STUDIES OF SOLUBILIZED SARCOPLASMIC RETICULUM

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The non-ionic detergent Triton X-100 solubilizes most of the protein and phospholipid from the sarcoplasmic reticulum of rabbit skeletal muscle. The calcium dependent ATPase of the sarcoplasmic reticulum is found in the soluble fraction whereas the basic ATPase activity remains insoluble in Triton X-100. Analytical ultracentrifugation and disc electrophoresis indicate that the solubilized material consists of particles having a molecular weight of approximately 80,000 daltons. These particles have a strong tendency to aggregate.

The sarcoplasmic reticulum of skeletal muscle is able to accumulate Ca^{++} when ATP is available for hydrolysis by the membranes (1). The requirement of ATP for calcium transport suggests that the sarcoplasmic reticulum can be studied by isolating the ATPase from the membranes and investigating its properties. Solubilization with detergents is an obvious first step in the isolation.

Materials and Methods. Triton X-100 was "B grade" (Calbiochem, Los Angeles) and all other materials were reagent grade. Fragmented sarcoplasmic reticulum (SR) was obtained by homogenizing and differentially centrifuging rabbit hind-leg white muscle (2, 5). The SR was purified by high ionic strength extraction according to the method of Masoro and Yu (3), with 10% sucrose added to the extraction medium. Sucrose (0.5 M) was also added to the final suspension medium (4).

The SR was dissolved in 1% (v/v) Triton X-100, 5 mM Histidine, pH 7.8, 10 μM Ethylene Diamine Tetra Acetic Acid (EDTA), and 10 μM Dithiothreitol (DTT) by agitation and incubation for fifteen min. The protein concentration in the ATPase experiments was 4.6 mg/ml in the initial solution. After incubation, the solubilized preparation was centrifuged at 40,000 x g for one

hr and the clear supernatant was decanted. All operations were performed at 2°. The supernatant (0.5 ml) was assayed for ATPase activity at 25° as previously described (5) with a $CaCl_2$ concentration of 0.1 mM. An identical assay was performed on supernatant that had been diluted one to ten in 5 mM Histidine, 10 μM EDTA, and 10 μM DTT, pH 7.8.

Protein was measured by means of a modified Folin assay (6) standardized against Bovine Serum Albumin. The modifications were: (a) the Folin-phenol reagent was used without dilution and (b) the heavy yellow precipitate formed by the Folin reagent's reaction with Triton X-100 was removed by centrifugation before the samples' absorbances were measured. Phospholipids were assayed according to the method of Bartlett (7).

Analytical ultracentrifugation was carried out in a Spinco AN-D rotor using the Backman model L 2-65 B ultracentrifuge with optical attachment. Disc electrophoresis was according to Hedrick and Smith (8), modified in that our reservoir buffer was 25 mM Tris-Glycine, pH 8.3, containing either 2% Triton X-100 or 10 mM Phenol as indicated. Our standard curves were constructed using the relative mobilities of gamma globulin, aldolase, albumin (monomer and dimer) and catalase.

Results. The solubilization procedure routinely dissolved between eighty and ninety percent of the SR protein, as determined by Folin assays of supernatant and precipitate fractions following centrifugation. A like percentage of the phospholipids was also solubilized. The calcium dependent ATPase activity of the solubilized fraction (supernatant) was initially 3.7 μmoles P_i liberated per minute per milligram protein. This activity declined over a week's time to 1.3 μmoles/min/mg, with the half life being 48 hr. The activity of the supernatant following 1:10 dilution was 7.8 μmoles/min/mg. The half life of the diluted sample's activity was also 48 hr. No basic ATPase activity could be found in the supernatant fraction, but it was retained by the precipitate.

Sedimentation velocity studies on the solubilized SR (Fig. 1a), revealed one fast peak (the solubilized particles) and a large, slow peak (presumably

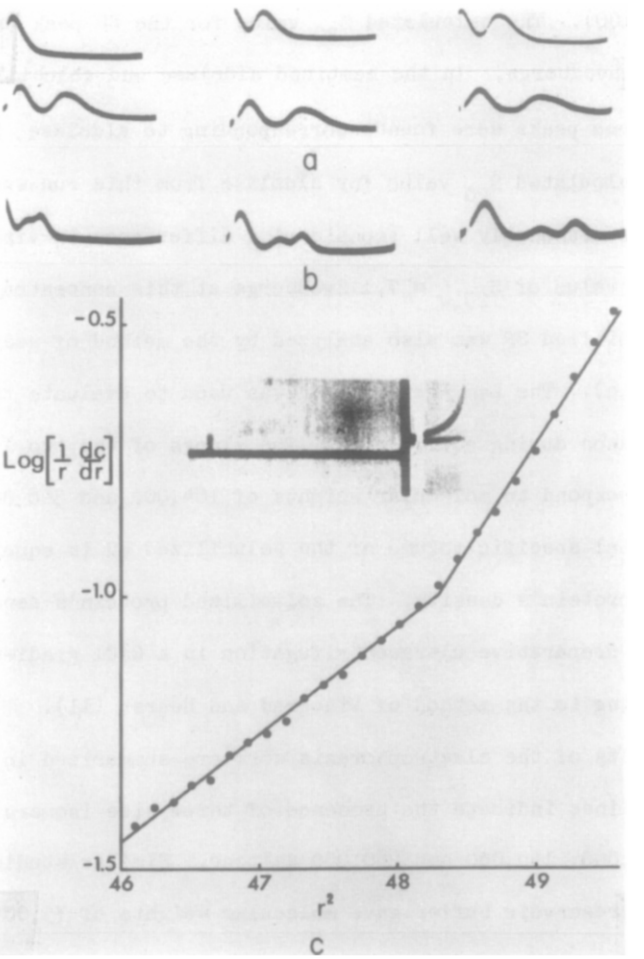

Figure 1: Analytical Ultracentrifugation

(a) Sedimentation velocity of Triton X-100 solubilized SR: SR was dissolved at a concentration of 10 mg protein/ml in 1% Triton X-100, 15 mM Tris-Maleate pH 7.0, and incubated at 25° for 10 min. Following centrifugation at 40,000 x g for 1 hr, the clear supernatant (5.8 mg protein/ml) was centrifuged at 60,000 rpm, 20°. Sedimentation was from left to right. Pictures were taken at 10 min intervals beginning at 25 min. Bar angle was 65° in the first two exposures, and 60° thereafter.

(b) Sedimentation velocities of aldolase and solubilized SR: Aldolase from rabbit skeletal muscle (5 mg/ml) was mixed with solubilized SR (3.9 mg protein/ml) in 1% Triton X-100, then treated as in (a). Photographs were taken at 35 min (bar angle = 65°), 54 min (bar angle = 65°) and 77 min (bar angle = 60°).

(c) Sedimentation equilibrium of solubilized SR: SR (3 mg protein/ml) was incubated in 2% Triton X-100, 15 mM Tris-Maleate pH 7.0 for 20 min at 25°, then centrifuged at 40,000 x g for 1 hr. The clear supernatant (1.0 ml) was centrifuged in a double sector cell at 20°. After 5 hr overspeed at 12,000 rpm, the speed was set a 8,000 rpm for 50 hr, when picture (inset) was taken (bar angle = 70°).

the Triton X-100). The calculated S_{20} value for the SR peak (average of three runs) was 5.3 Svedbergs. In the combined aldolase and solubilized SR experiment (Fig. 1b), three peaks were found--corresponding to aldolase, SR, and Triton X-100. The calculated S_{20} value for aldolase from this run was 6.7 Svedbergs, which compares reasonably well (considering differences in viscosity) with the published value of $S_{20,w} = 7.1$ Svedbergs at this concentration (9).

The solubilized SR was also analyzed by the method of sedimentation equilibrium (Fig. 1c). The Lamm method (10) was used to evaluate the Schlieren photographs taken during equilibrium. The slopes of the two lines shown in Figure 1c correspond to molecular weights of 164,000 and 328,000, assuming that the partial specific volume of the solubilized SR is equal to the reciprocal of the protein's density. The solubilized protein's density was determined by preparative ultracentrifugation in a CsCl gradient to be 1.22 gm/ml, according to the method of Vinograd and Hearst (11).

The results of the electrophoresis work are summarized in Figure 2. The intersecting lines indicate the presence of three size isomers with molecular weights of 85,000, 150,000 and 260,000 daltons. Similar studies using 10 mM Phenol in the reservoir buffer gave molecular weights of 75,000, 140,000 and 260,000 daltons while also revealing the presence of a highly (negatively) charged minor protein constituent with molecular weight 75,000 daltons.

Discussion. The analytical data presented here indicate that Triton X-100 disaggregates the SR into particles of approximately 80,000 daltons molecular weight. Figure 1c shows dimer and tetramer, whereas Figure 2 shows monomer, dimer and trimer. These particles have prolonged ATPase activity at significant levels when they are disaggregated (i.e. in the presence of Triton X-100), in contrast to preparations involving deoxycholate (4, 12). While other solubilization studies (3, 13) have indicated that the SR may be composed of small polypeptide units, our findings represent the solubilization of the smallest functioning units reported to date.

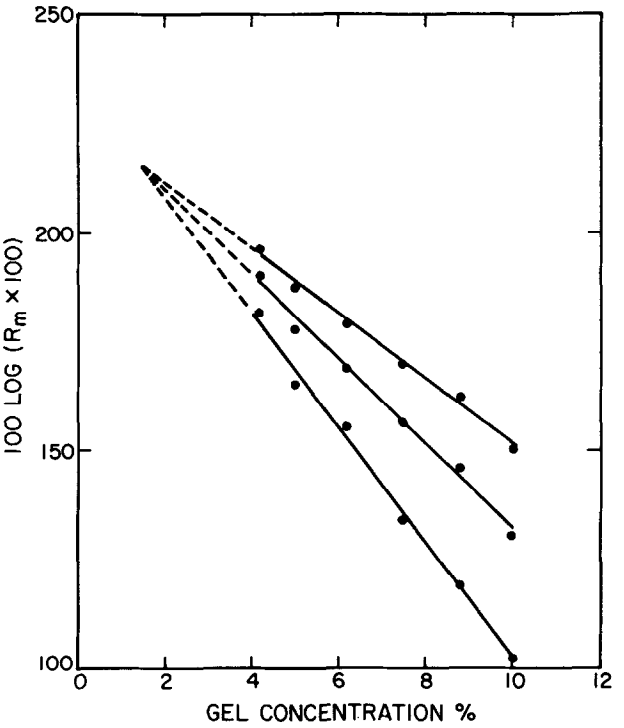

Figure 2: Analytical Disc Electrophoresis

Sarcoplasmic reticulum was solubilized in 1% Triton X-100 at a concentration of 0.76 mg protein/ml. After incubating for 10 min at 25°, the sample was centrifuged for one hr at 40,000 x g. The clear supernatant was filtered through 0.45 micron Millipore filter and then applied to the gels (0.1 ml of filtrate per gel). Electrophoresis was run at 2 mAmp per gel for two hr. Gels were stained with Amido Schwarz (0.5% in 7% acetic acid). Shown here is the average of four experiments.

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