FUNCTIONAL AND STRUCTURAL ROLES OF SARCOPLASMIC RETICULUM PROTEIN COMPONENTS

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1. Introduction

Sarcoplasmic Reticulum (SR) constitutes a membrane system highly specialized for Ca^{2+} transport. A most important component of this membrane is the Ca^{2+} dependent ATPase, which is tightly coupled to active transport of the divalent cation. Unfortunately, much uncertainty is found in the literature regarding the percentage of total SR protein accounted for by ATPase, as figures varying from 16% to 90% have been given [1–5]. Further uncertainty is related to the significance of other proteins associated with SR preparations [4,6].

With a variety of separation procedures and by comparative experiments we now show that the Ca^{2+} ATPase in fact accounts for 70–90% of the total SR protein and that other minor protein components are not involved in the mechanism of active Ca^{2+} transport. We estimate that, within a unit area of SR membrane the number of 106 000 dalton polypeptide chains identified with the ATPase enzyme exceeds by a factor of approx. three the number of particles visualized in freeze-fracture preparations.

2. Methods

SR was prepared from rabbit, lobster and chicken muscle as described previously [2]. Further purification was then carried out on a discontinuous sucrose gradient collecting the fraction sedimented between 26 and 29% sucrose after 2 h centrifugation at 76 000 *g*. The methods for protein determination, Ca^{2+} transport, ATPase activity, gel electrophoresis and freeze fracturing of SR membranes were previously reported [7]. The volume of SR vesicles excluded to [^{14}C] dextran was measured as described by Duggan and Martonosi [8].

3. Results and discussion

The electrophoretic resolution of the protein components of rabbit, lobster and chicken SR is shown in fig.1A. In all three preparations the main band corresponds to a protein of 106 000 daltons, previously identified with the ATPase enzyme [1–5]. In addition, the rabbit SR contains the two minor components named 'calcium binding protein' and 'calsequestrin' [4,6]. These two proteins are absent in the lobster and chicken preparations, which contain other minor bands migrating with different velocities. With regard to functional activity, it is shown in fig.2A that all three preparations take up calcium in the presence of ATP, with no relation to the presence of any of the minor protein components. In fact, the lobster SR, which contains the lowest amount of minor components is the most active.

It was suggested that one of the minor protein components of rabbit SR, 'calsequestrin', plays a role in binding calcium ions actively translocated into the lumen of the vesicles [4]. Therefore one may expect that the maximal levels of active calcium uptake are related to the amount of 'calsequestrin' present.

In this regard, using methods similar to those described by Meissner [9], we found that rabbit

SR preparations can be separated in fractions containing various amounts of 'calsequestrin' (fig.1B). Fraction 1 accounts for less than 1% of the SR preparation, its minimal yield limiting experimentation. Fractions 2, 3 and 4 account for 72%, 16% and 11% of the SR preparation and contain different amounts of 'calsequestrin' in each fraction. The relative ATPase contents are 90%, 81% and 56% respectively. All fractions display ATPase activity which is proportional to the relative content of ATPase protein as indicated by the electrophoretic gels. It is interesting to note that the SR vesicles with higher 'calsequestrin' content retain a lower ability to maintain calcium gradients in the *presence* of ATP

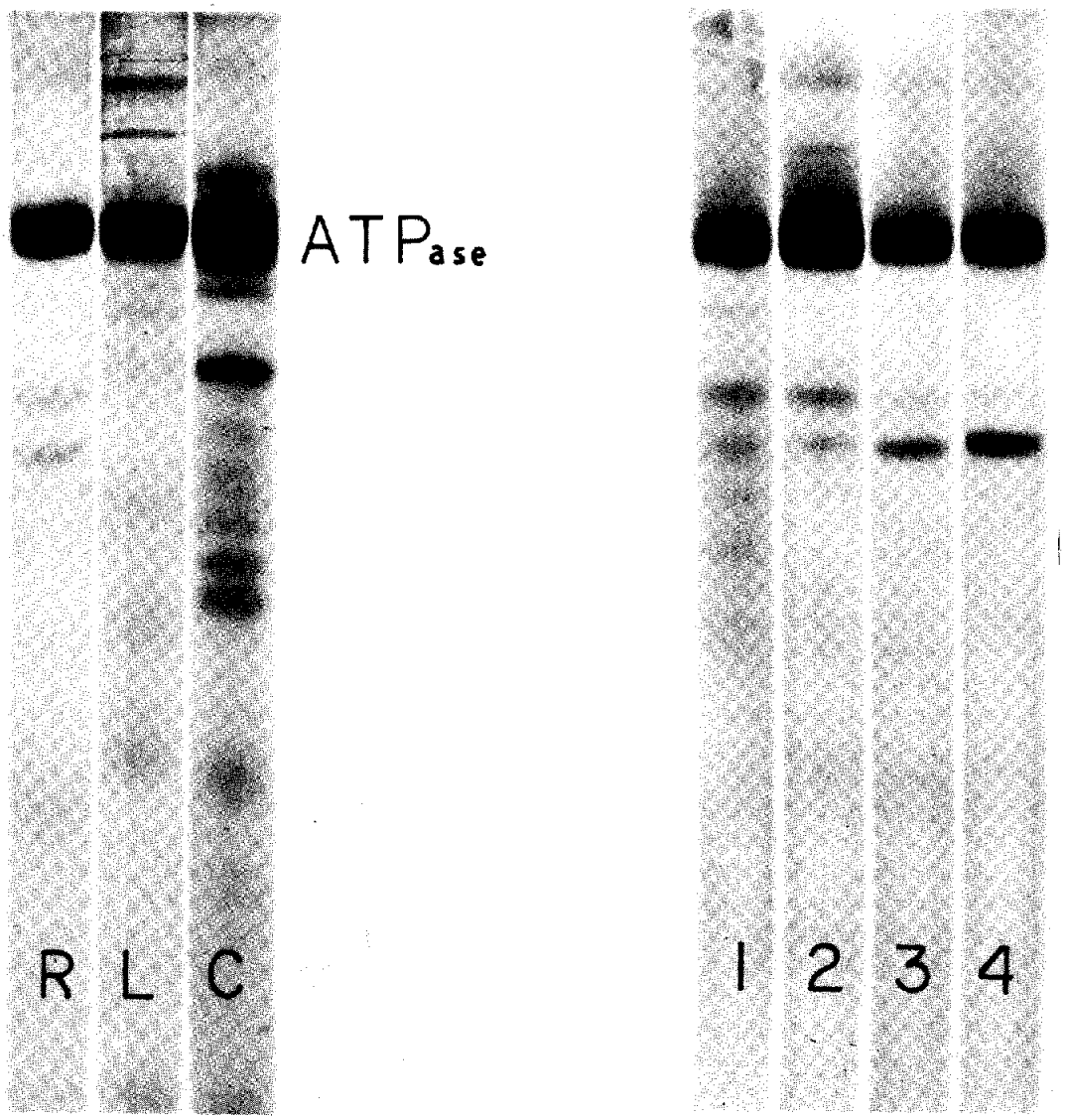

Fig.1. Electrophoretic separation of SR proteins solubilized in SDS. (a) SR from rabbit (R), lobster (L), and chicken (C) skeletal muscle. (b) fractions of rabbits SR obtained by 20 h centrifugation in a discontinuous multi-step sucrose gradient: 29% (1), 32% (2), 39% (3) and 43% (4).

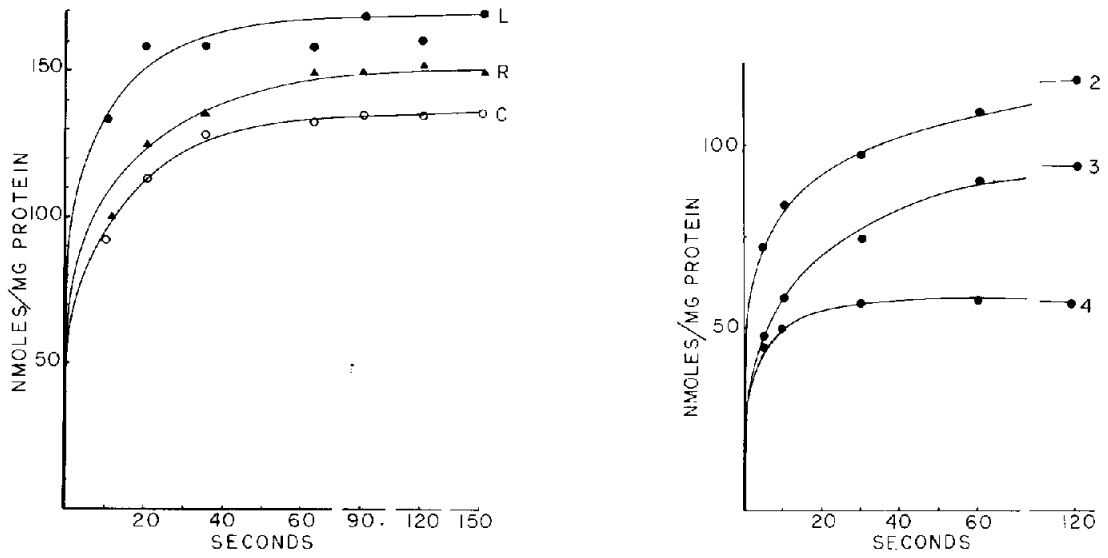

Fig.2. Calcium uptake by SR in the presence of ATP. Reaction mixture: 0.2–0.3 mg SR protein/ml, 20 mM MOPS buffer (pH 6.8), 20 mM KVl, 2 mM $MgCl_2$, 0.1 mM EGTA, 0.1 mM $CaCl_2$, and 2 mM ATP. Controls contained SR but no ATP. (a) Rabbit, lobster and chicken SR. (b) Fractions of rabbit SR obtained as for fig.1b.

(fig.2B), while displaying a greater calcium binding capacity in the *absence* of ATP (not shown).

These experiments and those performed with lobster and chicken SR indicate that the minor protein components are not a constant finding in different populations of SR vesicles and in different animal species. Furthermore, they are not necessary for *active* transport of calcium ion in SR vesicles. It is apparent that ATPase, in the presence of the membrane lipids and proteolipids, is the only protein involved in the Ca^{2+} pump.

Independent of the presence of the minor protein components, electron microscopic inspection of freeze-fracture specimens reveals the presence of particles in all SR preparations (not shown). The particles, characteristically present on the concave, but not on the convex faces, have been identified as the ATPase protein [10]. It was pointed out that the number of ATPase molecules and the number of particles per unit membrane area are of the same order of magnitude [7,11]. We are now better able to estimate this relation, having obtained more accurate figures for the fractional value of ATPase/total SR protein, and for the membrane area per protein unit weight.

The membrane area corresponding to 1 g SR protein (A_{tot}) is obtained by multiplying the surface area of one vesicle by the number of vesicles in 1 g SR protein:

$$A_{tot} = (4\pi r^2) \cdot \frac{(V_{tot})}{\frac{4\pi}{3} r^3}$$

where r is the average radius of one vesicle (750 Å) and V_{tot} is the total volume of the vesicles contained in a unit weight of SR protein, measured as the space excluded to [^{14}C] dextran. We found this volume to be 7.4 ± 0.2 ml/g protein in rabbit SR preparations in which the percentage of total protein accounted for by ATPase was 80–82%. In these SR preparations, our estimate for A_{tot} is 2.9×10^{14} μm^2/g SR protein. This value is very close to that calculated for a model membrane 62 Å thick* and composed of 1 g protein and 0.65 g lipid in analogy to SR (2.4×10^{14} μm^2 based on a density equal to 1.1).

From these figures it can be calculated that the number of polypeptide chains per unit area of SR

* The thickness of the SR membrane was determined by low angle X-ray diffraction [12].

membrane should be $1.41-1.75 \times 10^4/\mu m^2$. This value exceeds by a factor of 2.5–3.1 the density of particles observed in freeze fracture faces of the same SR preparations, which we found to be $5730 \pm 520/\mu m^2$. The discrepancy suggests that either a significant number of polypeptide chains are excluded from the fracture plane during freeze fracturing procedure, or a significant number of particles are oligomers including more than one polypeptide chain.

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References

[1] MacLennan, D. H. (1970) J. Biol. Chem. 245, 4508–4518.
[2] McFarland, B. H. and Inesi, G. (1970) Biochem. Biophys. Res. Commun. 41, 239–243; (1971) Arch. Biochem. Biophys. 145, 456–464.
[3] Martonosi, A. and Holpin, R. A. (1971) Arch. Biochem. Biophys. 144, 66–77.
[4] MacLennan, D. H. and Wong, P. T. S. (1971) Proc. Natl. Acad. Sci. U.S. 68, 1231–1235.
[5] Meissner, G. and Fleischer, S. (1971) Biochim. Biophys. Acta 241, 356–378.
[6] Ostwald, T. J. and MacLennan, D. H. (1974) J. Biol. Chem. 249, 974–979.
[7] Inesi, G. and Scales, D. (1974) Biochemistry 13, 3298–3306.
[8] Duggan, P. F. and Martonosi, A. (1970) J. Gen. Physiol. 56, 147–167.
[9] Meissner, G. (1975) Biochim. Biophys. Acta 389, 51–68.
[10] Deamer, D. W. and Baskin, R. J. (1969) J. Cell Biol. 42, 296–307.
[11] Inesi, G. (1972) Ann. Rev. Biophys. Bioengen. 1, 191–210.
[12] Worthington, C. R. and Liu, S. C. (1973) Arch. Biochem. Biophys. 157, 573–579.