SARCOPLASMIC RETICULUM FROM BARNACLE MUSCLE; COMPOSITION AND CALCIUM UPTAKE PROPERTIES

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1. Introduction

Sarcoplasmic reticulum (SR) in vertebrate skeletal muscle is a membrane system involved in the regulation of muscle contraction and relaxation, from which vesicles that transport Ca^{2+} coupled to ATP hydrolysis can be readily isolated. This vesicular system has been extensively studied during the last few years. However, less information is available concerning SR in invertebrate muscle. Microsomal fractions that actively transport Ca^{2+} when provided with ATP have been prepared from lobster [1,2] and from barnacle muscle [2]. A partial purification of the ATPase enzyme isolated from lobster muscle microsomes has also been described [3].

This report is a brief description of a sarcoplasmic reticulum vesicular fraction isolated from barnacle muscle. Protein and lipid compositions were studied, revealing a very simple system formed mainly by one protein, tentatively identified as the ATPase enzyme, and phospholipids as the major lipid class. This system displays a calcium-dependent ATPase activity and in the presence of ATP is able to take up calcium without oxalate in the medium.

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2. Materials and methods

Muscle fibers were isolated from the barnacle *Megabalanus psitaccus* and fragmented sarcoplasmic reticulum vesicles (SR) were prepared as described before [2]. SDS solubilization of SR vesicles was achieved by heating the samples for 5 min in a boiling water bath with 1% SDS, 10 mM DTT, 20 mM Tris-maleate, pH 7.0. Slab gel electrophoresis of the SR proteins was carried out as described in the legend to fig.1. Lipid fractions were extracted from lyophilized SR samples in chloroform methanol mixtures [5], and the extracts were analyzed for total lipids [6], phospholipids [7] and cholesterol [8]. The phospholipids were separated by two dimensional chromatography on TLC plates (0.25 mm, silica gel G, Merck) with chloroform : methanol : concentrated ammonia : water (80 : 80 : 2.7 : 7.3) and chloroform : methanol : acetone : glacial acetic acid : water (50 : 10 : 20 : 10 : 5), in the first and second dimension respectively. The phospholipid composition was determined by measuring the amount of P_i in the scraped spots previously digested in 70% perchloric acid [7]. ATPase activities were measured by determining the production of inorganic phosphate [9] in a medium containing 0.1 M KCl, 4 mM $MgCl_2$, 2 mM ATP, 50 mM Tris–HCl, pH 7.4 variable Ca^{2+} and EGTA concentrations. Protein concentrations used were in the range of 40 to 100 μg per ml. Ca^{2+} uptake was measured by the isotopic distribution method [10] using HA (0.45 μm) millipore filters, in the same medium described for ATPase assays. Blanks without ATP were always subtracted. All Ca^{2+} uptake experiments were done in the absence

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of oxalate, in order to mimic the conditions present in the in vivo system.

3. Results and discussion

SR membranes were obtained from barnacle muscle with yields of 1.0 mg protein per g wet muscle. Electron microscopic examination by negative staining of this preparation (data not shown) revealed a highly pure preparation of vesicles ranging from 120–160 nm in diameter, very similar to those described by Baskin [2]. The gel electrophoretic patterns of the SR membrane proteins from barnacle and rabbit white muscle are shown in fig.1. It is apparent in the gels that the major component in both systems corresponds to a protein of approx.106 000, which in the case of rabbit SR has been identified with the Ca^{2+}-transport ATPase [12]. No bands corresponding to the 55 000 mol. wt protein (calsequestrin) or the lower mol. wt. proteins found in rabbit SR [13] are present in barnacle SR. On comparison of the gel electrophoretic patterns of barnacle SR shown in fig.1 and lobster SR described elsewhere [3] some common features are apparent. Both systems have some bands of higher mol. wt than the ATPase as minor components and both lack calsequestrin, but the overall protein composition of barnacle SR is much simpler than lobster SR, since in the latter several bands of low mol. wt proteins were found. (However, caution must be taken in comparing gels obtained under different experimental conditions.)

Table 1 shows the lipid analysis of barnacle SR. The resulting profile is very similar to that obtained with rabbit SR [14]. Phospholipids constitute the major fraction of the total lipids, with phosphatidyl choline

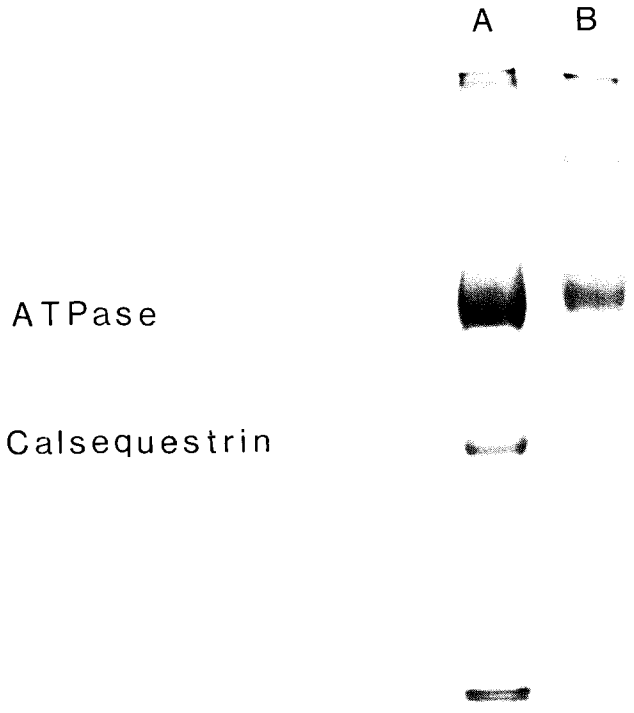

Fig.1. Slab gel electrophoresis of solubilized SR membrane proteins from rabbit white skeletal muscle (A) and barnacle (B). Rabbit SR was prepared as described elsewhere [11]. A slab gel apparatus (Hoefer Scientific Instruments Inc.) was used. 8% gels of 0.75 mm thickness were prepared according to the procedure described by Laemmli [4]. Twenty μg of solubilized membrane protein were used in each case. Gels were run at 1.6 mA per sample and were subsequently stained with Coomassie brilliant blue.

Table 1
Lipid composition of barnacle sarcoplasmic reticulum

Component		%
Total lipid (mg/mg protein)	0.56 ± 0.07 (15)	100
Phospholipids (mg/mg protein)	0.46 ± 0.01 (4)	82
Cholesterol (μg/mg protein)	40.4 ± 6.6 (20)	7
Phospholipid classes (% of total P_i) (Values are the average of two experiments)		
Phosphatidyl choline	70.0 ± 0.7	
Phosphatidyl ethanolamine	13.8 ± 0.5	
Phosphatidyl inositol	7.2 ± 0.5	
Phosphatidyl serine	5.0 ± 0.3	
Sphingomyelin	3.6 ± 0.0	
Cardiolipin	0.4 ± 0.1	

Experimental details are described in the text. Numbers represent mean ± SD; in parenthesis is the number of determinations.

and phosphatidyl ethanolamine as main components. Cholesterol is only a very minor fraction of the total lipids, a feature that seems to be a constant in all SR preparations so far studied and that is perhaps related to the strong inhibitory action of cholesterol on the purified ATPase enzyme [15].

The effect of varying the Ca^{2+} concentration on the ATPase activity is shown in fig.2. Very low Ca^{2+} concentrations, of the order of 1 μM, suffice to stimulate maximally the ATPase activity, which remains constant up to about 30 μM to be inhibited by increasing Ca^{2+} concentrations thereof. This behavior of the ATPase in barnacle SR is similar to that observed in SR isolated from vertebrate muscle [17], showing that in barnacle SR an inherent regulatory role of Ca^{2+} on enzymatic activity is also present. The optimum pH of the ATPase was found to be 7.4, the activity declining sharply to undetectable levels at either 6.5 or 8.0. The optimum assay temperature was between 27°C to 30°C, with a sharp inhibition at 35°C to reach complete suppression at 40°C (not shown). These results show that the ATPase activity in barnacle SR has very stringent requirements in terms of Ca^{2+} concentrations, pH and temperature.

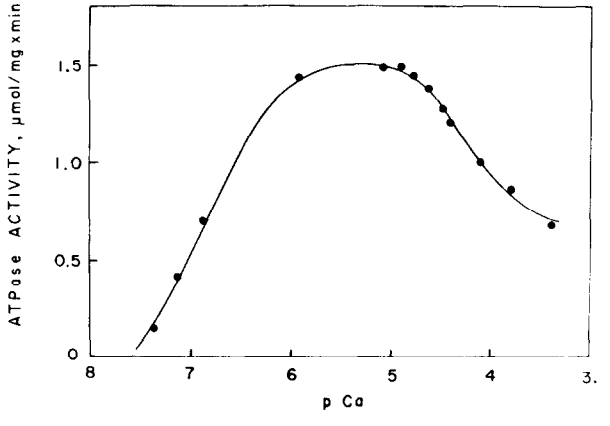

Fig.2. Ca^{2+}-dependence of the ATPase activity in barnacle SR. ATPase activities were measured as described under methods with variable Ca^{2+} and EGTA concentrations. Free Ca^{2+} concentrations were calculated with the use of a computer program [16], taking into account the endogenous Ca^{2+} concentrations of SR and of the chemicals, measured by atomic absorption spectroscopy. The assay temperature was 27°C.

Table II illustrates the values of Ca^{2+} uptake, in the absence of externally added precipitating agents, at different temperatures. Maximum uptake values were obtained at 17°C and 20°C, to decline at 25°C, suggesting that in these experimental conditions there is a considerable impairment of uptake as the temperature is raised above 20°C, despite the fact that the ATPase activity keeps increasing up to 30°C. However, no direct comparison of calcium uptake and ATPase activities at different temperatures is possible in these types of experiments, since further data on initial rates of ATPase activities are necessary to analyze in detail the apparent uncoupling effect of temperature. For the same reason, no calculations on the rates of ATP hydrolyzed vs. transported calcium were done. Unfortunately, with the techniques available to us during the present work it was not experimentally feasible to measure initial rates.

The kinetic profiles of Ca^{2+} uptake at 17°C and 27°C are shown in fig.3. In this case lower values of uptake than those measured in the experiment illustrated in table 2 were obtained. Similar variabilities in uptake values in the presence of oxalate were described in lobster SR [2], the reasons for this being unknown. The solid lines in each case represent a fitting of the experimental points with the saturation equations given in the legend to fig.3. It is interesting to note in this context that the time required to obtain half-maximum uptake values is 11 sec in both cases, so that the effect of increasing the temperature from 17°C to 27°C is to lower the uptake values by a constant factor at all times. A likely explanation for

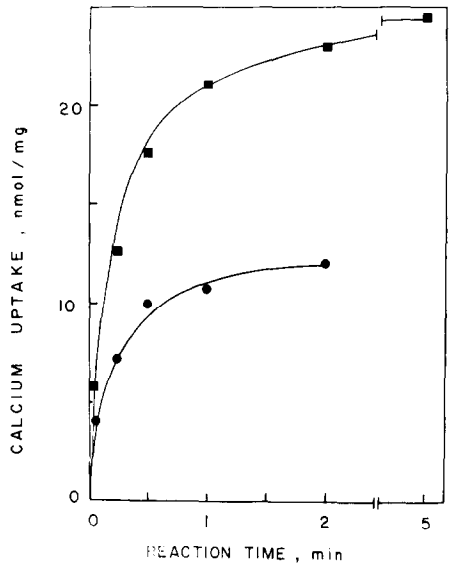

Fig.3. Ca^{2+} uptake in the absence of oxalate in barnacle SR. Experimental conditions as described in the text. Total Ca^{2+} concentration: 30 μM. Ca^{2+} uptake was measured either at 17°C (■) or 27°C (●). The solid lines represent the curves fitted to the experimental points by an equation of the general form:

$f(t) = AT/(k + t)$,

where A is the maximum uptake value in nmol per mg and k is given in seconds. The corresponding A values used at 17°C and 27°C were 25 and 13 nmol/mg. The k value used in both cases was 11 seconds.

Table 2
Effect of temperature on the uptake of calcium by SR from barnacle muscle

Temperature (°C)	Calcium uptake (nmol/mg)	%
0	14.7	35.5
17	48.2	100
20	46.5	96.5
25	35.5	73.6

Calcium uptake was measured as described in the text. Uptake values were determined after 15 sec incubation with a total calcium concentration of 6×10^{-5} M. Protein concentration: 0.1 mg per ml.

the observed decrease in uptake at temperatures higher than 20°C would be that above this temperature there was an increment in membrane leakiness.

Since the proposed physiological role of calsequestrin would be to provide the storage sites for Ca^{2+} [18], the absence of calsequestrin in both barnacle and lobster SR poses an interesting problem of how the transported Ca^{2+} is retained in invertebrate muscle SR, if this structure has a functional role similar to that of the vertebrate system. In fact, the absence of calsequestrin in both invertebrate systems might explain the rather low uptake values observed, from 20 to 50 nmol per mg at 17°C, which are consistent with previous data reported for lobster SR at the same Ca^{2+} concentrations as used in the present work [1].

In conclusion, we would like to stress the simplicity in terms of its composition of the barnacle SR preparation, which makes it a very attractive system to study the mechanism of ATP-dependent Ca^{2+} uptake. The physiological implications of the absence of calsequestrin on the overall SR function remain to be determined.

Acknowledgements

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