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SUBUNITS OF THE CALCIUM ION-PUMP SYSTEM OF SARCOPLASMIC RETICULUM

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Summary

Sarcoplasmic reticulum membranes with high content of Ca^{2+}-ATPase (80% of total protein) were dissolved in a non ionic medium and were submitted to isoelectric focusing in polyacrylamide gels. The membrane protein was resolved into six main bands whose isoelectric points range from 6 to 5. The mol. wt. of these peptides is about 100 000 as estimated by second dimension electrophoresis in sodium dodecyl sulfate-polyacrylamide system. The electrophoretic behaviour of the purified ATPase enzyme is similar to that of crude membranes.

Introduction

Sarcoplasmic reticulum membranes transport Ca^{2+} through a Ca^{2+}-pump system [1—4]. The biochemical features of this transport system have been extensively studied [4—6], but its molecular organization has not been characterized.

In this work we report the resolution of the Ca^{2+}-ATPase system into several components which may form the carrier complex responsible for the translocation of Ca^{2+} across the sarcoplasmic reticulum membrane.

Materials and Methods

Fragmented sarcoplasmic reticulum was isolated as described elsewhere [7]. The Ca^{2+}-ATPase enzyme was purified according to Warren et al. [8].

Electrophoresis in sodium dodecyl sulfate-polyacrylamide gels (10% acrylamide, 0.135% bisacrylamide) was carried out as described by Weber and Osborn [9].

Isoelectric focusing was carried out in columns (8 × 0.3 cm) of polyacryl-

Abbreviation: TEMED, *N,N,N′,N′*-tetramethyl-1,2-diaminoethane.

amide gels containing 4% acrylamide, 0.125% bisacrylamide, 8 M urea and 1.12% ampholytes (pH 3.5—10). The gels were allowed to polymerize for about 1 h after adding 0.1% *N,N,N',N'*-tetramethyl-1,2-diaminoethane (TEMED) and 0.033% ammonium persulfate. Preelectrophoresis proceeded for 30 min at 0.3 mA per gel. The cathodal and anodal electrolytes were 50 mM NaOH (or 0.4% ethylenediamine) and 50 mM H_2SO_4 (or 0.2% H_2SO_4), respectively. The gels were loaded with 80 μg of protein solutions (3.6 mg/ml) in 4.8% Triton X-100, 8 M urea, 1.6 mM EDTA (pH 7.4), 1% β-mercaptoethanol and 20 mM Tris (pH 7.4). Electrophoresis was run at 260 V for 18—24 h (end current 0.05 mA/gel). The gel columns were fixed in 50% CH_3OH for 12 h, stained with 0.25% Coomassie blue R in 45% CH_3OH and 9% CH_3COOH and destained in a solution containing 25% CH_3OH and 10% CH_3COOH.

To determine pH gradients, unfixed and unstained gels were cut in 0.5 cm slices which were suspended in 2.0 ml of boiled distilled water. The pH of the solutions was measured after 2 h equilibration.

Second dimension electrophoresis of isoelectric focused gels was performed for 10 h at 15 mA and pH 8.9 (50 mM Tris/glycine as buffer system) in sodium dodecyl sulfate-polyacrylamide slabs, 3.0 mm thick, containing 12% acrylamide, 0.38% bisacrylamide, 0.5% sodium dodecyl sulfate, 10% glycerol, 0.04% TEMED, 0.1% ammonium persulfate and 0.3 M Tris (pH 8.9). Isoelectric focused rods soaked in a solution containing 2.5% sodium dodecyl sulfate, 1% β-mercaptoethanol and 10 mM Tris (pH 7.4) were sealed to the slabs with 1% agarose in 0.3 M Tris (pH 8.9), 0.04% TEMED, 0.5% sodium dodecyl sulfate and 0.1% β-mercaptoethanol. The gel slabs were fixed for 12 h in a solution containing 25% CH_3OH and 10% CCl_3COOH, stained and destained as described above.

The gels were scanned at 540 nm in a gel scanner adapted to the Varian Techtron spectrophotometer, model 635.

Results and Discussion

Membranes of sarcoplasmic reticulum submitted to sodium dodecyl sulfate-polyacrylamide gel electrophoresis (Fig. 1) exibit a major band of about 100 000 daltons, the Ca^{2+}-ATPase system [5], and other minor peptides which probably represent contaminants, since it was possible to recover, from crude membranes, very active fractions devoided of such peptides [10,11].

The Ca^{2+}-ATPase system of isolated sarcoplasmic reticulum accounts for about 80% of the total protein as estimated from the absorbance profiles of the gels (Fig. 1). Since this value was not corrected for the deviations of the Beer's law, the amount of Ca^{2+}-ATPase may actually exceed that value.

Isoelectric focusing of the membrane protein separated 6—7 main bands whose isoelectric points range from 5.7 to 4.9 (Fig. 2). This general pattern is reproducible under various experimental conditions either by changing the electrolyte systems or the amount of protein loaded in the gels or the running time. The estimated values of the isoelectric points of the individual bands were consistently found to be in the range of 6 to 5.

Second dimension sodium dodecyl sulfate-polyacrylamide gel electrophoresis of isoelectric focused gels (Fig. 3) showed that about six peptides (isoelectric

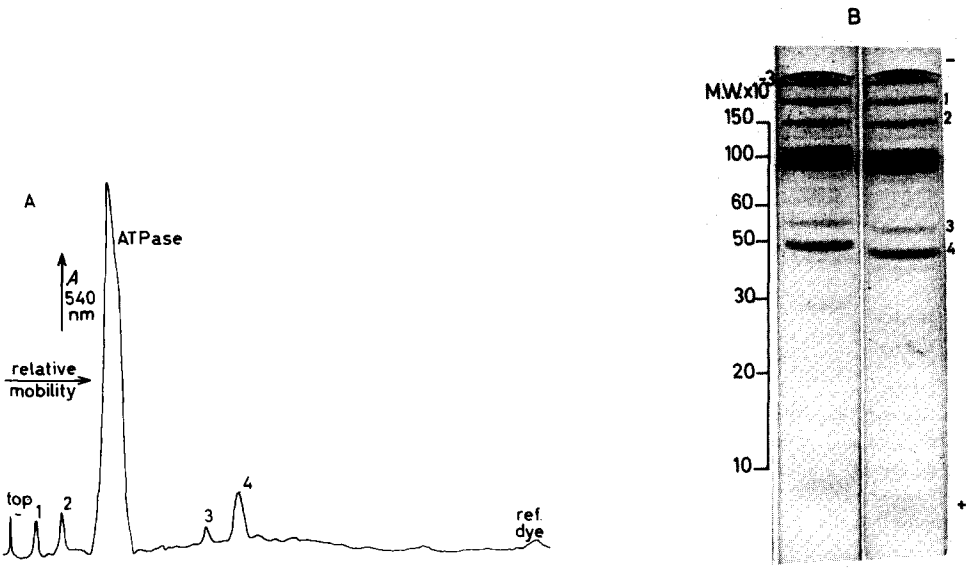

Fig. 1. Sodium dodecyl sulfate-polyacrylamide gel electrophoresis of sarcoplasmic reticulum membranes. A, densitometric trace of the gel rods in B. The ATPase enzyme (major band of mol. wt. 100 000) accounts for about 80% of the total protien. The quantitative calculations were made by triangulation of the peaks in A.

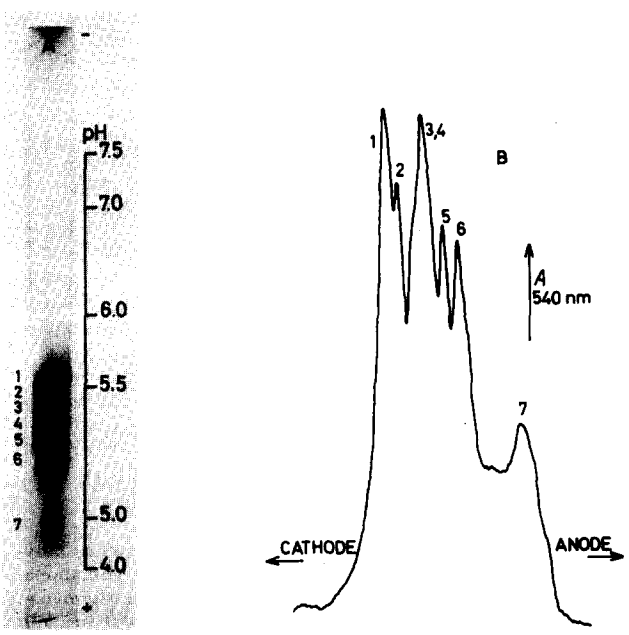

Fig. 2. Isoelectric focusing of the sarcoplasmic reticulum peptides. The isoelectric points of the separated bands range from 5.7 to 4.9. B, densitometric trace of the gel rod A.

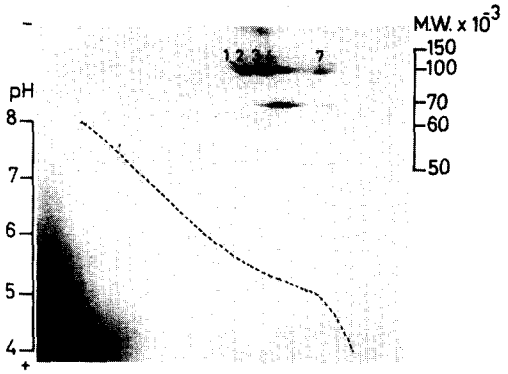

Fig. 3. Second dimension electrophoresis in sodium dodecyl sulfate-polyacrylamide gel slabs following isoelectric focusing. Gel rods of Fig. 2 were sealed to the top of sodium dodecyl sulfate-gel slabs. The profile of the pH gradient after isoelectric focusing is depicted. The numbering of the spots corresponds to the numbering in Fig. 2. The spot of lower mol. wt. probably corresponds to bands 5 and/or 6 in Fig. 2 and to band 4 in Fig. 1.

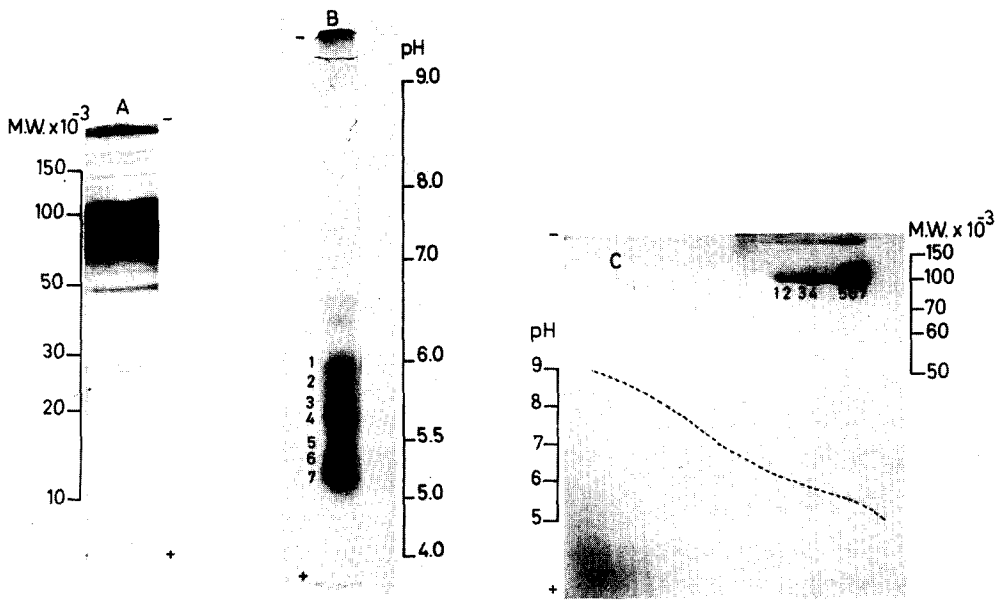

Fig. 4. Electrophoretic behaviour of the purified Ca^{2+}-ATPase enzyme. A, sodium dodecyl sulfate-polyacrylamide gel electrophoresis of the purified material. The gel was overloaded to show the contaminations. The ATPase band accounts for more than 95% of the total protein, as estimated by densitometry. B, isoelectric focusing of the purified material. The bands have isoelectric points between 6.0 and 5.0. C, second dimension electrophoresis in sodium dodecyl sulfate-gel slabs following isoelectric focusing. The numbering of the spots corresponds to the numbering in B. The profile of the pH gradient of gel B is depicted in C.

points with values from 6 to 5) have a mol. wt. of about 100 000, the mol. wt. commonly assigned to the Ca^{2+}-pump system of sarcoplasmic reticulum.

The electrophoretic profiles of the purified ATPase enzyme differ slightly from those described for "crude" membranes. Again, it was possible to discern about 6 bands with isoelectric points of values ranging from 6 to 5 (Fig. 4), but the distribution pattern is somewhat altered. The purification of the ATPase enzyme involves the use of the ionic detergent deoxycholate [8], most of which is removed by density gradient centrifugation. However, analyses of the lipid extracts, by thin-layer chromatography [12] showed spots which could be assigned to deoxycholate. Since this ionic detergent remains in the purified material, it may, thus, modify the values of the isoelectric points of the purified ATPase peptides relative to the values of the original ATPase peptides in sarcoplasmic reticulum. Furthermore, although the distributions of the phospholipid classes in both sarcoplasmic reticulum and ATPase preparations are similar (70% phosphatidylcholines, 17% phosphatidylethanolamines, 13% minor lipids), about 50% of the total phospholipid was removed during the purification procedure of the ATPase enzyme. Thus, the figure of 0.4 mg of phospholipid per mg of sarcoplasmic reticulum protein (28.5% of the total mass) was lowered to about 0.2 mg per mg of ATPase protein (17% of the total mass).

There are possible difficulties in the correct interpretation of these results, but which do not detract from the main significance of the work; thus I cannot a priori eliminate that some proteolytic cleavage of highly charged segments of the ATPase molecule may occur without changing its molecular weight. Translational modification of the enzyme by phosphorylation should not have occurred under the experimental conditions, and it should be of interest to test whether phosphorylation does in fact change the electrophoretic behaviour of the enzyme. I also believe that the different bands do not represent contaminating polypeptide chains which co-migrate because the ATPase enzyme as isolated is very homogeneous as evaluated from data on amino acid composition reported by various laboratories [13,14]. It is also improbable that the bands with different isoelectric migration reflect complexes of different lipids with the same homogeneous protein because basically the same results are obtained after about 50% of the lipid is removed during the purification of the enzyme. Furthermore, the concentration of Triton X-100 utilized led to clear solutions of the membrane material which suggest that most of the lipid, is dissociated from the protein.

The results suggest that the Ca^{2+}-ATPase system has several different polypeptides of similar molecular weights forming an enzymatic complex. Other investigators [15,16] showed that the Ca^{2+} transport as well as the phosphorylation reaction of the ATPase enzyme exhibit cooperative behaviour which suggests the involvement of various subunits on those functional capabilities. Furthermore, it was recently suggested on the basis of structural and functional features, that the Ca^{2+}-ATPase system may represent a multienzyme complex of several subunits [17]. Moreover, other investigators showed that the number of intramembrane particles of sarcoplasmic reticulum assigned to the ATPase system is about 1/3 of the number of 100 000 daltons peptides present in sarcoplasmic reticulum membrane [11].

Recent data [18] reveal that the smallest fully active complex of the Ca^{2+}-ATPase contains 3—4 polypeptide chains forming an oligomeric system. Furthermore, experiments on cross-linking suggest that the ATPase enzyme is organized in the membrane as a tetramer [19].

These results reinforce the previous idea [15—17] that the ATPase system may be formed by several subunits arranged in the membrane to form an hydrophilic channel through which the Ca^{2+} transport takes place.

Studies are in progress to further characterize the molecular components of the Ca^{2+}-pump system.

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