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The calcium ATPase of sarcoplasmic reticulum is inhibited by one Ca^{2+} ion

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Inhibition by calcium of the steady-state turnover of the calcium ATPase from sarcoplasmic reticulum of rabbit muscle follows a Hill slope of 0.8 ± 0.2 (pH 7.0, 0.1 M KCl, varying $[Mg^{2+}]$ and 2 μM A23187 ionophore). It is concluded that dissociation of the two Ca^{2+} ions from E-P·Ca_2 is sequential and that the inhibition arises from the binding of one Ca^{2+} to A-P·Ca_1.

ATPase, calcium; Inhibition; Calcium

1. INTRODUCTION

It is well known that the calcium ATPase of sarcoplasmic reticulum is activated by micromolar calcium and inhibited by millimolar calcium [1–4]. Micromolar calcium binds to the enzyme from the cytoplasmic surface to activate the enzyme for phosphorylation by ATP [1,5] and it is generally believed that inhibition occurs by the binding of two Ca^{2+} ions to a low affinity site after dissociation of 2 Ca^{2+} from the phosphoenzyme, which regenerates E-P·Ca_2 (eqn 1) [3,4,6]. This binding occurs from the lumenal side of the membrane and corresponds to a reversal of the normal pathway for hydrolysis; it prevents formation and hydrolysis of the free phosphoenzyme, E-P. Release of the two Ca^{2+} ions from

$$\text{E-P}\cdot\text{Ca}^2 \rightleftharpoons 2\ Ca^{2+} + \text{E-P} \longrightarrow \text{E} + \text{Pi} \qquad (1)$$

E-P·Ca_2 is rate-limiting for turnover at saturation with ATP and calcium under most conditions [7,8].

In principle, inhibition could occur by the binding of either 1 or 2 Ca^{2+} ions to E-P, to give E-P·Ca_1 or E-P·Ca_2, or by binding of 1 Ca^{2+} to E-P·Ca_1 to regenerate E-P·Ca_2 after the release of the first Ca^{2+}. It has been shown and confirmed that the release of the two Ca^{2+} ions from E-P·Ca_2 is sequential and ordered [9–11]. The reaction of $^{45}Ca^{2+}$ can be followed with intact vesicles by quenching the reactions with ADP in a rapid mixing apparatus, because E-P will not react with ADP after the $^{45}Ca^{2+}$ has been released into the interior of the vesicle. It was found with this assay that the first Ca^{2+} ion loses sensitivity to ADP with $k = 34\ s^{-1}$, with no inhibition by up to 40 mM internal Ca^{2+}, while internalization of the second Ca^{2+} occurs with $k = 17\ s^{-1}$ and is inhibited by internal Ca^{2+} with half-maximal inhibition at approximately 1.3 mM Ca^{2+} and a Hill slope of $n = 1.1$ [11].

We report here that catalysis of ATP hydrolysis by the calcium ATPase in the steady state is inhibited by a single Ca^{2+} ion at concentrations near millimolar.

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2. EXPERIMENTAL

Sarcoplasmic reticulum vesicles were prepared from rabbit muscle [11] and were found to hydrolyze ATP at rates of 7.0–8.4 μmol min^{-1} mg^{-1} in the presence of 2 μM A23187 ionophore, 1.5 mM ATP, 5 mM $MgSO_4$, and 25 μM $CaCl_2$, under the conditions described in table 1. Enzyme activity was measured spectrophotometrically by coupling ADP production to NADH oxidation with pyruvate kinase and lactate dehydrogenase [12].

3. RESULTS

Calcium at low concentrations of Mg^{2+} can inhibit the enzyme by competing with Mg^{2+} for binding with

Table 1

Inhibition of the sarcoplasmic reticulum ATPase by calcium

[ATP] (mM)	[Mg]	$K_{0.5}$(Ca) (mM)	n
0.1	optimum	0.63	0.9
0.1	20 × [Ca]	0.78	0.8
0.15	optimum	0.63	0.9
0.3	optimum	0.35	0.7
1.5	optimum	0.32	0.7
1.5	25 × [Ca]	0.50	0.8

Conditions: 0.1 M KCl, 2 μM A23187 ionophore, 40 mM Mops buffer (pH 7.0), 1 mM PEP, 0.15 mM NADH, 0.01 mg/ml sarcoplasmic reticulum vesicles, 0.025 mg/ml pyruvate kinase and 0.025 mg/ml lactate dehydrogenase at 25°C

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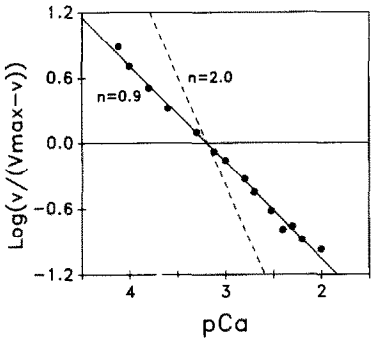

Fig.1. Hill plot for inhibition of calcium ATPase activity calcium. Conditions as in table 1, with 0.1 mM ATP and optimal $MgSO_4$.

ATP at the catalytic site; it forms a species Ca·E-P·Ca_2 that reacts very slowly, while high concentrations of Mg^{2+} inhibit activity at low calcium concentrations by binding at the calcium transport site [3,13]. In order to avoid inhibition by these mechanisms, rates were measured at the optimum concentration of Mg^{2+}, which was determined at each of a series of different concentrations of Ca^{2+}. Alternatively, the concentrations of Ca^{2+} and Mg^{2+} were increased together at a constant ratio in order to examine Ca^{2+} inhibition. The rate was found to be independent of [Mg^{2+}] over a considerable concentration range at [Ca^{2+}] > 0.1 mM and was determined at the optimum [Mg^{2+}] at lower [Ca^{2+}].

Fig.1 shows a Hill plot for inhibition of ATPase activity by calcium with 0.1 mM ATP and optimal concentrations of Mg^{2+}. The slope corresponds to $n = 0.9$. The table summarizes the results of similar experiments at different concentrations of ATP using both methods for variation of the Mg^{2+} concentration. The slopes of $n = 0.8 \pm 0.1$ are consistent with inhibition by a single Ca^{2+} ion in the presence and in the absence of activation by ATP, which gives an increase in rate of approximately two-fold at 1.5 mM. Values of $n = 1.3$ and 1.1 have been reported for inhibition of the hydrolysis of ATP and of acetyl phosphate, respectively, based on limited data [14].

4. DISCUSSION

The inhibition is attributed to binding of one Ca^{2+} ion to E-P·Ca_1, which regenerates E-P·Ca_2, with the rate constant k_{-1} (eqn 2). Binding of one Ca^{2+} to E-P is possible (k_{-2}), but is less likely to be responsible for the inhibition because the binding of two Ca^{2+} ions to E-P

$$\text{E-P}\cdot\text{Ca}_2 \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} \text{E-P}\cdot\text{Ca}_1 + \text{Ca}^{2+} \underset{k_{-2}}{\overset{k_2}{\rightleftharpoons}} \text{E-P} + 2\,\text{Ca}^{2+} \quad (2)$$

is cooperative [3,15]. Cooperativity requires that the second Ca^{2+} to bind, which is the first to dissociate, must have a higher affinity than the other Ca^{2+}, so that binding of one Ca^{2+} to E-P·Ca_1 is expected to give inhibition. If the concentration of Ca^{2+} is high enough for a single Ca^{2+} ion to bind to E-P to give E-P·Ca_1, it would be expected that a second Ca would also bind, because of this cooperativity; inhibition would then involve the binding of two Ca^{2+}, contrary to the experimental result.

The results show that dissociation of Ca from E-P·Ca_2 into the lumen of the vesicle, or into the medium with leaky vesicles or in the presence of ionophore, is sequential. It is not known at this time whether or not this is the same process as the sequential loss of sensitivity to ADP of the two Ca^{2+} ions of E-P·Ca_2 [9–11]. It should be emphasized that we are not yet able to provide a detailed interpretation of the mechanism of binding and dissociation of Ca^{2+} with the phosphoenzyme. This process is the most complex, and possibly the most important, in the reaction cycle of the ATPase.

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