Pre-steady-state kinetic study of the effects of K^+ on the partial reactions of the catalytic cycle of the plasma membrane Ca^{2+} -ATPase

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The effects of 100 mM K^+ on the partial reactions that take place during ATP hydrolysis by the calcium ion-dependent ATPase from plasma membrane (PM- Ca^{2+} -ATPase) were studied at 37 °C on fragmented intact membranes from pig red cells by means of a rapid chemical quenching technique. At 10 μ M $[\gamma\text{-}^{32}\text{P}]\text{ATP}$ plus non-limiting concentrations of Ca^{2+} and Mg^{2+} , K^+ increased the k_{app} of formation by 140 % to $84 \pm 11 \text{ s}^{-1}$ and the steady-state level of phosphoenzyme (EP) by 25 % to $3.4 \pm 0.17 \text{ pmol/mg}$ of protein. If added together with $[\gamma\text{-}^{32}\text{P}]\text{ATP}$ at the beginning of phosphorylation, K^+ was much less effective than if added earlier, indicating that it did not act on the phosphorylation reaction. Measurements of the $E_2 \rightarrow E_1$ transition by phosphorylation showed that in medium with Ca^{2+} and Mg^{2+} , K^+ increased the k_{app} of the transition by 55 % to $14 \pm 3 \text{ s}^{-1}$ and the apparent concentration of E_1 by 45 %, suggesting that

this may be the cause of the increased rate of phosphorylation observed in enzyme preincubated with K^+ . The presence of K^+ did not change the slow decay of EP without Mg^{2+} but activated the decay of EP made with Mg^{2+} , increasing its k_{app} by 60 % to $91 \pm 12 \text{ s}^{-1}$. In contrast with observations made during phosphorylation, if added at the beginning of dephosphorylation K^+ was fully effective in favouring decomposition of EP made in medium containing no K^+ . In the presence of either 3 mM ATP or 3 mM ATP plus calmodulin, which activate hydrolysis of CaE_2P , the effect of K^+ on dephosphorylation was conserved. Because the sites for K^+ are intracellular and the concentration of K^+ in normal red cells is above 100 mM, the effects described here must be taken into account to describe the catalytic cycle of the PM- Ca^{2+} -ATPase under physiological conditions.

INTRODUCTION

The calcium ion-dependent ATPase from plasma membrane (PM- Ca^{2+} -ATPase) pumps Ca^{2+} out of the cells against the electrochemical potential of Ca^{2+} by using energy from the hydrolysis of ATP. The splitting of ATP by the enzyme involves at least the series of partial reactions in Scheme 1, where E_1 and E_2 are conformers of the enzyme [1].

Though with different affinities, alkali metal ions such as K^+ , Na^+ , Rb^+ and NH_4^+ are equally effective in stimulating ATP hydrolysis and Ca^{2+} transport to an extent that ranges between 30 and 100 %, depending on the preparation and experimental conditions [1]. In human red cells these alkali metal ions activate the enzyme by combining at a single class of site(s) accessible

only from the interior of the cell and are not transported by the PM- Ca^{2+} -ATPase [2]. Although no regulatory role has been assigned to the univalent cations, in an intact cell the enzyme is almost saturated by intracellular K^+ , activating the enzyme to a sufficient extent to be taken into consideration for the analysis of the behaviour of the PM- Ca^{2+} -ATPase.

By means of a manual technique we have studied previously the effects of K^+ on the partial reactions of the PM- Ca^{2+} -ATPase from human red cells at 0 °C, concluding that K^+ increases the level and the rates of formation and breakdown of a phosphoprotein [3]. After that, the reactions of phosphorylation, dephosphorylation and the conversions of E_2 to E_1 and CaE_1P to CaE_2P of the PM- Ca^{2+} -ATPase have been studied under pre-steady-state conditions by means of a rapid chemical quenching technique [4–6].

In this paper we report experiments designed to re-examine the effects of K^+ on the partial reactions in the light of the novel information available now on the PM- Ca^{2+} -ATPase and on the effects of K^+ on partial reactions of the calcium ion-dependent ATPase from sarcoplasmic reticulum (SR- Ca^{2+} -ATPase) [7–9]. Experiments were performed on intact pig red cell membranes to preserve the natural lipid environment of the enzyme and at 37 °C to be confident that the behaviour observed *in vitro* resembles that of the PM- Ca^{2+} -ATPase in the cell. This is important after knowing the strong dependence of the PM- Ca^{2+} -ATPase on lipids [1] and the demonstration by Jones et al. [10] that the effects of K^+ on partial reactions of SR- Ca^{2+} -ATPase of cardiac muscle are temperature-dependent.

MATERIALS AND METHODS

Pig blood collected on acid/citrate/dextrose solution was used throughout. Red cell membranes depleted of Ca^{2+} and calmodulin

Scheme 1

Abbreviations used: CDTA, *trans*-1,2-diaminocyclohexane *N,N,N',N'*-tetra-acetic acid; EP, phosphoenzyme; PM- Ca^{2+} -ATPase, calcium ion-dependent ATPase from plasma membrane; SR- Ca^{2+} -ATPase, calcium ion-dependent ATPase from sarcoplasmic reticulum.

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were prepared following a modification of the procedure of Gietzen et al. [11,12] except that EGTA was used for *trans*-1,2-diaminocyclohexane *N,N,N',N'*-tetra-acetic acid (CDTA) and stored at -60°C until used. $[\gamma\text{-}^{32}\text{P}]\text{ATP}$ (specific radioactivity 1 mCi/ μmol) was prepared by the method of Glynn and Chappell [13] except that no unlabelled P_i was added to the incubation medium. $^{32}\text{P}\text{P}_i$ was provided by Comisión Nacional de Energía Atómica (Argentina). Calmodulin and enzymes and cofactors for the synthesis of $[\gamma\text{-}^{32}\text{P}]\text{ATP}$ were obtained from Sigma. Salts and reagents were of analytical reagent grade.

Phosphorylation and dephosphorylation experiments were performed at $37 \pm 3^{\circ}\text{C}$ in a rapid mixing apparatus adapted for chemical quenching (Intermekron AB, Uppsala, Sweden). Phosphorylation from $[\gamma\text{-}^{32}\text{P}]\text{ATP}$ was measured as described previously [5]. Unless otherwise indicated, during the experiments 2 ml of the membrane suspension at 5 mg/ml protein in 100 mM of either choline/HCl or KCl plus 0.7 mM CaCl_2 (0.2 mM Ca^{2+}), 0.5 mM CDTA, 0.5 mM MgCl_2 (0.49 mM Mg^{2+}) and 50 mM Tris/HCl (pH 7.4 at 37°C), in syringe I, was mixed with an equal volume of the suspending buffer with 20 μM $[\gamma\text{-}^{32}\text{P}]\text{ATP}$ in syringe II. Phosphorylation was started in the mixing chamber and finished by quenching the reaction mixture with 5 ml of a solution containing 10% (w/v) trichloroacetic acid, 5 mM ATP and 50 mM H_3PO_4 at 0°C . The suspension was centrifuged at 1750 *g* for 5 min at 5°C , and the sediment washed three times with 6 ml of a solution containing 7% (w/v) trichloroacetic acid and 50 mM H_3PO_4 . The washed precipitate was dissolved in 0.4 ml of 3% (w/v) SDS solution in 1 M Tris buffer. An aliquot of the solution was put aside for measuring protein; 0.25 ml was used for measuring radioactivity in a liquid-scintillation counter. The amount of phosphoenzyme (EP) was estimated from the difference between the amount of ^{32}P incorporated into the membrane protein in media with Ca^{2+} and in media of identical composition without Ca^{2+} . When preincubation time was a variable, phosphorylation was performed with three syringes and two mixing chambers as described previously [14]. During the experiments, 2 ml of the membrane suspension at 5 mg/ml protein in 100 mM choline/HCl, 0.5 mM CDTA and 50 mM Tris/HCl (pH 7.4 at 37°C), in syringe I, was mixed with an equal volume of the suspending buffer containing twice the desired concentration of Ca^{2+} , Mg^{2+} and either KCl or choline/HCl in syringe II. Preincubation took place in the lines between the first and the second mixing chambers. Phosphorylation began in the later chamber when 2 ml of the suspending buffer with 30 μM $[\gamma\text{-}^{32}\text{P}]\text{ATP}$ from syringe III was mixed with the membrane suspension. Dephosphorylation was performed with three syringes and two mixing chambers as described previously [6]. Phosphorylation took place in the lines between the first and the second mixing chamber and was interrupted in the second mixing chamber with 2 ml of the buffer containing an excess of either ATP or EGTA from syringe III. Dephosphorylation started at the second mixing chamber and finished when the reaction mixture was quenched with 7% (w/v) trichloroacetic acid plus 50 mM H_3PO_4 at 0°C .

The concentration of protein was estimated by the procedure of Lündahl [15]. Throughout this paper Ca^{2+} and Mg^{2+} refer to the free ionic species of these metals. The concentration of Ca^{2+} was measured as described previously [2] with an IS-561 Ca^{2+} -sensitive electrode. The concentration of Mg^{2+} was calculated by assuming the following apparent dissociation constants: $K_{\text{Ca-CDTA}} = 1.1 \times 10^{-11}$ M, $K_{\text{Mg-CDTA}} = 1.7 \times 10^{-8}$ M, $K_{\text{Ca-EGTA}} = 2.6 \times 10^{-7}$ M, $K_{\text{Mg-EGTA}} = 1.34 \times 10^{-2}$ M, $K_{\text{Ca-ATP}} = 5.4 \times 10^{-5}$ M and $K_{\text{Mg-ATP}} = 4.5 \times 10^{-5}$ M, neglecting the concentration of Mg^{2+} brought in as a contaminant of the solutions.

Except when otherwise stated, the curves drawn and the kinetic parameters' standard errors were those from the theoretical equations that fitted the data by least-squares non-linear regression by using the Gauss-Newton procedure [16]. The independent variable was assumed to be free of error and the variance of the dependent variable was assumed to be constant. In every experiment the measurements of EP level were made in at least duplicate and the individual values differ from the mean by less than 10%.

Experiments presented in the Results section were chosen as representative of two to four experiments. The concentration of active PM- Ca^{2+} -ATPase varies between membrane preparations and this is reflected in the quantities measured. For this reason the control experiments were run simultaneously with those with K^{+} and on the same membrane preparation.

RESULTS

The effects of K^{+} on phosphorylation

As reported previously [5], phosphorylation of the PM- Ca^{2+} -ATPase in the presence of Mg^{2+} at 37°C is fast. Consequently, in the experiment in Figure 1 the concentration of EP in medium without K^{+} increased along a simple exponential curve with $k_{\text{app}} = 35 \pm 3 \text{ s}^{-1}$ up to a steady value of 2.7 ± 0.14 pmol/mg of protein. When 100 mM K^{+} was added, the concentration at which the PM- Ca^{2+} -ATPase activity is optimal, EP reached a higher maximum value of 3.4 ± 0.17 pmol/mg of protein with $k_{\text{app}} = 84 \pm 11 \text{ s}^{-1}$. The increase in the rate of formation and the steady level of EP at 37°C agree with results reported earlier from experiments made at 0°C [3].

We have shown previously that when the PM- Ca^{2+} -ATPase is phosphorylated in the presence of calmodulin, the concentration of EP rises with time sharply along a curve that passes through a maximum and then levels off at a value similar to that in the absence of calmodulin [5], as shown in the inset to Figure 1.

Figure 1 The effect of K^{+} on the time course of phosphorylation of the PM- Ca^{2+} -ATPase

Membranes preincubated in buffer with choline (O) or buffer with K^{+} (●) were phosphorylated with 10 μM $[\gamma\text{-}^{32}\text{P}]\text{ATP}$. The equation that fitted the experimental points was $[\text{EP}] = [\text{EP}]_{\text{ss}}(1 - e^{-kt})$, where $[\text{EP}]_{\text{ss}}$ is the steady-state concentration of EP, t is time and k is the apparent rate constant. (Inset) The effect of K^{+} on the time course of phosphorylation in the presence of calmodulin. Membranes preincubated in buffer containing 120 nM calmodulin with (●) and without (O) K^{+} were phosphorylated in media with 10 μM $[\gamma\text{-}^{32}\text{P}]\text{ATP}$. The curves were fitted by eye.

Figure 2 Effect of preincubation time with and without K^+ on the level of EP at 2.5 ms

Membranes in 100 mM choline/HCl, 0.5 mM CDTA and 50 mM Tris/HCl (pH 7.4 at 37 °C) in one of the syringes at 37 °C were mixed with an equal volume of 0.4 mM Ca^{2+} , 0.5 mM CDTA, 1 mM $MgCl_2$, 50 mM Tris/HCl (pH 7.4 at 37 °C) plus 200 mM choline/HCl (○) or 200 mM KCl (●) and kept for different times in the millisecond range. The concentration of Ca^{2+} after mixing was 0.2 mM. Phosphorylation was started by mixing 1 volume of 30 μ M [γ - ^{32}P]ATP in the same buffers with 2 volumes of the preincubated membranes and the reaction allowed to proceed for 2.5 ms. The equation that fitted the experimental points was $[EP] = [EP](1 - e^{-kt})$, where $[EP]$ is the concentration of EP at infinite preincubation time, t is time and k is the apparent rate constant.

Results show that in the presence of calmodulin K^+ was still effective in increasing the level of EP.

The effects of K^+ added before phosphorylation

The experiments shown in Figure 1 were performed after the general procedure that we use regularly for phosphorylation. Accordingly, during the experiments from Figure 1 the membranes were kept in buffer with Mg^{2+} and Ca^{2+} , with and without K^+ , at 37 °C for 30 min before phosphorylation. This raised the question of whether K^+ could act on the dephosphoenzyme during preincubation to stimulate the synthesis of EP. If this were so, K^+ added together with ATP at the start of phosphorylation should be less effective than during the experiments shown in Figure 1. Membranes were preincubated in buffers with Mg^{2+} and Ca^{2+} plus either choline or K^+ at 37 °C for 30 min and then phosphorylated by the addition of 10 μ M [γ - ^{32}P]ATP plus sufficient concentrated solution of either choline or KCl to give a final concentration of 100 mM. To minimize the effects of K^+ on partial reactions of the catalytic cycle, phosphorylation was made to last for 2.5 ms. When choline was present instead of K^+ throughout the experiment, the EP concentration was 0.67 ± 0.03 pmol/mg of protein. When K^+ was present only during phosphorylation because it was added at the same time as [γ - ^{32}P]ATP, the EP concentration increased by 8 % to 0.72 ± 0.04 pmol/mg of protein. When K^+ was present throughout the experiment, the EP concentration increased by 57 % to 1.05 ± 0.05 pmol/mg of protein. This change in effectiveness, depending on whether K^+ reached the enzyme before or during phosphorylation, suggested that acceleration of phosphorylation was due not to a direct effect of K^+ on the reaction but rather on a previous one, during preincubation.

The effects of K^+ on the $E_2 \rightarrow E_1$ transition

Before phosphorylation, K^+ could have accelerated the $E_2 \rightarrow E_1$ transition (reaction 6 in Scheme 1). To investigate this possibility we tested the effect of K^+ on the transition of E_2 into E_1 by

Figure 3 Effect of K^+ on the time course of dephosphorylation of EP made in media without added $MgCl_2$

The membranes were phosphorylated in media with choline/HCl (○) or KCl (●) plus 50 mM Tris/HCl (pH 7.4 at 37 °C) 1.2 mM $CaCl_2$ (0.2 mM Ca^{2+}) and 1 mM EGTA. Dephosphorylation was started by mixing 1 volume of 0.9 mM ATP in the same media with 2 volumes of the phosphorylated membranes. The equation that fitted the experimental points was $[EP] = [EP]_r \exp(-k_r t) + [EP]_s \exp(-k_s t)$, where k is the apparent rate constant, t is time and the subscripts r and s stand for rapid and slow respectively. The values that gave the best fit were: (i) in buffer with choline: $[EP]_r = 49.7 \pm 4.8\%$; $k_r = 90 \pm 10$ s $^{-1}$; $[EP]_s = 50.3 \pm 4.3\%$; $k_s = 0.7 \pm 0.3$ s $^{-1}$; $[EP]$ at 100% was 1.91 ± 0.18 pmol/mg of protein; and (ii) in buffer with K^+ : $[EP]_r = 61.2 \pm 2.4\%$; $k_r = 134 \pm 7$ s $^{-1}$; $[EP]_s = 38.8 \pm 4.1\%$; $k_s = 0.7 \pm 0.4$ s $^{-1}$; $[EP]$ at 100% was 1.42 ± 0.11 pmol/mg of protein.

measuring its time course by phosphorylation as described previously [14]. For this purpose, membranes were preincubated with Ca^{2+} plus Mg^{2+} , with and without 100 mM K^+ , for different lengths of time and then phosphorylated for 2.5 ms (instead of 8 ms as in [14]). Provided that (i) the conditions for phosphorylation are the same, (ii) CaE_1 is the only enzyme species phosphorylated by ATP, and (iii) reaction 6 is much slower than any of the other reactions leading to CaE_1P , the concentration of EP after 2.5 ms should be directly related to the initial rate of phosphorylation and hence reflect the abundance of E_1 [14]. Experiments such as these are complex and difficult to perform so that the collected data were not very precise. Nevertheless results in Figure 2 show that the levels of EP of the PM- Ca^{2+} -ATPase that had been in contact with K^+ during preincubation were higher than those of the control at any preincubation time.

The curves in Figure 2 represent exponentials with maximal values of 1.03 ± 0.05 and 0.71 ± 0.02 pmol/mg of protein, corresponding to enzyme preincubated with and without K^+ respectively. Values of k_{app} were of 9 ± 1 and 14 ± 3 s $^{-1}$ in the absence and presence of K^+ respectively. On the basis of the reasoning above, the results in Figure 2 suggest that K^+ accelerated the transformation between conformers from E_2 to E_1 , increasing by 45 % the apparent concentration of E_1 .

The effects of K^+ on dephosphorylation

We have shown elsewhere [11] that, as judged by the lack of Na^+ -dependent phosphorylation of the Na^+/K^+ -ATPase, media containing CDTA can be prepared substantially free of contaminant Mg^{2+} . We have also found that EP made in medium with CDTA decomposes very slowly in contrast with EP prepared in medium with a non-limiting concentration of Mg^{2+} , which decomposes rapidly [6]. Time courses of dephosphorylation of EP prepared in media without $MgCl_2$, CDTA and K^+ are biphasic as shown in Figure 3. Our interpretation is that because these media contain

Figure 4 Effects of K^+ plus ATP on the time course of dephosphorylation of EP made in media with Mg^{2+}

The membranes were phosphorylated in buffer with choline (○) or buffer with K^+ (●). Dephosphorylation was started by mixing 1 volume of 0.9 mM MgATP in buffer with choline or K^+ with 2 volumes of the phosphorylated membranes. The equation that fitted the experimental points was $[EP] = [EP]e^{-kt}$. The values of 100% [EP] in buffer with choline or K^+ were 2.74 ± 0.13 and 3.48 ± 0.19 pmol/mg of protein respectively. The values of k that gave the best fitting were 91 ± 12 and 57 ± 6 s^{-1} in buffer with and without K^+ respectively.

limiting concentrations of Mg^{2+} as a contaminant, phosphorylation of the PM- Ca^{2+} -ATPase leads to formation of Mg^{2+} -bound EP plus Mg^{2+} -free EP. Accordingly, the rapid component of the curve represents the decomposition of Mg^{2+} -bound EP whereas the slow component represents the decomposition of Mg^{2+} -free EP [6]. The results in Figure 3 show that when K^+ was present the slow component of the curve remained unchanged and the rate of the rapid component increased, suggesting that K^+ accelerated the decomposition of EP acting on the Mg^{2+} -bound EP. To study in more detail the effects of K^+ on dephosphorylation, EP was made in medium with 0.5 mM $MgCl_2$ with and without 100 mM K^+ , chased with 0.3 mM ATP and the course of decay was followed for up to 30 ms, as shown in Figure 4. In the presence of K^+ , EP dephosphorylated along a single exponential curve with $k_{app} = 91 \pm 12$ s^{-1} , a value 59% higher than that of the control without K^+ .

In the experiments in Figures 3 and 4, K^+ was present throughout. To see whether it was sufficient for K^+ to be present during dephosphorylation to accelerate the decay of EP, membranes were preincubated at 37 °C in 0.2 mM Ca^{2+} , 0.5 mM $MgCl_2$, 50 mM Tris/HCl (pH 7.4 at 37 °C) plus 100 mM either choline/HCl or KCl and then phosphorylated by the addition of 20 μ M $[\gamma\text{-}^{32}\text{P}]\text{ATP}$. After 4 ms, EP was chased with 0.3 mM ATP plus 100 mM KCl and the amount of EP measured at 0 and 4 ms after chasing. The ratio $[EP_{t=4}]/[EP_{t=0}]$ was 0.49 for the PM- Ca^{2+} -ATPase in the presence of K^+ throughout the experiment and 0.47 for the enzyme that received K^+ during dephosphorylation only. The fact that the decay of EP was the same in both conditions showed that, during dephosphorylation only, K^+ was fully effective in activating this reaction and hence that K^+ accelerated dephosphorylation of EP made without K^+ .

The effects of K^+ on decomposition of EP in the presence of 3 mM ATP and 3 mM ATP plus calmodulin were studied. The results in Table 1 show that as judged by the fraction of total EP remaining after 4 ms of dephosphorylation, combination of ATP with low affinity to the PM- Ca^{2+} -ATPase accelerates the hydrolysis of EP [6]. Calmodulin enhances this acceleration by ATP [6]. The results in Table 1 also show that under any of the conditions tested, decomposition of EP was accelerated by K^+ .

Table 1 The effects of K^+ on dephosphorylation in media with ATP and ATP plus calmodulin

Membranes preincubated in buffer with Ca^{2+} plus Mg^{2+} with either choline or K^+ plus 0 or 120 nM calmodulin (CaM) were phosphorylated with 20 μ M $[\gamma\text{-}^{32}\text{P}]\text{ATP}$ for 500 ms. The EP formed was chased with either 3 mM ATP or 2 mM EGTA when ATP was absent, and the amount of EP was measured at 0 and 4 ms. The ratios $[EP_{t=4}]/[EP_{t=0}]$ were obtained from the values of EP after 4 and 0 ms of dephosphorylation respectively.

Dephosphorylation medium containing	$[EP_{t=4}]/[EP_{t=0}]$
0 mM ATP	0.72
0 mM ATP plus K^+	0.61
3 mM ATP	0.52
3 mM ATP plus K^+	0.41
3 mM ATP plus 120 nM CaM	0.33
3 mM ATP plus 120 nM CaM plus K^+	0.17

DISCUSSION

This paper reports results of experiments designed to test the effects of addition of K^+ on the partial reactions of the catalytic cycle of the PM- Ca^{2+} -ATPase at 37 °C by means of a rapid chemical quenching technique. The study was performed with the hope of collecting information allowing us to understand the mechanism of the activation of the enzyme by univalent cations. It has been argued that alkali metal cations could stimulate Ca^{2+} -ATPases by promoting the formation of a new phosphorylated intermediate different from EP [17]. The demonstration in this paper that K^+ accelerated to the same extent the decay of EP, regardless of whether it was formed with or without K^+ , represents a new and strong argument in favour of the idea that all the actions of K^+ were exerted on the catalytic cycle of the PM- Ca^{2+} -ATPase. Because the effects described here occurred at a K^+ concentration close to that in the cytoplasm, they must be taken into account when considering the behaviour of the PM- Ca^{2+} -ATPase under physiological conditions.

Results show that if K^+ was added before phosphorylation, it increased the apparent rate constant of EP formation by 140% and the level of EP by 25%. If added together with $[\gamma\text{-}^{32}\text{P}]\text{ATP}$ at the beginning of phosphorylation, K^+ did not change this reaction significantly. Incubation of the enzyme with K^+ before phosphorylation raised by about 55% the k_{app} of the $E_2 \rightarrow E_1$ transition observed with Ca^{2+} plus Mg^{2+} and by 45% the apparent concentration of E_1 . Experimental results are therefore in keeping with the idea that the acceleration of the $E_2 \rightarrow E_1$ transition by K^+ probably led to the observed increase in the rate of phosphorylation.

Provided that EP was made in Mg^{2+} -containing medium, its decomposition was nearly 60% faster if K^+ was present. This, together with the lack of effect of K^+ on EP made without Mg^{2+} , could indicate that K^+ accelerated the hydrolysis of CaE_2P , whose formation from CaE_1P depends on Mg^{2+} . These results, however, do not allow us to discard the alternative that K^+ acted by increasing further the rate of the conversion of CaE_1P into CaE_2P (reaction 4 in Scheme 1) in the Mg^{2+} -bound enzyme, raising the concentration of CaE_2P that decomposes rapidly.

ATP at relatively high concentration (3 mM) accelerates decomposition of CaE_2P [6]. Under these conditions activation by K^+ was apparent. Furthermore, in medium with calmodulin, which makes hydrolysis of CaE_2P with 3 mM ATP proceed at the highest rate, K^+ was still effective in accelerating the reaction.

The changes in the kinetics of the partial reactions observed on addition of K^+ could be interpreted as the expression of a more general effect of K^+ in the condition of the PM- Ca^{2+} -ATPase. In

fact, because all the effects of K^+ are exerted from the inner surface of the cell membrane and the concentration of K^+ in the cytoplasm of the red cells is well above that of the $K_{1/2}$ for activation, the PM- Ca^{2+} -ATPase in the intact cell is permanently activated by K^+ . This being so, a possible interpretation of the results in this paper is that during isolation and storage of the red cell membranes in the absence of K^+ , the enzyme underwent a slight or local denaturation. By this way of thinking, addition of K^+ should drive the PM- Ca^{2+} -ATPase towards a condition close to its native state, which would be that endowed with the kinetic properties shown in the Results section. In connection with this, it is suggestive that incubation of SR- Ca^{2+} -ATPase in the absence of substrate and activators leads to reversible changes in the properties of the enzyme [18,19].

Finally, results in this paper support the conclusion that during activation of the PM- Ca^{2+} -ATPase by K^+ , partial reactions of the transformation of E_2 into E_1 and decomposition of EP are accelerated. It has been reported that univalent alkali cations increase the rate of formation and decomposition of the EP of SR- Ca^{2+} -ATPase from skeletal and cardiac muscle [10,20–23]. In addition, Medda et al. [7], Moutin and Dupont [8], and more recently Lee et al. [9] showed that K^+ increases the apparent rate of the E_2 to E_1 transition of the SR- Ca^{2+} -ATPase from skeletal muscle, shifting the equilibrium towards E_1 . In the light of those results and the findings in the present paper, it would seem that the mechanisms of activation of the PM- Ca^{2+} -ATPase and of the SR- Ca^{2+} -ATPase by K^+ are similar.

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