when the rates were small enough to fulfill initial rate conditions after 93 ms.

The steady-state concentration of occluded \(\text{Rb}^+\) was measured by
mixing the rapid-mixing apparatus equal volumes of a suspension of Na+/K+-ATPase (80–100 µg of protein/ml) in reaction media with the same environment containing 20 µM ATP and different concentrations of 86Rb+. Reaction mixtures were incubated for 3.5 s before being injected into the cooling-and-washing chamber. Control experiments using aging times from 3 to 10 s showed no variation in the amounts of occluded rubidium, indicating that 3.5 s was enough to reach steady state.

The steady-state concentration of phosphoenzyme was measured by the same procedure as that used for occlusion except that [γ-32P]ATP was used. After 5.6 s, the reaction was quenched by mixing 3 volumes of the reaction mixture with 2 volumes of an ice-cold solution of 25 g/100 ml trichloroacetic acid and 50 mM H3PO4.

ATPase activity was determined according to Schwarzbaum et al. (8), measuring the amount of [32P]P released from [γ-32P]ATP. The reaction medium was identical as that used for the occlusion and dephosphorylation experiments. Enzyme concentration was 10 µg of protein/ml. Incubation times varied between 5 and 40 s to avoid the hydrolysis of more than 10% of the ATP, thus ensuring initial rate conditions. Blanks were measured in the media where all Na+ was replaced by K+.

Theoretical equations were adjusted to the results by weighted non-linear regression based on the Gauss-Newton algorithm using commercial programs (Excel, Sigma-Plot, and Mathematica for Windows). Weighting factors were calculated as the reciprocal of the variance of experimental data.

[γ-32P]ATP was either purchased as such or synthesized using the procedure of Glynn and Chappel (10), except that no unlabeled orthophosphate (P) was added. Carrier-free [32P]P [86Rb]RbCl, and [γ-32P]ATP were from PerkinElmer Life Sciences. ATP, enzymes, and reagents for the synthesis of [γ-32P]ATP were from Sigma. All other reagents were of analytical grade.

**RESULTS AND DISCUSSION**

Comparison between the Initial Rates of Dephosphorylation and Occlusion—We measured the time courses of dephosphorylation and of Rb+ occlusion in parallel experiments using the same enzyme preparation in media of the same composition and temperature. All time courses were determined after the addition of Rb+ to enzymes that perform steady-state Na+-ATPase activity. Results are shown in Fig. 2, A (dephosphorylation) and B (Rb+ occlusion).

We calculated the initial velocities of Rb+ -dependent dephosphorylation (v0 dep) and Rb+ occlusion (v0 occ) from the absolute values of the initial slopes of their time courses. These are plotted as a function of Rb+ concentration in Fig. 3. It can be seen that both velocities rose with [Rb+] along parabolic curves (panel A). This strongly suggests that both curves are the initial part of sigmoid functions that do not approach saturation even at 1000 µM [Rb+]. A striking feature of the results in Fig. 3 is that for Rb+ concentrations less than 250 µM, dephosphorylation is faster than occlusion (v0 dep > v0 occ). This difference is cancelled at concentrations higher than 250 µM, from which v0 occ becomes progressively larger than v0 dep. At 1000 µM Rb+, v0 dep is almost twice as fast as v0 dep. This is to be expected if at sufficiently high [Rb+] dephosphorylation occurs via the EPRb2 → E (Rb2+) pathway.

In panel B, the initial part of v0 dep and v0 occ as a function of [Rb+] have been zoomed in to plot values between 0 and 35 µM Rb+. It is clear that v0 dep increases with [Rb+] even at the lowest concentrations tested. This indicates that binding of a single Rb+ to EPRb2 is sufficient to promote dephosphorylation. Therefore, these results are compatible with our hypothesis that k41 > k40.

The meaning of the shapes of v0 dep and v0 occ at low [Rb+] can be analyzed considering that

\[
\nu_{\text{dep}} = \frac{v_{\text{dep}} - k_{40}E_{2}P_{0}}{k_{41}[E_{2}]PRb + k_{42}[E_{2}PRb_{2}] - k_{40}E_{2}P_{0}} \quad \text{(Eq. 5)}
\]

and

\[
\nu_{\text{occ}} = \alpha k_{41}[E_{2}]PRb + 2k_{42}[E_{2}PRb_{2}] \quad \text{(Eq. 6)}
\]

where α is a coefficient whose value will be either 1 or 0 depending on whether or not E2PRb leads to the occlusion of a single Rb+. Note that, under the conditions of our experiments, the initial rate of Rb+-depend-
ent dephosphorylation will be the difference between the rates of breakdown and of formation of EP. Because before the addition of Rb⁺ the enzyme performs Na⁺-ATPase activity in steady state, the rate of dephosphorylation will be exactly balanced by that of phosphorylation. For this reason the term −k_{depho}P₀ appears in Equation 5.

If each state of EₚP is expressed as in Equations 2–4, then at very low [Rb⁺], Equations 5 and 6 can, respectively, be approximated by

\[ v_{depho,Rb}^0 = EₚP₀ \left( k_{41} - k_{40} \frac{[Rb⁺]}{Kₐ₁} + (k_{42} - k_{40}) \frac{[Rb⁺]^2}{K_{Rb1}K_{Rb2}} \right) \]  

(Eq. 7)

and

\[ v_{occ}^0 = EₚP₀ \left( \alpha k_{41} \frac{[Rb⁺]}{Kₐ₁} + 2k_{42} \frac{[Rb⁺]^2}{K_{Rb1}K_{Rb2}} \right) \]  

(Eq. 8)

which show that both v_{occ}^0 and v_{depho,Rb}^0 include a linear and a parabolic term in [Rb⁺]. In the case of v_{depho,Rb}^0, the initial slope will be positive (as in the results in Fig. 3B) only if k_{41} > k_{40}; that is, if there is activation by a single Rb⁺. In the case of v_{occ}^0, a positive initial slope indicates α > 0. If one Rb⁺ were occluded in Eₚ, α would be equal to 1, and the initial slope of v_{occ}^0 would be slightly larger than that of v_{depho,Rb}^0 (cf. linear terms in Equations 7 and 8). This is in stark contrast with our results in which the initial slope of v_{occ}^0 is about one-third that of v_{depho,Rb}^0, indicating the absence of stoichiometric occlusion.

Effects of Rb⁺ Concentration on ATPase Activity, Phosphoenzyme, and Occluded Rb⁺ in Steady State—In the experiment whose results are shown in Fig. 4, ATPase activity was measured in media containing 10 μM ATP and from zero to 1000 μM Rb⁺. It can be seen that the response to [Rb⁺] is biphasic, with an initial activation that shows no signs of sigmoidicity and a maximum at around 40 μM Rb⁺ (see the inset) followed by a decrease to an asymptotic value that is lower than that observed at zero [Rb⁺]. We have shown previously (11) that inhibition by Rb⁺ such as that seen in the second phase in Fig. 4 is not caused by displacement of Na⁺ from the sites from which it activates the ATPase.

The results in Fig. 4 are similar to those obtained by other authors (12, 13). They also agree with the observation by Vilsen (14) who found an analogous response to K⁺ using 3 mM ATP in a mutant of rat kidney Na⁺/K⁺-ATPase with very low affinity for ATP.

The most likely cause of the activation by Rb⁺ is that k_{41} > k_{40}. The lack of sigmoidicity in the activation phase supports the idea that activation reflects the binding of only one Rb⁺ to the phosphoenzyme. Notice that if k_{41} = k_{40} ATPase activity would continuously decrease with [Rb⁺]. This is so because the only effect of Rb⁺ would be to shift the rate-limiting step from that governed by k_{40} to Eₚ to Eₚ transition, whose value at 10 μM ATP is 1.3 s⁻¹ (5). This mechanism would account for the inhibition by Rb⁺ shown in Fig. 4 and was first proposed by Post et al. (12) in their pioneering work on occlusion.

The steady-state levels of EP and of Rb_{occ} (Figs. 5 and 6, respectively) are sigmoid functions of Rb⁺ concentration. Comparison of these
results with those in Fig. 3 shows that the apparent affinity for Rb + is much higher for the steady-state results (K0.5 ≈ 100–200 μM) than for the effects of Rb + on the initial velocities. The reason of this discrepancy is that initial velocity only measures the rate of Rb + binding, whereas the steady-state measurements include the effect of the formation and breakdown of occluded Rb +. Because the rate of deocclusion is very low at 10 μM ATP, the enzyme accumulates in its occluded state, which acts as a sink.

In Fig. 7 we plotted the sum of the concentrations of EP and of occluded enzyme (½ Rbocc) as a function of [Rb +]. It can be seen that the sum reaches a minimum at about 200 μM Rb +. This result is consistent with the idea that at intermediate Rb + concentrations there is an accumulation of enzyme states that are neither phosphorylated nor occluded. These states might correspond to those, like Es, that participate in the activation of dephosphorylation by a single Rb +.

The experimental data of the steady-state values of ATPase activity (Fig. 4), phosphorylation (Fig. 5), and occlusion (Fig. 6) were fitted to the following equation,

\[ Y = \frac{Y_0 K_1 K_2 + Y_1 K_2 [Rb^+] + Y_2 [Rb^+]^2}{K_2 + K_3[Rb^+] + [Rb^+]} \]  

(Eq. 9)

where Y0 and Y1 are the values of the steady-state property being measured in the absence and presence of saturating concentrations of Rb +, respectively, Y1 is the contribution of Y at intermediate Rb + concentrations, and K1 and K2 are apparent constants for the dissociation of Rb + from the enzyme.

As can be seen by the continuous lines that fit the experimental points, Equation 9 gave an excellent description of the three steady-state results. Inspection of the best-fitting values of the parameters in Table 1 shows the following. (i) For Rb + occlusion, Y0 and Y1 tended to values that were not significantly different from zero. This is consistent with the lack of states with only one Rb + occluded per enzyme. (ii) Y1 > Y0 > Y2 for ATPase activity determines the existence of the enzyme, and that the activity at non-limiting Rb + concentration is less than the Na + -ATPase activity (Fig. 4). (iii) In the case of EP levels, Y0 > Y1 > Y2, which fits with the continuously decreasing curve of EP versus [Rb +]. (iv) Y2 for Rb + occlusion is twice the value of Y0 for EP, as it is to be expected if all the enzyme went from EP to E2(Rb2) as [Rb +] went from zero to infinity. (v) The ratio Y1/2 for Rbocc is equal to the value of E2(E2(Rb2)) at saturating [Rb +]. Under this condition Y1/2 times the apparent rate constant for the E2(Rb2) → E1 + 2Rb + step at 10 μM ATP (1.3 s⁻¹) approaches closely the value of Y2 for the ATPase activity. This fits the idea that at saturating [Rb +] all the reaction flow takes place through this step. (vi) The values of K1 and K2 for each steady-state property tested are sufficiently close to make it likely that the three properties obey the same mechanism. In fact, if the average value of K1 and K2 for the three properties are used for fitting Y0, Y1, and Y2, the resulting curves describe very well the experimental data (results not shown).

The Value of k41—in contrast with k40 or k42, there is no experimental condition that allows estimating k41 separately from k40 or k42 (cf. Equation 3 with Equations 2 and 4). In view of this we used an indirect method to estimate k41 based on assuming rapid-equilibrium binding of Rb + to E2P, in which case the initial rate of Rb +-dependent dephosphorylation will be

\[ v^0_{\text{dephos,Rb}} = E_2P_0 \frac{(k_{41} - k_{40})K_{Rb2}[Rb^+] + (k_{42} - k_{40})[Rb^+]^2}{K_{Rb1}K_{Rb2} + K_{Rb2}[Rb^+] + [Rb^+]^2} \]  

(Eq. 10)

When [Rb +] ≪ K Rb2 Equation 10 will approach

\[ v^0_{\text{dephos,Rb}} = E_2P_0 \frac{(k_{41} - k_{40})[Rb^+] + \beta[Rb^+]^2}{K_{Rb1} + [Rb^+]} \]  

(Eq. 11)

where

\[ \beta = \frac{k_{42} - k_{40}}{K_{Rb2}} \]  

(Eq. 12)

Equations 11 and 12 show that, knowing the values of E2P0 and of k40, the values of k41, K Rb1, and \( \beta \) can be estimated by fitting Equation 11 to the experimental results of \( v^0_{\text{dephos,Rb}} \). When this is done using the experiment in Fig. 3, the best-fitting value of k41 is 24 ± 16 s⁻¹ or 18 ± 11 s⁻¹ depending on the value of E2P0 used (see Table 2). These values lay between the usually accepted values for k40 (2.5 s⁻¹) and k42 (250–500 s⁻¹; for references see Table 3).

Comparison between the Experimental Results and the Simulations of the Scheme in Fig. 1—The experimental results presented so far are consistent with our hypothesis that the binding of a single Rb + to E2P activates dephosphorylation without leading to the stoichiometric

### Table 1

| Parameter | ATPase activity | EP | Rbocc |
|-----------|-----------------|----|-------|
| Y0        | 4.06 ± 0.13     | 2.309 ± 0.010 | 0 |
| Y1        | 7.8 ± 1.8       | 0.90 ± 0.47    | 0 |
| K1        | 2.38 ± 0.24     | 0.026 ± 0.026  | 4.642 ± 0.098 |
| K2        | 43 ± 31         | 312 ± 56       | 74 ± 16 |
| K3        | 85 ± 55         | 44.1 ± 6.1     | 143 ± 16 |

### Table 2

The best-fitting values of the parameters of Equation 11

| Parameter | \( E_2P_0 \) |
|-----------|--------------|
| \( k_{41}(s^{-1}) \) | 24 ± 16 |
| \( K_{Rb1} (\mu M) \) | 702 ± 450 |
| \( \beta (s^{-1} \mu M^{-1}) \) | 0.12 ± 0.03 |

a E2P calculated from the model in Fig. 1 at [Rb +] = 0 using the rate constants in Table 3.

b EP measured in the absence of Rb +.

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**FIGURE 7.** The sum of the steady-state levels of EP (Fig. 5) plus ½ Rbocc (Fig. 6) as a function of [Rb +]. The continuous line is a plot of the same variables obtained from the continuous lines of Figs. 5 and 6. The short dashed, dotted, and long dashed lines are, respectively, simulations of the scheme in Fig. 1 for the conditions (i), (ii), and (iii) and the values of the rate constants given in Table 3.
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TABLE 3

| Values of the kinetic parameters used for simulations of the scheme in Fig. 1 for different conditions |
|-----------------------------------------------------------------------------------------------------|

We assumed that the binding of the two Rb\(^+\) to E\(_{P}\) and that binding of ATP to E\(_{P}(\text{Rb}_2)\) takes place in rapid equilibrium. The values of the total enzyme concentration (E\(_x\)) and of k\(_{oc}\) were obtained by fitting the solutions of the model to the results of EP and ATPase activity at [Rb\(^+\)] = 50 µM, fixed thereafter. Only K\(_{Rb1}\), k\(_{41}\), and (except in condition k\(_{oc}\) = k\(_{oc}\) and α = 0) k\(_{44}\), were adjusted; the rest of the values were kept constant as given in the table.

| Parameter | Units | Condition tested | Fitting k\(_{41}\) | Fitting k\(_{44}\) |
|-----------|-------|------------------|------------------|------------------|
| E\(_x\)   | nmol mg\(^{-1}\) | 2.369 | 2.369 | 2.369 |
| k\(_{41}\) | s\(^{-1}\) μM\(^{-1}\) | 10 | 10 | 10 |
| k\(_{42}\) | s\(^{-1}\) | 100 | 100 | 100 |
| k\(_{43}\) | s\(^{-1}\) | 2 | 2 | 2 |
| k\(_{44}\) | s\(^{-1}\) | 75 | 75 | 75 |
| k\(_{oc}\) | s\(^{-1}\) | 25 | 25 | 25 |
| k\(_{40}\) | s\(^{-1}\) | 2.405 | 2.405 | 2.405 |
| k\(_{41}\) | s\(^{-1}\) | 2.405 | 35.52 | 47.41 |
| k\(_{42}\) | s\(^{-1}\) | 500 | 500 | 500 |
| k\(_{50}\) | s\(^{-1}\) | 0.13 | 0.13 | 0.13 |
| k\(_{5a}\) | s\(^{-1}\) | 30 | 30 | 30 |
| k\(_{b}\) | µM | 0.3396 | 11.07 | 6.525 |
| k\(_{Rb1}\) | µM | 1279 | 1599 | 1206 |
| k\(_{ATP}\) | µM | 4916 | 6396 | 4823 |

* Kaufman et al. (5) and Schwarzbaum et al. (8).
* Fixed, considering that Forbush (15) suggests a value much higher than 100 s\(^{-1}\) and that Heyse et al. (16) give a value higher than 1000 s\(^{-1}\).
* Kaufman et al. (5) and González-Lebrero et al. (17).
* k\(_{Rb2}\) was fixed as 4 x k\(_{Rb1}\) (see González-Lebrero et al. (17)).

It could be argued that for our experimental results, the slope of v\(_{occ}\) = f([Rb\(^+\)]) when [Rb\(^+\)] → 0 is not zero because one Rb\(^+\) is occluded in E\(_x\) (α = 1) but that a faction of the occluded Rb\(^+\) is washed out during its measurement. If this were so, the rate of deocclusion would be the same as the rate of E\(_x\) to E\(_1\) reaction (k\(_{oc}\)). The best value of this constant (11 s\(^{-1}\)), see Table 3) falls far below the values of deocclusion rate that can be obtained by the washing of occluded Rb\(^+\) during its measurement. A more likely explanation may be that Rb\(^+\) occlusion does take place but in only one of the microscopic states of E\(_P\)Rb\(_1\). An example of these states would be species of E\(_P\) that bind one Rb\(^+\) and one Na\(^+\), as has been suggested by Vilsen (14).

It is not known if the ATPase activated by a single Rb\(^+\) is able to drive active transport. This cannot be discarded in view of the observation by Beaugé et al. (18) who showed that a response similar as that observed in Fig. 4 is elicited by extracellular K\(^+\). The data presented here were collected in a non-compartmentalized system that does not allow deciding if transport occurs. Even if a compartmentalized system were available, to measure the stoichiometry of net K\(^+\) pumping with 10 µM ATP (and, hence, with a very low pump-to-leak ratio) is a very difficult task. For this reason, all the reliable measurements of stoichiometry of transport in the Na\(^+\)/K\(^+\) pump have been performed with optimal concentrations of ATP and Na\(^+\) and K\(^+\) (19). In conclusion, results in this paper indicate the presence of an until now unknown mode of behavior of the Na\(^+\)/K\(^+\)-ATPase, whose further study may help to understand the mechanism of active transport of Na\(^+\) and K\(^+\).

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