Identification of Two Intrinsic Proteins Uniquely Associated with the Terminal Cisternae of the Sarcoplasmic Reticulum

Kevin P. Campbell and Adil E. Shamoo

Department of Radiation Biology and Biophysics
University of Rochester School of Medicine and Dentistry
Rochester, New York 14642

Introduction

The membranes directly involved in excitation-contraction coupling in skeletal muscle are the transverse tubular membrane and the junctional sarcoplasmic reticulum membrane[1]. It is generally accepted that depolarization at the transverse tubular membrane initiates the release of calcium from the terminal cisternae of the sarcoplasmic reticulum[2]. There have been recent studies toward the understanding of the electrophysiological properties of the membranes involved in excitation-contraction coupling[3]. However, little is known about the membrane proteins or their role in excitation-contraction coupling. In this study, we have shown that sarcoplasmic reticulum vesicles derived from the terminal cisternae contain two Intrinsic membrane proteins which are probably unique to the terminal cisternae.

Materials and Methods

A combination of differential and isopycnic zonal ultracentrifugation was used to isolate light and heavy sarcoplasmic reticulum vesicles (LSR, HSR). LSR and HSR were obtained from the 30-32.5% (w/w) and 39-42.5% (w/w) region of the sucrose gradient, respectively. Our method is similar to that of Meissner[4] and Caswell[5]. Deoxycholate (DOC) treatment (5-10 mg protein/ml, 0.1 mg DOC/mg protein, 1 M NaCl and 1 mM DTT) was done essentially according to the method of MacLennan[6]. Triton treatment was carried, out using the method, of Ikemoto[7]. Electron microscopy was performed by Dr. Franzini-Armstrong[8]. Gel electrophoresis profiles were obtained by the method of Swank and Munkres[9] (SDS-Urea PAGE) and Laemmli[10] (SDS-PAGE). The procedure of Fairbanks[11] was used for glycoprotein staining. SR calcium oxalate loading and sucrose gradient separation was carried out according to the method of Levitsky[12]. Cytochrome ($a+a_3$) was assayed using the dual wavelength method of Levitsky[12].

Results

In thin section electron microscopy LSR appear as empty vesicles whereas the HSR appear as vesicles filled with electron dense material, similar to that seen in the terminal cisternae of the sarcoplasmic reticulum. A further description of LSR and HSR will appear in a paper in preparation[8].

Reprinted from *Calcium Binding Proteins and Calcium Function*
(R.H. Wasserman et al., eds.).

SDS-Page shows that the LSR (Fig. 1) contains essentially one protein having a MW of approx. 102,000 (Ca^{2+}+MG^{2+}-ATPase). Five main protein bands are consistently observed on SDS-Page of HSR (Fig. 1) having MWs of approx. 102,000 (Ca^{2+}+MG^{2+}-ATPase), 64,000 (calsequestrin), 55,000 (high affinity calcium binding protein), 34,000 and 30,000. SDS-Urea PAGE gives qualitatively the same protein composition except that the SR proteolipid is visible in gels of both LSR and HSR. The results of staining the gels of HSR with Schiff's reagent indicate that the two intrinsic proteins have little or no carbohydrate.

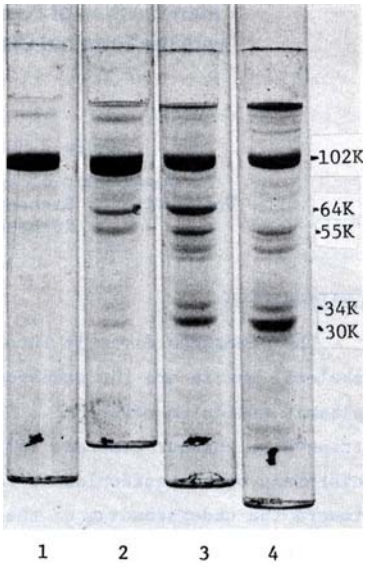

Figure 1. Laemmli SDS-PAGE of light (1), intermediate (2), heavy SR vesicles (3) and of the insoluble fraction of -DOC-treated HSR (4) showing the enrichment of the 30,000 and 34,000 dalton proteins.

Deoxycholate,at low ratios of detergent to protein, has been used by MacLennan[6] to selectively solubilize the extrinsic membrane proteins from SR vesicles. DOC treatment of the LSR results in the further purification of Ca^{2+}+Mg^{2+}-ATPase. DOC treatment of HSR results first, in the extraction of calsequestrin and high affinity calcium binding protein into the soluble fraction; and second, in the enrichment of the 30,000 and 34,000 dalton peptides in the insoluble fraction (Fig. 1). Triton treatment of the HSR also results in the enrichment of these two proteins in the Insoluble fraction. Using additional DOC followed by ammonium acetate fractionation yields two fractions from HSR both have the ATPase peptide but one is enriched in the 30,000 dalton peptide and the other in the 34,000 dalton peptide.

Mitochondrial contamination as measured by cytochrome (a+a_3) heme content is less than 1% in LSR and less than 3% in HSR. Calcium oxalate-loaded HSR, separated from mitochondrial contamination, has been shown to contain the 30,000 and 34,000 dalton proteins.

Discussion

Isopycnic zonal ultracentrifugation has been used to Isolate light and heavy sarcoplasmic reticulum vesicles from rabbit skeletal muscle. LSR and HSR differ in their protein composition as seen by SDS-PAGE and morphology as seen by thin section electron microscopy. The biochemical and morphological data indicate that most of the LSR and HSR are derived from the longitudinal and

terminal regions of the SR, respectively. DOC treatment of LSR and HSR reveals that the HSR contains two intrinsic proteins which are unique to the HSR.

Since the two intrinsic proteins account for a small percentage of the HSR protein, experiments were conducted to rule out the possibility that they might arise from contamination. The level of cytochrome ($a+a_3$) Indicates that the level of mitochondrial contamination in the HSR is quite low. In order to further rule out mitochondrial contamination, we actively loaded HSR with calcium oxalate in the presence of azide to inhibit mitochondrial calcium accumulation. We were then able to separate the; loaded HSR from mitochondrial fragments using a step sucrose gradient. The isolated calcium oxalate loaded HSR was then shown to contain both intrinsic proteins.

Since our preparation does contain some transverse tubular contamination, mostly attached to the HSR in the form of dyads, we have not ruled out that one or both of these proteins are located In the T-tubule membrane and/or the junction between the T-system and the terminal cisternae.

We are presently carrying out further experiments on the localization of these proteins and the relationship of these proteins to the mechanism of calcium release.

Acknowledgements

This paper is based on work supported by US-ERDA contract and supported in part by NIH, MDA and Upjohn Company, Report # UR-3490-1108. Adil E. Shamoo is an Established Investigator of the American Heart Association.

References

1. Franzini-Armstrong, C. (1975) Fed. Proc. 34: 1382-1389.
2. Ebashi, S. and Endo, M. (1968) Progr. Biophys. Mol. Biol. 18: 123-183.
3. Fuchs, F. (1974) Annual Rev. Physiol. 36: 461-502.
4. Meissner, G. (1975) Biochim. Biophys. Acta 389: 51-68.
5. Caswell, A. H., et al. (1976) Arch. Biochem. Biophys. 176: 417-430.
6. MacLennan, D. H. (1970) J. Biol. Chem. 245: 4508-4518.
7. Ikemoto, N. (1975) J. Biol. Chem. 250: 7219.
8. Campbell, K. P., Franzini-Armstrong, C. and Shamoo, A. E. (manuscript in preparation).
9. Swank, R. T. and Munkres, K. D. (1971) Anal. Biochem. 39: 462-477.
10. Laemmli, U.K. (1970) Nature 227: 680-685.
11. Fairbanks, G., et al. (1971) Biochem. 10: 2606-2617.
12. Levitsky, D. O., et al. (1976) Biochem. Biophys. Acta 443: 468-484.