POSSIBLE FUNCTIONAL STATES OF THE ENZYME OF THE SARCOPLASMIC CALCIUM PUMP

M. MAKINOSE

Max-Planck-Institut für medizinische Forschung, Abteilung Physiologie, Heidelberg, 69 Heidelberg, Jahnstrasse 29, GFR

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1. Introduction

The calcium accumulation performed by the sarcoplasmic vesicles isolated from the skeletal muscle is a unique example for an active ion transport process by biomembranes. The high transport activity on the one hand and the nearly complete absence of other enzymatic activities on the other hand, made it possible to perform detailed kinetic studies of calcium translocation and of the coupled ATP (NTP) hydrolysis. In this respect the sarcoplasmic membrane proved to be superior to most other membranes with transport activity. On the basis of such studies, a number of reaction mechanisms of the sarcoplasmic calcium pump were proposed by several authors with more or less experimental support [1–8].

In the last years, it was demonstrated that the calcium accumulation by the sarcoplasmic vesicles is a reversible process [9–11]. The translocation of calcium in both directions across the membrane is provided by the formation of an activated intermediate– a phosphoprotein [12,13]. Recently, it has been shown that, even in the absence of energy-rich phosphate donators, the vesicle protein can be phosphorylated with orthophosphate after formation of a steep concentration gradient of calcium ions across the membrane [12,13]. This phosphoprotein has the same chemical properties as those of the phosphoproteins obtained in the presence of energy rich phosphate donators and was assumed to be an acyl phosphate.

These observations indicate directly that the sarcoplasmic membrane acts as an energy converter which transduces osmotic energy into chemical energy and vice versa. They caused us to verify the supposed reaction steps of the sarcoplasmic calcium pump.

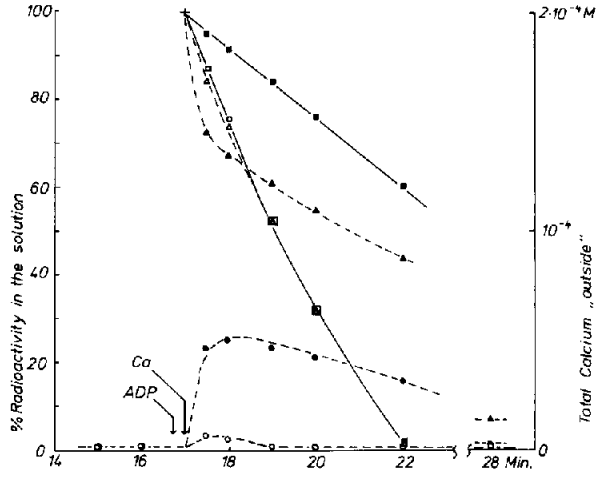

Fig. 1. Calcium fluxes during calcium accumulation by the sarcoplasmic vesicles. At first, the vesicles were loaded with 0.4 μmoles per mg protein of hot (circles) or cold (triangles) calcium in assays containing 20 mM histidine, 7 mM $MgCl_2$, 40 mM KCl, 5 mM ITP, 5 mM orthophosphate, 0.2 mM calcium and 0.5 mg vesicle protein per ml assay. After 17 min, the same amount of cold or hot calcium was added to the hot or the cold assays respectively. Shortly before the second calcium addition, 0.5 mM ADP was added to identical assays (full symbols). ○ and ● : Started with hot calcium; △ and ▲ : Started with cold calcium (left ordinate). □ and ■ : Calculated concentration of total external calcium (right ordinate).

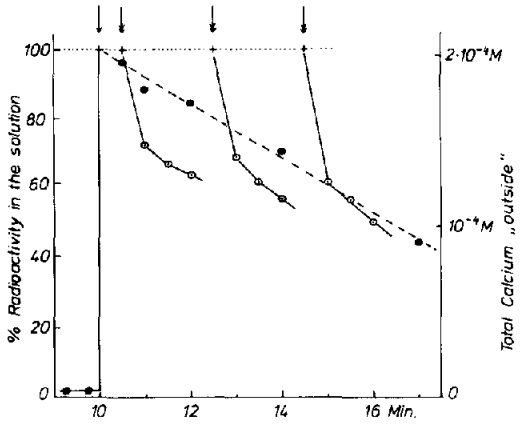

Fig. 2. Influx and net uptake of calcium in the presence of ADP. Under the same condition as in fig. 1, the vesicles were preloaded with cold calcium (0.4 μmoles per mg protein). At 10 min, 0.5 mM ADP and 0.2 mM calcium were added (↓). (•) Show the decrease of total calcium concentration in the external solution (right ordinate). ↓: addition of ^{45}Ca. (⊙) Show the decrease of the radioactivity from the external solution (left ordinate).

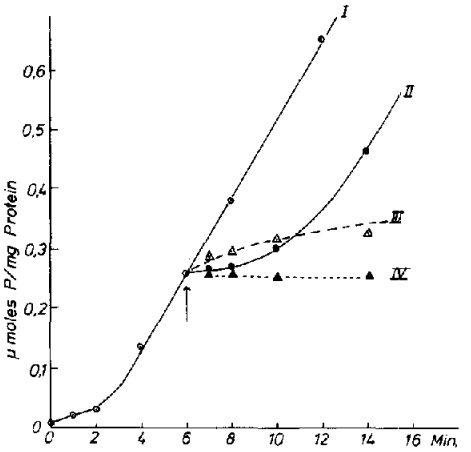

Fig. 3. Orthophosphate incorporation into the NTP fraction during the calcium transport. The initial assay (curve I) contains 20 mM histidine, 5 mM ITP, 7 mM $MgCl_2$, 40 mM KCl, 5 mM [^{32}P] orthophosphate, 0.2 mM $CaCl_2$, and 0.5 mg/ml vesicle protein. After 6 min, 0.2 mM $CaCl_2$ (curve II) or 0.5 mM ADP (curve III) or both of 0.2 mM $CaCl_2$ and 0.5 mM ADP (curve IV) were added to the aliquots of the initial assay.

2. Experimental

The sarcoplasmic vesicles were prepared according to Hasselbach and Makinose [15]. The methods for determination of calcium exchange [14], NTP–NDP exchange [2,4] and orthophosphate incorporation in NTP fraction [10] have been described elswhere.

3. Results and discussion

When sarcoplasmic vesicles are loaded with calcium (0.4 μmoles per mg protein) in a solution containing ITP and PO_4 (fig. 1.), the addition of a second portion of calcium to the assay induces not only the resumption of calcium uptake but also a simultaneous outflux of calcium from the vesicles. In the absence of ADP, this efflux of calcium is very small so that the rate of calcium accumulation is nearly identical with the rate of calcium influx. In the presence of 0.5 mM ADP, however, the rate of calcium accumulation is strongly reduced [14] while the rate of calcium influx and that of calcium efflux are strongly enhanced. The rate of calcium influx is even higher than in the absence of ADP. It should be pointed out that under these very conditions – i.e., in the presence of ADP and of external calcium – the NTP–NDP exchange reaction is activated [1, 2, 4].

Fig. 1. gives the impression that the exchange between external and internal calcium takes place only for a short period of time after the addition of the second portion calcium. This is, however, not the case. As shown in fig. 2, when the external calcium is labelled by addition of a negligible small amount of ^{45}Ca of very high specific activity at various time intervals after the addition of the second portion of calcium, a rapid disappearance of radioactivity after each labelling is observed, i.e., the exchange of calcium between inside and outside takes place throughout the observed time interval. Under the prevailing experimental conditions, the main fraction of the accumulated calcium is stored as calcium phosphate crystals inside of the vesicles. The calcium, which forms the core of the crystals can scarcely be exchanged with calcium dissolved in the internal milieu and, consequently, with that in the external solution around the vesicles. Hence, the rapidly exchangeable part of the accumulated calcium is relatively small. This is presumably the reason for the apparent cessation of the calcium exchange within the short time span (fig. 1).

In order to assure the correlation between calcium exchange and phosphate liberation, orthophosphate

Table 1
The exchange rate of calcium and phosphate (nmoles/mg protein.min).

	-ADP	+ADP
Calcium		
Influx	128	220
Efflux	24	180
Accumulation (influx–efflux)	104	40
PO_4-incorporation into organic phosphate	10	0
NTP–NDP exchange	—	312

Conditions: 20 mM histidine; 7 mM $MgCl_2$; 40 mM KCl; 5 mM ITP; 5 mM orthophosphate; 0.2 mM calcium and 0.5 mg vesicle protein per ml assay; 2 mM ADP was added; preloading of calcium is 0.4 μmoles per mg protein; pH 7; room temperature.

incorporation into the NTP fraction was measured under the same conditions as described in fig. 1. Fig. 3. shows that the orthophosphate incorporation takes place when the external calcium concentration is lowered by the calcium accumulation in the starting assay. The addition of the second portion of calcium leads to an intermission of orthophosphate incorporation until the external calcium concentration is lowered again (Fig. 3, Curve II). On addition of 0.5 mM ADP or together with 0.2 mM calcium, the incorporation of orthophosphate ceases completely. In other words, under the conditions, under which the calcium fluxes and NTP–NDP exchange reaction are stimulated, the orthophosphate incorporation into NTP is completely suppressed.

These experimental results allow to establish the sequence of two essential steps in the reaction chain of calcium translocation for which the following scheme has tentatively been proposed [1–8]:

$$2\,Ca_o + NTP + E_1 \overset{Mg}{\rightleftharpoons} Ca_2E \sim P + NDP \qquad \text{(step 1)}$$

$$Ca_2E \sim P \overset{Mg}{\rightleftharpoons} 2\,Ca_i + PO_4 + E_2 \qquad \text{(step 2)}$$

$$E_2 \rightleftharpoons E_1 \qquad \text{(step 3)}$$

where Ca_o and Ca_i are the external and internal calcium concentration respectively. The described results

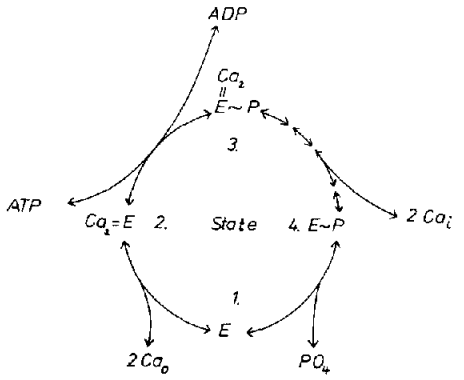

Fig. 4

indicate that the phosphorylated carrier releases the calcium to the internal space of the vesicles before the bound phosphate is lost. If the phosphorylated enzyme lost the phosphate simultaneously with the calcium or even before it, one should expect an activation of orthophosphate incorporation into the NTP fraction when the calcium exchange is enhanced. This is, however, not the case (table 1). It means that step 2 in the reaction scheme includes two sub-steps:

$$Ca_2\,E \sim P \quad E \sim P + 2\,Ca_i \qquad \text{(step 2a)}$$

$$E \sim P \quad E_2 + PO_4 \qquad \text{(step 2b)}$$

Taking these facts into account, functional circulation of the carrier enzyme for the calcium translocation could be written as shown in fig. 4. Certainly, some intermediate states could exist between the four assumed states. In the forward reaction, when calcium is accumulated, the enzyme binds two calcium ions on the external surface of the vesicle membrane (state 1–2). It reacts with NTP and forms phosphoprotein (state 2–3). The phosphorylated intermediate releases calcium ions in the inside of the vesicles (state 3–4) and is subsequently dephosphorylated (state 4–1). State 1 and 2 takes place on the external surface and state 4 on the internal surface of the vesicle membrane. The localisation of the state 3 remains to be defined.

As was already reported [11,12], in the absence of NTP and NDP, the labelling of the vesicle protein by [^{32}P] orthophosphate is enhanced by increasing of Ca_i as well as lowering of Ca_o. As a consequence, in

the foreward reaction, the enzyme should bind external calcium before it reacts with ATP as formulated in fig. 4. However, this does not exclude that additional reaction steps may occur in parallel or in series with the discussed sequence.

The vesicle protein in state 1 binds calcium apparently with high affinity. Affinity constants between $10^6 - 10^7$ M^{-1} were given by several authors [8, 16, 17]. In state 4, this high affinity to calcium disappears. The enzyme reaches state 1 from state 4 by losing phosphate and regains the high affinity for the calcium ions.

Obviously, the formation of the high energy phosphate bond in the vesicle protein diminishes the high calcium affinity of the calcium transport enzyme.

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