

The Effect of Calcium and Phosphate on the Biphasic Calcium Uptake by the Sarcoplasmic Reticulum

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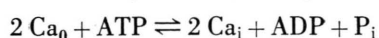
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Sarcoplasmic Reticulum, Calcium, Phosphate, Flow Dialysis

The amplitude of the fast uptake and the initial rate of the slow uptake increase with increasing free calcium concentrations, up to $30\ \mu\text{M}$. In that range, both processes are correlated to each other. At higher concentrations, the slow uptake is more inhibited than the fast uptake. The fast uptake shows a maximum amplitude which remains unchanged in the presence of phosphate. The slow uptake leads to a nearly complete depletion of the external calcium, and its rate is proportional to the phosphate concentration, even at physiological range. The sarcoplasmic ATPase liberates inorganic phosphate and the slow uptake is an autocatalytic process.

Introduction

In a previous paper, the flow dialysis procedure was used to study the kinetic of the Ca^{2+} -uptake by the fragmented sarcoplasmic reticulum (FSR). It was shown that the calcium accumulation by the vesicles displays two phases which were called the fast and the slow uptake, respectively. The former is an exponential function of time, the latter presents the characteristics of an autocatalytic reaction. A set of reactions is autocatalytic when it is inhibited by a substrate or activated by a final product. During the ATP-driven calcium uptake the concentration of external calcium decreases, while that of both internal calcium and total inorganic phosphate increases. The inhibition of the SR-ATPase by high calcium concentrations has been recognized by several authors^{2–4}. The phosphate is neither actively accumulated nor does it affect the ATPase activity. Therefore, phosphate was considered as a simple calcium precipitating agent which is passively dragged into the vesicles⁴. More recently, however, it was discovered that the phosphate participates as a substrate to the reverse direction of the reaction described by the overall equation:



and therefore should inhibit the inward movement. Both activating and inhibiting effects were taken into account by introducing the solubility product of calcium phosphate into the above equation.

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Under this assumption Ca_0 at equilibrium decreases with increasing $[\text{P}_i]$ and the rate of Ca uptake increases likewise. The present work will show that the rate of the slow uptake phase increases linearly with the phosphate concentration. Moreover, the phosphate liberated by the ATPase during the slow uptake is sufficient to explain the autocatalytic aspect of the second phase.

Material and Methods

The fragmented sarcoplasmic reticulum was extracted from the crayfish tail according to the slightly modified procedure of MacLennan^{1,5}. The adaptation of the non-equilibrium dialysis technique to kinetical purposes has been described previously¹. The protein content was measured by the biuret method in the presence of Na-deoxycholate, and the inorganic phosphate liberated by the ATPase was determined by the phosphomolybdate method⁶. When non-specified, the medium contained 5 mM MgCl_2 , 50 mM imidazol (pH 7.45), 100 mM KCl, 0.05 mM CaCl_2 , 5 mM ATP, and the reaction was initiated by the addition of 100–200 $\mu\text{g}/\text{ml}$ reticulum protein. In these conditions about 28% of the solution calcium is free. This value was either measured by the differential dialysis method⁷, or calculated by using the stability constants of Schwarzenbach⁸. The linear functions were fitted with a linear regression program.

Results

The amplitude of the fast uptake and the initial rate of the slow uptake are phenomenologically related to the initial concentration of ionized cal-



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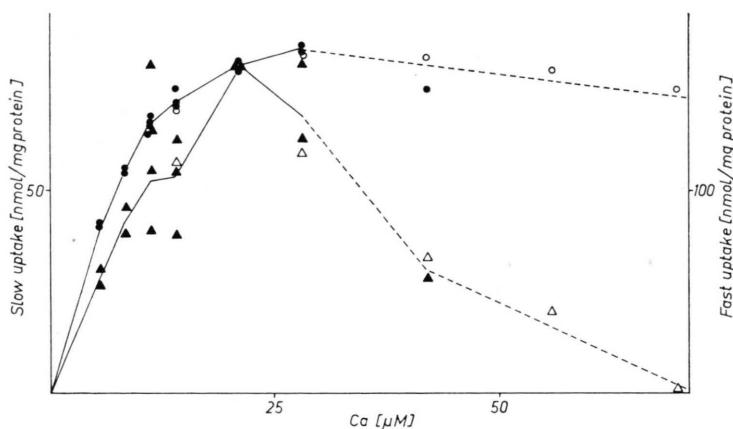


Fig. 1. Calcium dependence of slow and fast uptake. The medium contained 5 mM MgCl_2 , 50 mM imidazol (pH 7.45), 100 mM KCl, 5 mM ATP, 160 $\mu\text{g/ml}$ reticulum protein and various concentrations of radioactive CaCl_2 . ●, calcium accumulated during the fast uptake (○, 150 $\mu\text{g/ml}$ reticulum protein). ▲, calcium accumulated during the 3 min following the fast uptake, in the same experiments. △, calcium accumulated during the 4 min following the fast uptake, for 150 $\mu\text{g/ml}$ reticulum protein. Only the data represented by full symbols and corresponding to calcium concentrations under 30 μM were used for the statistical fit.

cium up to 30 μM . In this concentration range the relation is reminiscent of a saturation curve with a maximal capacity of approximately 200 nmol per mg protein. The data of the slow uptake have a considerable dispersion. Nevertheless, both processes are correlated to each other. The correlation coefficient equals 0.747, the probability of non-correlation is 0.1% (Fig. 1). When the external concentration of free calcium exceeds 30 μM , the fast uptake reaches a plateau or seems to decrease slightly, while the slow uptake is strongly inhibited. As indicated in Table I, the same inhibition is observed in the presence of phosphate.

Table I. Calcium dependence of the uptake rate 3 min after the addition of the vesicles, for the same initial concentration of phosphate. First column: The medium contains 5 mM MgCl_2 , 50 mM Tris (pH 7.45), 100 mM KCl, 5 mM ATP, 1 mM P_i , and 100 $\mu\text{g/ml}$ reticulum protein. Second column: The medium contains 5 mM MgCl_2 , 50 mM imidazol (pH 7.45), 100 mM KCl, 5 mM ATP, 2 mM P_i , and 100 $\mu\text{g/ml}$ reticulum protein.

Initial calcium concentration [μM]	Uptake rate [nmol·min ⁻¹ ·mg ·protein ⁻¹]	
	$\text{P}_i=1\text{ mM}$	$\text{P}_i=2\text{ mM}$
14	320	
56	115	335
100	75	160
140	—	50

The phosphate activates the slow uptake even at low concentrations, while the fast uptake remains practically unchanged (Fig. 2). In order to show this specific action of the anion, the incremental amounts of calcium accumulated when the concentrations of phosphate are raised from 0 to 0.5 mM or from 1 to 2 mM are reported in Fig. 3. No change

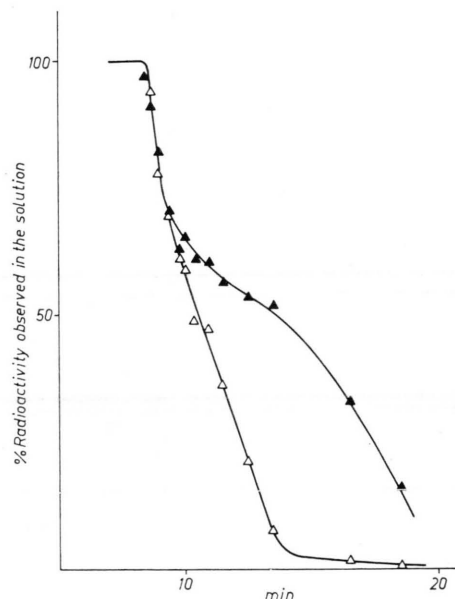


Fig. 2. Activation of calcium uptake by the phosphate. ▲, -▲-, The medium is that described in the methods section. △, -△-, The medium is that described in the methods section, plus 0.5 mM phosphate.

is observed during the fast phase. The additional uptake reaches its maximum 6–7 min after the addition of reticulum. Then the effect of phosphate becomes less and less detectable, because the slow uptake always leads to a nearly complete removal of the calcium from the medium¹.

Since the fast uptake is not activated by phosphate, it becomes less and less observable with increasing amounts of phosphate and calcium. In order to resolve the action of phosphate, the effect of the calcium must be circumvented. For this purpose, the data of the slow uptake rate and of the phosphate concentration respectively were

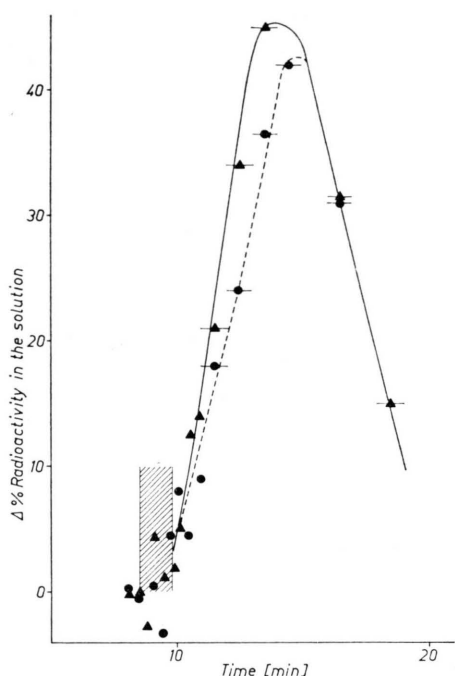


Fig. 3. Activation of calcium uptake by the phosphate. Each curve is a comparison between two experiments where the medium differs only for the initial phosphate concentration. The curves are time functions and represent the per cent of radioactivity in the solution for the first experiment minus the per cent of radioactivity in the solution for the second experiment. ▲, —▲—, The first medium is that described in the methods section, the second contains 0.5 mM phosphate; ●, —●—, the first medium is that described in the methods section except that the calcium concentration is 0.2 mM and the phosphate concentration 1 mM; the second medium contains 2 mM phosphate. The etched surface corresponds to the interval of maximal activity of the fast uptake.

divided by the concentration of ionized calcium present in the solution at the corresponding time. Both ratios are linearly correlated in a wide range of calcium and phosphate concentrations (Fig. 4). The calculated slope is 0.019 min^{-1} , the correlation coefficient 0.985 and the non-correlation probability far below 0.1%.

Discussion

Both calcium uptake and SR ATPase activity depend on the concentration of free external calcium considerably. This dependence varies according to the concentration range of calcium under consideration. Below $1 \mu\text{M}$ the calcium activates the enzyme with a Hill coefficient of $1.8^{9,10}$. Between 1 and

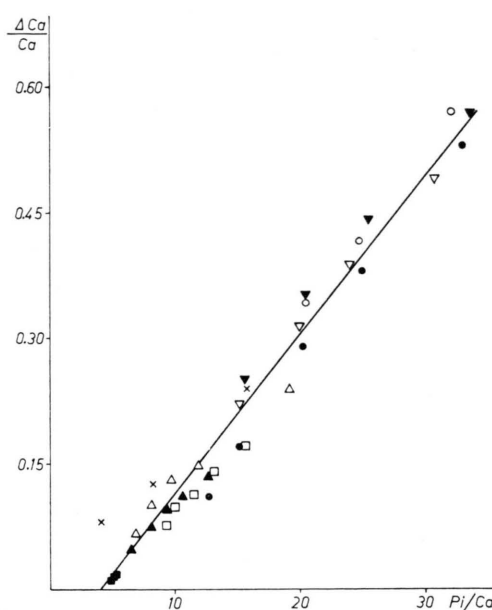


Fig. 4. The relationship between the role of the slow uptake and the concentration of calcium. Ca^{2+} -uptake rates and phosphate concentrations are divided by the calcium concentration as they are present in the assay at the moment of observation. The values of the uptake rate recorded here are the tangents of the uptake curve measured at different time intervals of several experiments. The concentration of calcium decreases with time, while the phosphate concentration is increased by the activity of the reticulum ATPase. This increase is taken in account here. The initial concentration of calcium and phosphate are respectively: ×, $50 \mu\text{M}$ and $0 \mu\text{M}$; ▲, △, $200 \mu\text{M}$ and $1000 \mu\text{M}$; ▼, ▽, $200 \mu\text{M}$ and $2000 \mu\text{M}$; ●, ○, $350 \mu\text{M}$ and $2000 \mu\text{M}$; ■, □, $500 \mu\text{M}$ and $2000 \mu\text{M}$; and $500 \mu\text{M}$ and $4000 \mu\text{M}$.

$30 \mu\text{M}$ the ATPase activity and the uptake rate in the presence of oxalate and EGTA remain unchanged^{2,9,10}, while the fast uptake amplitude increases and reaches a plateau. The latter observation was confirmed by Makinose using rabbit SR¹¹. The correlation observed between the fast uptake amplitude and the slow uptake initial rate, the dependence of these values on the initial concentration of ionized calcium, are phenomenological relations. Their significance is not yet understood. The amplitude of the fast uptake tends asymptotely to a maximal value. According to other results not reported here the amplitude is also proportional to the vesicular protein concentration for the same initial calcium concentration. When the external free calcium concentration exceeds $30 \mu\text{M}$, one observes a drop of both ATPase activity¹⁰ and slow uptake, while the fast uptake is hardly inhibited. This observation corresponds to the finding that at

low ATP concentrations the slow uptake is much more reduced than the fast uptake¹.

The amplitude of the fast uptake is not affected by phosphate. However, the resolution of the non equilibrium dialysis does not permit to rule out a possible activation of the fast uptake rate. An argument against this possible activation is the fact that for the slow uptake, both uptake rate (this work) and uptake amplitude after 15 min¹² increase linearly with the phosphate concentration. The slope of the curve represented in Fig. 4 reveals a linear relationship between the rate of calcium uptake and the phosphate concentration and could simply correspond to the diffusion constant of the phosphate through the vesicular membrane. In that case, the passive transport of the phosphate would

be the regulating step of the uptake process. The calcium can cross the membrane rapidly, but the transport of a single type of ions would lead to the creation of an electrical potential and therefore would be rapidly slowed down. However, the observed competition between phosphate and oxalate in a very narrow concentration range suggests a mechanism more complicated than a simple diffusion¹³. The main result of this study is that even in the concentration range found in the resting living muscle¹⁴, the phosphate can activate the calcium uptake. The increase of the inorganic phosphate concentration during prolonged muscular work might enhance the activity of the sarco-plasmic pump.

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