Regulation of the Conformational Transition in the Ca-ATPase from Sarcoplasmic Reticulum by pH, Temperature, and Calcium Ions*

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The effects of pH, temperature, and Ca\textsuperscript{2+} ion concentration on the equilibrium and the rates of interconversions between the two conformations of the dephosphorylated Ca-ATPase from sarcoplasmic reticulum were studied by means of a fluorescein-labeled enzyme. An equilibrium constant, $K_{E_1,E_2}$, was estimated from the fluorescence shifts induced by vanadate and Ca\textsuperscript{2+} which stabilize the states $E_1$ and $E_2$, respectively. Ca\textsuperscript{2+}-induced fluorescence quenching increased markedly, and vanadate-induced fluorescence enhancement decreased sharply, upon raising the temperature from 10 to 40 °C. We infer from these results that the conformation transition $E_2 \rightarrow E_1$ is exothermic with an enthalpy change $\Delta H^\circ = -20.5$ kcal/mol (at pH 7.0). Similarly, an increase in the pH of the medium from pH 5.5 to 8.0 decreased the Ca\textsuperscript{2+}-induced fluorescence quenching and increased the vanadate-induced fluorescence enhancement. The results imply that in the absence of Ca\textsuperscript{2+} and in the presence of Mg\textsuperscript{2+} at 15-32 °C most of the enzyme exists in the $E_1$ conformation below pH 6.0, and in the $E_2$ conformation above pH 8.0.

In the presence of vanadate, the rates of the conformation transitions in response to Ca\textsuperscript{2+} measured at acidic pH were extremely slow. An increase in the pH of the medium and of temperature markedly enhanced the rate of conformational change in response to the addition or removal of Ca\textsuperscript{2+} in the presence of vanadate.

The Ca\textsuperscript{2+} concentration required to induce half-maximal fluorescence changes was slightly decreased by increasing the temperature and binding of only one Ca\textsuperscript{2+} was necessary to induce the conformational transition. The role of protons, Ca\textsuperscript{2+} ions, and of temperature in the control of the conformational transition is discussed.

There is ample evidence that the Ca-ATPase from sarcoplasmic reticulum undergoes conformational changes in response to Ca\textsuperscript{2+} binding to the external high affinity binding sites of the enzyme. These Ca\textsuperscript{2+}-induced changes are reflected by an increase in the intrinsic tryptophan fluorescence (1), by a fluorescence quenching of fluorescein bound in the active center (2), by changes in the rate of inactivation by SH reagents (3), and by a stabilization against inactivation at acidic pH (4) and by dicyclohexylcarbodiimide (5). Several models that describe the partial reactions of ATP hydrolysis by the enzyme have been proposed (for reviews, see Refs. 6 and 7). The Ca\textsuperscript{2+}-induced conformational transition of the dephosphorylated enzyme ($E_0 \rightarrow E_1$) is usually recognized as the slowest step in the turnover of the enzyme and there is evidence that ATP at high concentrations stimulates this transition (8, 9). It is generally accepted that binding of Ca\textsuperscript{2+} ions to the external high affinity binding sites stabilizes the $E_1$ conformation of the enzyme; however, there are no direct measurements of the equilibrium between these two conformations in the absence of Ca\textsuperscript{2+}.

We have reported recently that fluorescein 5'-isothiocyanate modifies the ATP binding site of the Ca-ATPase. We have also demonstrated that Ca\textsuperscript{2+} ions quench the fluorescence of the bound fluorescein and since these fluorescence changes were found to be specific to Ca\textsuperscript{2+}, to be abolished by dicyclohexylcarbodiimide and to require micromolar concentrations of Ca\textsuperscript{2+} we suggested that they reflect conformational changes induced in the enzyme by Ca\textsuperscript{2+} binding (2, 10).

In previous works and in the accompanying paper, it was demonstrated that vanadate ions inhibit Ca-ATPase activity (11), phosphatase activity (12), and phosphorylation by inorganic phosphate (13) in the SR-Ca-ATPase from skeletal muscles. It has also been shown that vanadate inhibition is antagonized by Ca\textsuperscript{2+} (11, 13) and that preincubation of the enzyme with vanadate inhibited Ca\textsuperscript{2+} binding to the high affinity binding sites on the enzyme (13). Based on these results, it was suggested that vanadate ions preferentially bind to the $E_2$ conformation of the enzyme forming a stable $E_2$:Mg\textsuperscript{2+}:vanadate complex (13). Vanadate ions at micromolar concentrations also induced a large fluorescence enhancement of the fluorescein-labeled enzyme in the total absence of Ca\textsuperscript{2+} ions and the effect was antagonized by Ca\textsuperscript{2+} ions (13). This antagonistic effects of Ca\textsuperscript{2+} and of vanadate plus Mg\textsuperscript{2+} ions on the fluorescence of FITC-SRV suggested that vanadate shifts the conformational equilibrium in an opposite direction to Ca\textsuperscript{2+} in agreement with the inhibition studies in native SRV. It was also observed that the effects of vanadate on Ca\textsuperscript{2+} binding, on phosphorylation by $P_i$, and on the interactions with pyrophosphate were almost identical in native-SRV and in FITC-SRV. These similarities in the effects of Ca\textsuperscript{2+} and vanadate on native and FITC-SRV suggest that the dynamic conformational equilibrium $E_1 \rightleftharpoons E_2$ in the FITC-modified enzyme which is reflected by the fluorescence changes is similar to the conformational equilibrium $E_1 \rightleftharpoons E_2$ in the native enzyme. The possibility of stabilizing one form ($E_1$) by Ca\textsuperscript{2+} ions and the other form ($E_2$) with vanadate allows one to measure the equilibrium between the two forms in the absence of Ca\textsuperscript{2+}.

This paper describes the use of this technique to measure the effect of pH and temperature on this equilibrium. The influence of Ca\textsuperscript{2+} ion concentrations, temperature, and pH on the rates of the conformation transitions is described and the

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1 The abbreviations used are: SRCA-ATPase, the Ca\textsuperscript{2+} and Mg\textsuperscript{2+}-dependent ATP hydrolysing enzyme from sarcoplasmic reticulum membranes; SRV, sarcoplasmic reticulum vesicles; FITC, fluorescein 5'-isothiocyanate; FITC-SRV, FITC-modified sarcoplasmic reticulum vesicles; EGTA, ethylene glycol bis(β-aminoethyl ether)-N,N,N',N'-tetraacetic acid.
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implications for the mechanism of action of the enzyme are discussed.

EXPERIMENTAL PROCEDURES

Fragmented sarcoplasmic reticulum vesicles were prepared from rabbit muscles as previously described (14). Membranes were labeled with fluorescein by incubation (10 min at 23 °C) with 30 μM FITC followed by removal of the unbound reagent on Sephadex G-50 columns, as described before (2, 10). Fluorescence measurements were carried out in a Perkin-Elmer MPF 44 spectrofluorimeter as described before (2, 19). Fluorescein-labeled membranes (40-50 μg) were suspended in a cuvette containing in 2.5 ml 50 mM Tris-maleate at the desired pH, 100 mM KCl, 5 mM MgCl\textsubscript{2}, and 0.1 mM EGTA, and the fluorescence changes were followed. The excitation and emission wavelengths were 495 and 525 nm, respectively.

Chemicals were obtained from Sigma Chemical Co.

RESULTS

The Effect of Temperature on the $E_1 \rightleftharpoons E_2$ Equilibrium—Fig. 1 demonstrates the effect of the temperature on the Ca\textsuperscript{2+}-induced fluorescence quenching in the absence and in the presence of vanadate. In the absence of vanadate, the amplitude of the Ca\textsuperscript{2+}-induced fluorescence quenching increased markedly between 10 and 40 °C, and was approximately doubled every 10 °C. Conversely, the vanadate-induced fluorescence enhancement decreased between 10 and 40 °C. The sum of the Ca\textsuperscript{2+}-induced fluorescence quenching (in the absence of vanadate) plus the vanadate-induced fluorescence enhancement was essentially the same at all the temperatures tested, (and was similar to the extent of the Ca\textsuperscript{2+}-induced fluorescence quenching in the presence of vanadate except for temperatures >40 °C). The results imply that in the presence of excess Ca\textsuperscript{2+}

\[
\frac{E_2}{E_1} = \frac{a}{a+b}
\]

when $a = $ Ca\textsuperscript{2+}-induced fluorescence quenching in the absence
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The first order rate constants of the Ca\textsuperscript{2+}-induced fluorescence quenching at pH 7.0 in the presence of vanadate were derived from the fluorescence traces in Fig. 1.

Similarly, one can calculate the equilibrium constant \( K_{E_2/E_1} \) simply as the product \( a/b \). The results in Fig. 1 suggest that in the absence of Ca\textsuperscript{2+} at low temperature the enzyme existed mainly at the \( E_1 \) conformation whereas at high temperature most of the enzyme is in the \( E_2 \) conformation. Fig. 2a shows the temperature dependence of \( K_{E_2/E_1} \), which yields an enthalpy value \( \Delta H^\circ = +20,500 \) cal/mol. Fig. 2b shows that at low pH, which as shown below shifts the equilibrium to \( E_2 \), higher absolute values of the equilibrium constant were obtained, but the temperature dependence and calculated enthalpy change of +20,500 cal/mol remained the same at all pH values. Fig. 1 demonstrates also that the rates of the fluorescence changes induced by addition of vanadate and then Ca\textsuperscript{2+} increased markedly between 10 and 40 °C, indicating very high activation energies for the conformation changes due to binding and release of vanadate ions. The curve in Fig. 3 shows the temperature dependence of the initial rate of the Ca\textsuperscript{2+}-induced fluorescence quenching in the presence of vanadate at pH 7.0. It yields an activation energy of 25 kcal/mol. At pH 8.0, we obtained an activation energy of 35.4 kcal/mol for the Ca\textsuperscript{2+}-induced fluorescence quenching and similar values were obtained for the vanadate-induced fluorescence enhancement (not shown). These high activation energies are

Fig. 3. Temperature dependence of the rate constant \( k_c \).

The first order rate constants of the Ca\textsuperscript{2+}-induced fluorescence quenching at pH 7.0 in the presence of vanadate were derived from the fluorescence traces in Fig. 1.

Fig. 4. The effect of pH on the Ca\textsuperscript{2+}-induced conformation transition in the presence and absence of vanadate. 40 \( \mu \)g of fluorescein-labeled SRV were incubated in a reaction mixture at the pH indicated at 32 °C as in Fig. 1. 200 \( \mu \)M ammonium vanadate, 400 \( \mu \)M Ca\textsuperscript{2+}, or 1 mM EGTA was added when indicated. Other conditions were as described under “Experimental Procedures.”

Fig. 5. The effect of pH on the conformation of the Ca\textsuperscript{2+}-ATPase at 15 and 32 °C. The Ca\textsuperscript{2+}-induced fluorescence quenching obtained in the presence of 200 \( \mu \)M vanadate at 32 °C (circles) and in the absence of vanadate at 32 °C (triangles) and at 15 °C (squares) was measured in Fig. 4 and plotted as a function of pH in a. In b, the fraction of the \( E_2 \) conformation \( (E_2/E_1 + E_2) \) was calculated from the ratio of the fluorescence quenching in the absence and presence of vanadate as explained in the text and plotted as a function of the pH of the medium.
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consistent with the slow rates of interaction of vanadate with the enzyme.

The pH Dependence of the \(E_1 = E_2\) Equilibrium—Fig. 4 demonstrates the effect of pH on the Ca\(^{2+}\)-induced confor-

Fig. 4 also demonstrates that increasing the pH markedly stimulated the rates of the vanadate-induced fluorescence enhancements and of the Ca\(^{2+}\)-induced fluorescence quenching (in the presence of vanadate). Fig. 6, a and b, shows the effect of pH on the first order rate constants of these conformational changes. The initial rate of the Ca\(^{2+}\)-induced fluorescence quenching was increased by approximately 10-fold per 1 pH unit. The relevance of these findings to the interactions between Ca\(^{2+}\) ions and protons will be discussed below.

The Effect of Ca\(^{2+}\) Concentration on the Rate and the Extent of the Conformation Transition—Fig. 7 demonstrates the Ca\(^{2+}\) concentration dependence of the extent of fluorescence quenching at pH 7.0 in the absence of vanadate at 15
El and appreciably, if at all, the conformational equilibrium between turnover cycle. This conclusion is further supported by the FITC modification influences the conformational equilibrium tophane fluorescence in ATP binding and not in other intermediary steps of the or Ca2+ binding fluorescence enhancements in response to Ca2+ and the same FITC-SRV are very similar dependence on Ca2+ ion concentration was obtained in both states of the SR Ca2+-ATPase by following the fluorescence changes in these experiments may be a pH-induced shift of the conformational equilibrium between the phosphorylated and dephosphorylated states of the enzyme; the proton dissociation constants of the free and the protonated enzyme states respectively.

This scheme readily explains the pH dependence of the conformational equilibrium if \( K_{H1} > K_{H2} \) and \( K_{H1} > K_C \) or in other words if \( E_1 \) is a stronger acid than \( E_2 \). This hypothesis is consistent also with several previous observations. Thus, the optimal conditions for phosphorylation of the enzyme by inorganic phosphate are at acidic pH of 5.5-6.0 and a relatively high temperature (18) which, according to the data presented here, are the conditions under which most of the enzyme exists at the \( E_1 \) conformation. However, the maximal phosphorylation yield by \( \gamma^{32}\text{P}\text{ATP} \) is obtained at alkaline pH (19) and at 4 °C. Similarly, a decrease in the apparent affinity for inorganic phosphate observed at high pH (20) is consistent with the shift of the equilibrium to \( E_1 \) at high pH values.

In elegant experiments, de Meis and Tume (21) have demonstrated net ATP synthesis catalyzed by the Ca-ATPase which was induced by a pH shift from pH 5 to 8 in the absence of a Ca2+ concentration gradient. If the pH influences the equilibrium between the phosphorylated and dephosphorylated intermediates in the same way, this would provide an explanation for their results. The driving force for ATP formation in these experiments may be a pH-induced shift of the equilibrium from \( E_2 \) – P to \( E_1 \) – P followed by phosphate transfer to ADP (21).

The possibility that the pH effect on the fluorescence signals reflects the ionization of the bound fluorescein seems very unlikely since as mentioned above we have measured an apparent dissociation constant of 5.7 from the pH dependence of the total fluorescence yield for the bound chromophore, a value significantly lower than the pK values obtained for the conformational equilibrium. However, it seems likely that fluorescein protonation is the reason for the drop in the fluorescence signals below pH 6.0 described in Fig. 5a.

The possible interrelations between Ca2+ ions and protons is of particular interest in light of the results presented here and in relation to previous works. Thus, it was previously demonstrated that the apparent Ca2+ binding affinity of both and the low affinity Ca2+ binding sites increased with a decrease in the proton concentration of the medium (22-24), indicating a Ca2+-proton competition. Also, the recent demonstrations of proton release coupled to Ca2+ binding by Madeira (25) and by Chiesi and Inesi (26) are consistent with displacement of protons by Ca2+ at the active site. This hypothesis of Ca2+-proton competition is presented schematically below:

![Diagram](https://example.com/diagram.png)

**Fig. 8.** The Ca2+ concentration dependence of the rates of the conformational transition. Fluorescein-labeled SRV were preincubated 5 min with 200 μM ammonium vanadate before the addition of Ca2+ in the same reaction mixture described specifically in Fig. 7 and the initial rates of the fluorescence quenching in response to Ca2+ were calculated and plotted as a function of the free Ca2+ concentrations.

and at 30 °C. Half-maximal fluorescence quenching was obtained with 3 μM free Ca2+ at 15 °C and with 4 μM free Ca2+ at 30 °C. These results suggest that the apparent Ca2+ binding affinity is slightly decreased by increasing the temperature in this range. The effect of the temperature on the extent of the fluorescence quenching was already demonstrated in Fig. 1.

Fig. 8 shows the effect of the free Ca2+ concentration on the initial rates of the fluorescence quenching in the presence of vanadate at 15 and at 30 °C at pH 7.5. At both temperatures, a slope of 0.9 was obtained, suggesting that binding of one Ca2+-induced conformation transition. The high Ca2+ concentration requirement will be discussed below.

**DISCUSSION**

In this report, we described measurements of the conformational equilibrium between the two dephosphorylated states of the SR Ca2+-ATPase by following the fluorescence changes in a fluorescein-labeled ATPase preparation. We have shown before that FITC modifies covalently and specifically the ATP binding site of the enzyme (2, 17). This modification introduces both a negative charge as well as a relatively bulky group at the active site and could in principle influence the conformation and the interconversions between the different states of the enzyme.

However, the observation that the FITC modification does not inhibit phosphorylation by inorganic phosphate (13, 17) or Ca2+ binding (13) and the observation that FITC-SRV pumps Ca2+ in the presence of acetyl phosphate as substrate (17) suggested that the modification described specifically in ATP binding and not in other intermediary steps of the turnover cycle. This conclusion is further supported by the observation that the interactions of vanadate with native and FITC-SRV are very similar (13). The possibility that the FITC modification influences the conformational equilibrium was investigated by following Ca2+-induced changes in tryptophan fluorescence (1) in native and in FITC-SRV. The same fluorescence enhancements in response to Ca2+ and the same dependence on Ca2+ ion concentration was obtained in both preparations. Therefore, FITC does not seem to influence appreciably, if at all, the conformational equilibrium between \( E_1 \) and \( E_2 \) and the results obtained with modified enzyme can be taken as relevant also to the unmodified enzyme.

The data presented in this report suggest that in the absence of Ca2+ there is a dynamic equilibrium between two conformations of the dephosphorylated Ca-ATPase, \( E_1 \rightarrow E_2 \), which is strongly affected by both the pH and the temperature of the medium; the \( E_2 \) conformation is stabilized at acidic pH and by high temperature and the \( E_1 \) conformation is stabilized at alkaline pH and by low temperature. The pH effect on the conformational equilibrium may be described schematically as follows:

\[
\begin{align*}
\text{K}_{H_1} & \quad \text{K}_{H_2} \\
E_1 & \quad \text{E}_2 \\
\text{K}_C & \quad \text{K}_\text{CH}
\end{align*}
\]
In this model, we have assumed that Ca$^{2+}$ ions and protons compete for the high affinity Ca$^{2+}$ binding site in $E_1$ and have ignored Ca$^{2+}$ binding to $E_2$.

According to this model, the pH dependence of the apparent Ca$^{2+}$ dissociation constant, $K_{DH}$, can be described by the following equation (see Appendix):

$$K_{DH} = \frac{K_{Ca}(\text{pH})}{K_{Ca}(\text{pH}) - K_{V}}$$

where $K_{Ca}$ is the true pH-independent Ca$^{2+}$ dissociation constant. By assuming the following values: $K_{Ca} = 10^{-7}$ M, $K_{H} = 10^{-9}$ M, and $K_{V} = 10^{-6}$ M, and by using a value of $3 \times 10^{-8}$ for $K_{DH}$ at pH 7.0 (Fig. 7), we derived from the equation a value of close to 3 for $K_C$ and a corresponding value of close to 300 for $K_{CH}$.

By introducing these values for $K_{DH}$, $K_{H}$, and $K_{V}$ into the equation, we calculated the predicted values of $K_{DH}$ at different pH values and the calculated values are in rather good agreement with apparent Ca$^{2+}$ dissociation constants derived according to Almeida and de Meis (24) as shown in Table I.

Figs. 1 and 4 demonstrate that vanadate slows down the rates of the conformational changes induced by addition and by removal of Ca$^{2+}$ ions. Figs. 4 and 6 show that only at acid pH values did vanadate markedly decrease the rates of the conformational changes and that decreasing the proton concentration increased the rates of the conformational changes in the presence of vanadate. These results would be expected if the rate limitations in the presence of vanadate are the binding and dissociation of vanadate and not the conformational equilibria and if the binding and dissociation of vanadate from $E_2$ are much faster than the binding and dissociation from $E_0$. Since changes in the ionization states as well as formation of higher vanadate V complexes (dimers, trimers, etc.) are known to occur between pH 5 and 8 (27), it seems possible that the changes in the response rates reflect differences in the rates of binding and dissociation between different ionization forms of vanadate.

The pronounced effect of the temperature on the equilibrium constant $K_{DH}$, which was demonstrated in Fig. 2, suggests that the conformation transition from $E_2$ to $E_1$ is an exothermic reaction which involves heat release of about 20 kcal/mole. This conclusion is consistent with previous calorimetric measurements which demonstrated a large heat release upon Ca$^{2+}$ binding to sarcoplasmic reticulum vesicles (28). This negative enthalpy change suggests that the conformation transition $E_2 \rightarrow E_1$ is associated with a decrease in the entropy of about 70 cal/mole$ \cdot$ degree (the calculated $\Delta S$ varies from $-77.4$ cal/mole$ \cdot$ degree at 10$^\circ$C to $-66.0$ cal/mole$ \cdot$ degree at 40$^\circ$C). These large entropy changes indicate that the conformation transition is associated with marked internal structural changes in the Ca-ATPase protein.

Previous studies of the effect of temperature on the Ca-ATPase demonstrated breaks in the Arrhenius plots of the Ca-ATPase and Ca$^{2+}$ uptake reactions at around 18-20$^\circ$C (29, 30). These breaks were observed even in soluble ATPase preparation in which the natural lipids had been exchanged and a similar temperature dependence was observed also in a hydrogen exchange reaction of the peptide bonds. This suggests a temperature-induced transition in the structure of the Ca-ATPase protein around 20$^\circ$C. We have observed no such breaks either in the Arrhenius plots of the rate of the Ca$^{2+}$-induced fluorescence quenching (Fig. 3) or in the effect of temperature on the equilibrium constant $K_{DH}$ (Fig. 2). This indicates that the temperature-induced transition affects a subsequent step in the reaction of the enzyme.

Previous studies have demonstrated that temperature influenced the binding of Ca$^{2+}$ (31) and the ratio of Ca$^{2+}$ transported to ATP hydrolyzed (32, 33). At 0$^\circ$C, only one high affinity Ca$^{2+}$ binding site was observed but two high affinity binding sites were detected at 22$^\circ$C (31). In this paper, we demonstrate that an increase in the temperature slightly decreased the affinity to Ca$^{2+}$ (Fig. 7) which is consistent with the effect of temperature on the conformational equilibrium shown in Fig. 2. The observed decrease in affinity from 3$\mu$M at 15$^\circ$C to 4$\mu$M at 30$^\circ$C is slightly smaller than expected from the calculated shift of the conformational equilibrium (4.8$\mu$M). The results shown in Fig. 8 suggest that at least in the presence of vanadate binding of only one Ca$^{2+}$ equivalent seems to be sufficient to induce the conformation transition. It may be noted that also in the (Na-K)-ATPase only one K$^+$ equivalent was required for inducing a similar conformation change (15). This result may suggest a sequential binding mechanism of the two Ca$^{2+}$ ions to the enzyme. Such an interpretation is consistent also with recent Ca$^{2+}$ binding measurements which suggest a cooperative sequential binding of the two Ca$^{2+}$ equivalents to the enzyme (34).

The markedly lower apparent Ca$^{2+}$ affinity seen in the kinetic measurements (Fig. 8) compared to the high Ca$^{2+}$ binding affinity in the equilibrium measurements (Fig. 7) may be reflection of low affinity Ca$^{2+}$ binding to $E_2$ and can be interpreted according to the following scheme:

$$K_{Ca} \frac{E_1}{E_2} \frac{E_2}{E_1} \frac{K_{Ca}}{K_{Ca}}$$

The equilibrium Ca$^{2+}$ binding measurements will reflect mainly the saturation at the high affinity Ca$^{2+}$ binding site on $E_1$ and proceed as follows: $E_2 \rightarrow E_1 \rightarrow E_1Ca$. However, if the rate of the conformation transition $E_2Ca \rightarrow E_1Ca$ is much faster than $E_2 \rightarrow E_1$ (for $a \gg a_C$) then it could be expected that at high Ca$^{2+}$ concentration the rate of the conformation transition will be limited by Ca$^{2+}$ binding to $E_2$ and will proceed as follows: $E_2 \rightarrow E_2Ca \rightarrow E_1Ca$. This interpretation is consistent with the expected low Ca$^{2+}$ binding affinity of $E_2$ and...
it is also consistent with the idea that Ca$^{2+}$ and protons compete for the same binding site and that at the $E_1$ conformation the Ca$^{2+}$ affinity is high and the proton affinity low and conversely in the $E_2$ conformation the Ca$^{2+}$ affinity is reduced and the proton affinity increased by several orders of magnitude. In the normal mode of action of the pump, it is believed that Ca$^{2+}$ is released from the phosphorylated $E_2$ state to the inside. Since our experiments were carried out with intact vesicles, we have to assume that the passive permeability of the membrane was large enough to allow Ca$^{2+}$ to get in during the time of the measurements.

Finally, it should be mentioned that the results reported here suggest striking analogies between the SR Ca-ATPase and the (Na-K)-ATPase. Thus, previous studies in the (Na-K)-ATPase have demonstrated a preferential interaction of vanadate with the $E_2$ conformation (35), similar effects of vanadate ions on the rates of the conformation transitions (36), and also a similar pH effect on the conformational equilibrium of the dephosphorylated enzyme (37). These similarities suggest that the two enzymes are also similar in structure and in their mechanism of action.

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APPENDIX

Derivation of $K_{Ca}^{3}$

The Ca$^{2+}$-H$^{+}$ competition model:

$$
K_{CH} \quad K_{C} \quad K_{Ca}
$$

where:

$$
K_{H_1} = \frac{[H^+][E_1]}{[E_1][H]}
$$

$$
K_{H_2} = \frac{[H^+][E_2]}{[E_2][H]}
$$

$$
K_{C} = \frac{[E]}{[E]}
$$

$$
K_{CH} = \frac{[E][H]}{[E][H]}
$$

$$
K_{Ca} = \frac{[E][Ca]}{[E][Ca]}
$$

The fraction of $E_1$:Ca will be:

$$
f = \frac{[E][Ca]}{[E][E] + [E][H] + [E][E][H] + [E][Ca]}
$$

From Equations 1-6, $f$ can be expressed as:

$$
f = \frac{[Ca]}{[Ca] + K_{Ca}\left(\frac{K_{HC}([H^+] + K_{HC} + K_{C} K_{HC}([H^+] + K_{HC})}{K_{HC} K_{HC}}\right)}
$$

and therefore:

$$
K_{HC} = K_{Ca}\left(\frac{K_{HC}([H^+] + K_{HC} + K_{C} K_{HC}([H^+] + K_{HC})}{K_{HC} K_{HC}}\right)
$$

This equation describes the dependence of the apparent Ca dissociation constant $K_{HC}$ as a function of the pH, the true Ca dissociation constant for $E_1$, the proton dissociation constants $K_{HC}$ and $K_{HC}$, and the conformational equilibrium constant $K_{HC}$.

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