Properties and Characterization of a Highly Purified Sarcoplasmic Reticulum Ca\textsuperscript{2+}-ATPase from Dog Cardiac and Rabbit Skeletal Muscle*

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Sarcoplasmic reticulum (SR) Ca\textsuperscript{2+}-ATPase was purified from dog cardiac and rabbit skeletal muscle using Triton X-100 at optimal ratios of 0.5 for cardiac and 0.5 to 1.0 for skeletal SR. The yields of Ca\textsuperscript{2+}-ATPase were 4 to 5 and 1 to 2.2 mg/100 mg of cardiac and skeletal SR protein, respectively. The enzyme activities were 547 ± 67 µmol ADP/mg/h for cardiac and 1192 ± 172 µmol ADP/mg/h for skeletal Ca\textsuperscript{2+}-ATPase. Removal of excess Triton X-100 increased the enzyme activities to 719 ± 70 and 1475 ± 206 µmol ADP/mg/h, respectively. The residual content of Triton X-100 for cardiac and skeletal Ca\textsuperscript{2+}-ATPase was 20 and 5 mol/mol of enzyme, respectively. Maximum levels of phosphoenzyme were 4.4 ± 0.2 and 5.6 ± 0.6 mmol/mg in each case. A single protein band of 100 kDa was obtained for each purified Ca\textsuperscript{2+}-ATPase by sodium dodecyl sulfate-polyacrylamide gel electrophoresis. The preparations were stable at ~80 °C for 5 months in the presence of 1 mM Ca\textsuperscript{2+}. The phospholipid content of the purified enzyme was 2-fold greater than that of native cardiac and skeletal SR microsomes. Repeated washing of the purified enzyme preparation did not alter the phospholipid content or the specific activities.

Our purpose for purification of dog cardiac and rabbit skeletal SR Ca\textsuperscript{2+}-ATPase was to compare the properties of the two enzymes in similar stages of purity. SR vesicles isolated from muscle tissues possess Ca\textsuperscript{2+} transport activity which is stoichiometrically coupled to membrane-bound Ca\textsuperscript{2+} ATPase (1–4). In the past, we had found that several intrinsic functional properties of the Ca\textsuperscript{2+}-ATPase differ in SR vesicles from various muscle types (5–8). In order to distinguish between the effects of the intrinsic properties and vesicular structure on the SR function, it is essential to purify the enzymes. Purification of rabbit skeletal SR Ca\textsuperscript{2+}-ATPase was first achieved by MacLennan (9) with Na deoxycholate and later by Ikemoto et al. (10) who used Triton X-100. Purification of cardiac SR Ca\textsuperscript{2+}-ATPase has been extremely difficult. There are only three reports on the purification of cardiac SR Ca\textsuperscript{2+}-ATPase; one, some years ago, by Levitsky et al. (11) for pigeon heart SR Ca\textsuperscript{2+}-ATPase and the others, more recently, by Van Winkle et al. (12) and Bidlack et al. (13) for dog heart SR enzyme.

The present communication describes a simple procedure for purification of dog cardiac and rabbit skeletal SR Ca\textsuperscript{2+}-ATPase with high activity using Triton X-100. The procedure utilizes simple differential ultracentrifugation of a Triton X-100-solubilized SR and precipitation of the Ca\textsuperscript{2+}-ATPase protein by a 2-fold dilution of the detergent. No additional steps, such as fractional precipitation with ammonium acetate (9), sucrose gradient centrifugation (11, 12), or Sepharose column chromatography (10), are needed for separation of the contaminating proteins or for repurification of the solubilized enzyme. Our procedure is rapid and reproducible and yields a purified Ca\textsuperscript{2+}-ATPase which is stable and has a very high specific activity.

MATERIALS AND METHODS

Purification of SR Ca\textsuperscript{2+}-ATPase—Dog cardiac and rabbit skeletal SR vesicles were prepared by the method routinely used in this laboratory (5, 14) which is a modification of our original procedure (15). These preparations were the starting material for subsequent solubilization and purification of the Ca\textsuperscript{2+}-ATPase. A suspension of SR vesicles (20–30 mg of protein/ml) in 20 mM Tris maleate, 9.6 mM KCl and 0.2 mM sucrose could be quick-frozen in dry ice/acetone and stored at ~80 °C for several months without loss of activity. Storage in a ~80 °C freezer without prior quick-freezing resulted in a loss of activity in the purified Ca\textsuperscript{2+}-ATPase.

The membranes of the SR preparation were solubilized with Triton X-100. The optimum ratio of Triton X-100/SR protein was 0.5 by weight. Ratios of 1 and 1.5 were also tried, but produced less satisfactory results. Triton X-100 was added slowly to the SR suspension (58 mg in 15 ml) in a 100 mM Tris/HCl buffer (pH 8.5) containing 0.3 M sucrose, 1 mM dithiothreitol, 0.5 mM KCl, 20 mM CaCl\textsubscript{2}, and 5 mM ATP. The mixture was stirred gently at 0 °C for 5 min with the aid of a magnetic stirrer, followed by centrifugation at 124,000 × g (Beckman L5-50, Rotor 50) for 1 h. The resultant supernatant was centrifuged at 165,000 × g for another hour, and the supernatant was diluted by slow addition of an equal volume of ice-cold 100 mM Tris/HCl buffer (pH 8.5) containing 0.5 M KCl, 0.3 M sucrose, 1 mM dithiothreitol, and 20 mM CaCl\textsubscript{2}. The diluted preparation was stirred gently for 5 min at 0 °C with a magnetic stirrer, after which it was centrifuged at 135,000 × g for 1 h and the supernatant discarded. The residual material in the centrifuge tubes (eight) consisted of a dense white pellet with a soft, light brown upper layer. The upper layer was dislodged by the addition of 1 ml of 20 mM Tris maleate buffer (pH 6.8) containing 0.5 M KCl, 0.3 M sucrose, 1 mM CaCl\textsubscript{2}, and 1 mM dithiothreitol followed by gentle shaking or suspension using a Pasteur pipette. The suspended material was transferred to the other centrifuge tubes in sequence and the combined material transferred to a Dounce homogenizer. The procedure was repeated with an additional 1 ml of buffer solution. The suspension was homogenized with 10 strokes using a Teflon pestle. This preparation could be stored at ~80 °C for several months without loss of activity. The yield was 4 to 5 mg for cardiac and 1 to 2.2 mg for skeletal Ca\textsuperscript{2+}-ATPase based on 100 mg of starting SR protein.

In some experiments, the excess Triton X-100 was removed by dilution of the suspension to 10 ml with the same solution and centrifugation at 113,000 × g for 1 h. There was no loss of activity with up to 12 resuspension–centrifugation cycles.

Protein Determination—Protein was measured by the Lowry...
method (16) using bovine serum albumin as standard. However, the Tris, KCl, sucrose, and dithiothreitol in the solution for the purified Ca\(^{2+}\)-ATPase, the Triton X-100 present in the solubilization and precipitation steps, and the SDS, EDTA, and \(\beta\)-mercaptoethanol present in the sample prepared for gel electrophoresis all interfere with the Lowry procedure (17, 18).

To overcome this problem, the approach we have used combines two methods: the first, which used Na deoxycholate and protein precipitation by trichloroacetic acid, was designed to remove interfering sulfhydryl reagents (17). The second procedure increases the concentration of NaOH and phenol reagent (both from conventional 0.09 to 0.17 ml) to offset the precipitation of the phenol reagent by Triton X-100 (18). The procedure was as follows: 100 \(\mu\)l of 1% Na deoxycholate was added to 25 to 400 \(\mu\)l of the protein suspension and mixed, and the protein was precipitated by the addition of 1 ml of 10% trichloroacetic acid followed by 1 ml of the same trichloroacetic acid solution to rinse off the precipitate formed on the internal wall of the test tubes. The suspension was centrifuged at 3,000 rpm (Beckman TJ-6) for 10 min. For the SDS-dialyzed sample originally containing \(\beta\)-mercaptoethanol (see below), the precipitate after the centrifugation was washed once again with 10% trichloroacetic acid. The pellets were dissolved in 0.5 ml of 1 N NaOH followed by the addition of 2 ml of 0.01% alkaline cytochrome solution. After 10 min at room temperature, 0.5 ml of 1 N phenol reagent was added. After 30 min, the samples were read at 750 nm. When Triton X-100 was present, the tubes were centrifuged to remove the precipitate formed before reading the absorption. Standard curves were prepared using bovine serum albumin dissolved in a solution containing 0.25 M KCl, 30% sucrose, 3% Triton X-100, and 1% \(\beta\)-mercaptoethanol and treated with Na deoxycholate followed by precipitation of the enzyme with trichloroacetic acid as described above. The recovery of protein was 100% when less than 100 \(\mu\)g of bovine serum albumin was used. We found this procedure to be consistent and reproducible at all stages in the purification of the Ca\(^{2+}\)-ATPase.

Determination of Triton X-100 Content—Triton X-100 binding to the Ca\(^{2+}\)-ATPase was measured by using [\(^1\)H]Triton X-100 (100 cpm/mm) in the purification procedure. A suspension of about 3.5 mg of the purified Ca\(^{2+}\)-ATPase in 10 ml of the final buffer was centrifuged at 113,000 \(\times\) g for 1 h. The resultant pellet was homogenized in 1 to 2 ml of buffer solution, aliquots of which were used for determination of protein, Triton X-100, and Ca\(^{2+}\)-ATPase activity. For \(H\) counting, samples (20, 40, and 60 \(\mu\)l) were mixed with 0.5 mg of skeletal SR carrier protein in 0.5 ml of the same buffer, dissolved in 1 ml of 0.01 N NaOH and mixed with 16 ml of Aquasol-2. The amount of Triton X-100 which was present was calculated from a [\(^1\)H]Triton X-100 standard curve, prepared, and counted under identical conditions as the experimental sample. The volumes of the homogenized suspensions for subsequent washing of the Ca\(^{2+}\)-ATPase were all adjusted to 10 ml with the buffer solution, and after each additional washing, larger volumes were used for the various assays to ensure a sufficient amount of protein for each assay.

Determination of Total Phospholipids—Total phospholipids bound to the microsomal SR and purified Ca\(^{2+}\)-ATPase proteins were extracted by the method of Folch et al. (19). Before the extraction, Na deoxycholate was added and the preparation was washed twice with 10% trichloroacetic acid, as described above, to ensure complete precipitation of the phospholipid-bound protein and removal of the contaminating ATP, ADP, and inorganic phosphate. The phosphorus in the extracted phospholipids was determined by the method of Bartlett (20).

Solubilization of Microsomal SR and Purified Ca\(^{2+}\)-ATPase for SDS Gel Electrophoresis—The microsomal SR preparation was dialyzed at room temperature overnight against a 20 mM Tris/HCl buffer (pH 7.5) containing 1% SDS and 1% \(\beta\)-mercaptoethanol. It was necessary to remove the CaCl\(_2\) from the purified Ca\(^{2+}\)-ATPase by the addition of 0.5 ml of EDTA to the dialysis buffer solution. In the absence of EDTA, the Ca\(^{2+}\)-ATPase formed aggregates and did not enter the gel. The presence of EDTA and CaCl\(_2\) did not affect the gel pattern of the original, Ca\(^{2+}\)-free microsomal SR. Protein was determined by the Lowry method, as described above.

SDS polyacrylamide gel electrophoresis was carried out according to the method of Laemmli (21). Samples (25 \(\mu\)g) were run on 7.5% slab gels followed by staining in Coomassie blue. Destained gels were scanned in a Biomed Instruments soft laser densitometer at 520 nm.

Assay of Ca\(^{2+}\)-ATPase Activity—The Ca\(^{2+}\)-ATPase was assayed by the linked-enzyme method (22). In 2.5 ml of the buffer solution described above, 0.5 ml of the test tubes. The suspension was centrifuged at 10,000 \(\times\) g for 20 min. The membrane preparations were filtered (Millipore filter type HA, pore size, 0.45 \(\mu\)m), and the filter was washed twice with 7 ml each of 30 mM Tris/HCl buffer solution (pH 7.0) containing 2 mM EDTA. The filter was placed in a scintillation vial, dried in a 60°C oven, and immersed with 7 ml of Aquasol-2. The amount of Ca\(^{2+}\) retained by the enzyme was determined by measuring the radioactivity of Ca\(^{2+}\), which was corrected for the background level of the ATP-independent Ca\(^{2+}\) binding to the protein. For the skeletal microsomal SR or the purified Ca\(^{2+}\)-ATPase, the reaction time was 2 min.

RESULTS

Data on the purification of the cardiac and skeletal SR Ca\(^{2+}\)-ATPase preparations are summarized in Table I and Figs. 1-3.

Cardiac SR Ca\(^{2+}\)-ATPase—For the cardiac SR Ca\(^{2+}\)-ATPase purification, the first supernatant resulting from the centrifugation at 124,000 \(\times\) g for 1 h of the Triton X-100-solubilized microsomal SR revealed a Ca\(^{2+}\)-ATPase activity which was 96% of the total ATPase activity. An additional 1 h of centrifugation at 165,000 \(\times\) g did not result in any loss of enzyme activity. A subsequent 2-fold dilution of the Triton X-100-containing supernatant followed by centrifugation at 113,000 \(\times\) g for 1 h resulted in a pellet containing about a 4-fold increase in the Ca\(^{2+}\)-ATPase specific activity. The SDS gels showed that, in the starting microsomal SR, the 100-kDa Ca\(^{2+}\)-ATPase was about 30% of the total protein (Fig. 1A); whereas, after the dilution step, the Ca\(^{2+}\)-ATPase content in the pellet was greater than 90% and was contaminated with only trace amounts of proteins of 92, 66, 55, and 51-57 kDa (Fig. 1A2). Further washing did not improve the purity of this preparation (Fig. 1A4). The two successive centrifugations at 124,000 \(\times\) g and 165,000 \(\times\) g generally yielded a Ca\(^{2+}\)-ATPase which was contaminated with a much greater amount of the 92-kDa protein. The purified Ca\(^{2+}\)-ATPase could be stored in the presence of 1 mM CaCl\(_2\) at 0°C for a day and at \(-80^\circ\)C for 5 months without...
Purification of SR Ca\(^{2+}\)-ATPase with Triton X-100

**TABLE I**

<table>
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<tr>
<th>Fraction</th>
<th>Source</th>
<th>Ca(^{2+})-ATPase</th>
<th>Mg(^{2+})-ATPase</th>
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<th>Ca(^{2+}) accumulation</th>
<th>Mitochondrial ATPase</th>
<th>Yield</th>
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<td></td>
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<td>(\mu\text{mol ADP/mg h})</td>
<td>(\mu\text{mol ADP/mg h})</td>
<td>(\mu\text{mol/mg})</td>
<td>(\mu\text{mol/mg})</td>
<td>(\mu\text{mol ADP/mg h})</td>
<td>mg/100 mg SR protein</td>
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<td>Starting microsomal SR</td>
<td>Cardiac</td>
<td>84.2 ± 19.5</td>
<td>4.5 ± 2.1</td>
<td>1.4 ± 0.2</td>
<td>2656</td>
<td>5.6 ± 1</td>
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<td>19.4</td>
<td>1.7 ± 0.1</td>
<td>2791</td>
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<td>SR Triton X-100(^d)</td>
<td>Cardiac</td>
<td>138.5</td>
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<td>2.2</td>
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<td>First supernatant(^e)</td>
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<td>5.9</td>
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<tr>
<td>Second supernatant(^f)</td>
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<td>132.2</td>
<td>5.7</td>
<td>1.3</td>
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<tr>
<td>Purified Ca(^{2+})-ATPase(^g)</td>
<td>Cardiac</td>
<td>547.4 ± 66.9</td>
<td>3.3 ± 0.9</td>
<td>86</td>
<td></td>
<td>0</td>
<td>4.2 ~ 5.9</td>
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<tr>
<td></td>
<td>Skeletal</td>
<td>1192.3 ± 172.4</td>
<td>2.5 ± 2.7</td>
<td>145</td>
<td></td>
<td>0</td>
<td>1.5, 2.2^d</td>
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<tr>
<td>Washed Ca(^{2+})-ATPase(^h)</td>
<td>Cardiac</td>
<td>719.4 ± 70.1</td>
<td>6.3 ± 2.9</td>
<td>4.4 ± 0.2</td>
<td>79</td>
<td>0</td>
<td>2.0 ~ 4.9</td>
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<td>(n = 3)</td>
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<tr>
<td></td>
<td>Skeletal</td>
<td>1473.6 ± 205.5</td>
<td>3.5 ± 3.7</td>
<td>5.6 ± 0.6</td>
<td>14</td>
<td>0</td>
<td>0.5, 1.2^c</td>
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\(^a\) Determined in 500 \(\mu\text{M}\) ATP, 34.6 \(\mu\text{M} \text{Ca}^{2+}\), 100 \(\mu\text{M} \text{KCl}\), and 2.5 \(\mu\text{M} \text{Mg}^{2+}\).

\(^b\) 20 min of reaction.

\(^c\) 0.5 mg of Triton X-100 was added to each milligram of SR protein.

\(^d\) After centrifugation of SR Triton X-100 mixture at 124,000 \(\times g\) for 1 h.

\(^e\) After centrifugation of the first supernatant at 165,000 \(\times g\) for 1 h.

\(^f\) After 2-fold dilution of the detergent in the second supernatant and centrifugation at 133,000 \(\times g\) for 1 h.

\(^g\) Initial ratio of Triton X-100 to SR protein was 1:0.

\(^h\) The purified Ca\(^{2+}\)-ATPase was washed with final buffer and pellet isolated by centrifugation.

loss of activity. The enzyme was free of both rotenone-inhibitable NADH oxidative activity and hydrolytic activity for phosphoenolpyruvate. As expected, the purified SR Ca\(^{2+}\)-ATPase did not have Ca\(^{2+}\)-transport activity.

The purified Ca\(^{2+}\)-ATPase contained a higher content of phospholipids than the starting microsomal SR, 49.5 ± 4.4 versus 22.7 ± 3.4 \(\mu\text{g}\) of phospholipid \(\mu\text{g/mg}\) of protein. The Triton X-100 content was also high, 130 mol/mol of protein. Successful washings of the preparations did not change the phospholipid content, but significantly decreased the bound Triton X-100 to a minimum level of about 20 mol/mol of protein. It is important to emphasize that the washings and removal of Triton X-100 did not alter the Ca\(^{2+}\)-ATPase activity (Fig. 2).

During development of this procedure for purification of the cardiac SR Ca\(^{2+}\)-ATPase, it was necessary to use Triton X-100 at a concentration of 0.5 mg/mg of SR protein. When the detergent was used in lower ratios, little solubilization of the membrane-bound Ca\(^{2+}\)-ATPase occurred (data not shown). The specific activity as a function of detergent concentration of three pellets obtained at different stages of purification are shown in Fig. 3. We found that the optimum concentrations of the two “substrates” for the Ca\(^{2+}\)-ATPase, ATP and Ca\(^{2+}\), which were required to protect the enzyme, were 5 and 20 mM, respectively. It was essential that high Ca\(^{2+}\) be maintained in the solubilization medium to reproducibly obtain purified Ca\(^{2+}\)-ATPase with high specific activity. Solubilization of the microsomal SR in the absence of CaCl\(_2\) caused a total loss of enzyme activity; solubilization in CaCl\(_2\) without ATP resulted in a 50% decrease in the activity. The use of ATP carried with it an unavoidable disadvantage, viz. ATP caused precipitation of denatured proteins from the Triton X-100 solution within 1 h after solubilization. Therefore, unnecessary delay should be avoided between centrifugations, and after the final centrifugation, the soft pellet of the purified Ca\(^{2+}\)-ATPase should be washed immediately with an ATP-free buffer. It is important to store the purified Ca\(^{2+}\)-ATPase in the presence of CaCl\(_2\) without which the enzyme loses 50% of its activity at 0 °C overnight.

Skeletal SR Ca\(^{2+}\)-ATPase—The Triton X-100 purification procedure also yielded a final purified Ca\(^{2+}\)-ATPase from skeletal SR. In the starting microsomal SR, the 100-kDa Ca\(^{2+}\)-ATPase was about 45% of the total proteins (Fig. 1B). After the dilution step, the enzyme was about 60% of the total proteins (Fig. 1B), in contrast to cardiac Ca\(^{2+}\)-ATPase which was 90% pure. Washing of this enzyme fraction yielded a purified Ca\(^{2+}\)-ATPase, which constituted 85% of the total proteins (Fig. 1B). The purified Ca\(^{2+}\)-ATPase contained more phospholipids than the original microsomes: 28.5 ± 2.5 versus 15.9 ± 0.1 \(\mu\text{g}\) of phospholipid \(\mu\text{g/mg}\) of protein. The bound Triton X-100 was 95 mol/mol of protein. Successive washings substantially decreased the Triton X-100 bound to a minimum value of about 5 mol/mol of protein but affected neither the phospholipid content nor the Ca\(^{2+}\)-ATPase activity (Fig. 2).
**Purification of SR Ca\(^{2+}\)-ATPase with Triton X-100**

**Fig. 2.** Contents of bound Triton X-100, phospholipids, and Ca\(^{2+}\)-ATPase activity with respect to washing of the enzyme. Inset, Ca\(^{2+}\)-ATPase activity in micromoles of ADP/mg/h.

**Fig. 3.** Specific Ca\(^{2+}\)-ATPase activity of three pellets separated at different stages of purification versus the initial ratio of Triton X-100 to SR protein (w/w). O—O, first pellet from the microsomal SR-Triton X-100 mixture after centrifugation at 124,000 × g for 1 h; △—△, second pellet from the first supernatant centrifuged at 165,000 × g; ▲—▲, third pellet from the 2-fold diluted second supernatant centrifuged at 113,000 × g. The broken line indicates activity after one washing of each pellet.

was found that the optimum ratios of Triton X-100 to SR protein for purification of skeletal SR were from 0.5 to 1.0. The specific activities of the purified Ca\(^{2+}\)-ATPase and at two previous steps in the purification as a function of detergent concentration are shown in Fig. 3.

**DISCUSSION**

We have developed a method for purification of dog cardiac SR Ca\(^{2+}\)-ATPase which yields a preparation of an average activity of 947 μmol of ADP/mg/h, more than three times greater than the activity of the enzyme purified by the method of Van Winkle et al. (12), which utilized Na deoxycholate for initial solubilization of the starting microsomal SR. In our hands, the method of Van Winkle et al. (12) also gives a preparation with the yield and activity as reported. However, the first supernatant obtained after the initial solubilization with Na deoxycholate often contains protein particulates. Once this particulate material was removed, the supernatant did not yield protein material with high Ca\(^{2+}\)-ATPase activity in the subsequent steps. The yields of the purified Ca\(^{2+}\)-ATPase, which we obtained in our simplified procedure, are about the same as that obtained by Van Winkle et al. (12). However, our ability to obtain a purified enzyme with approximately 7-fold increase in activity in about 3 h has distinct advantages. Bidlack et al. (13) have recently reported a purification method for dog cardiac SR using a very small amount of Na deoxycholate for two stepwise solubilizations of the SR microsomes. The yields and activities of their purified Ca\(^{2+}\)-ATPase are, however, not improved compared to those of Van Winkle et al. (12). The only other published purification procedure for obtaining cardiac SR Ca\(^{2+}\)-ATPase (from pigeon hearts) with a similar range of activity, 720-960 μmol of P\(_i\)/mg/h, is that by Levitsky et al. (11), who purified the Ca\(^{2+}\)-ATPase by a Na deoxycholate/ammonium acetate procedure (9) from Ca\(^{2+}\)-oxalate-loaded SR vesicles separated on sucrose gradients.

Our choice of the nonionic detergent Triton X-100 for the initial solubilization of the cardiac microsomal SR was initially based on the fact that the detergent in high amount can effectively solubilize the membrane-bound skeletal SR Ca\(^{2+}\)-ATPase without denaturing the enzyme (10). Although Na deoxycholate is often used in the purification of skeletal SR Ca\(^{2+}\)-ATPase and has also been used in attempts to purify the cardiac SR Ca\(^{2+}\)-ATPase (11-13), this ionic detergent formed a white precipitate in the presence of 20 mM CaCl\(_2\) which rendered the purification process difficult. This high concentration of CaCl\(_2\) was required for protection of enzyme activity.

In the process of purification with Triton X-100, the vesicular cardiac and skeletal microsomes were solubilized and reaggregated, but the Ca\(^{2+}\)-transport activity lost. Reaggregation of the solubilized protein resulted in a higher content of phospholipids/mg of protein, and, perhaps by the same process, Triton X-100 was retained by the enzyme. A large amount of the Triton X-100 appears to be loosely bound, since it was easily removed by washing; however, removal of the excess Triton X-100 did not alter the phospholipid content. Triton X-100, when present at concentrations greater than 100 mol/mol of Ca\(^{2+}\)-ATPase, appeared to suppress enzyme
activity but the full activity could be recovered by removal of the excess detergent. The detergent, bound to the enzyme in the amount of 5-10 mol/mol, did not appear to affect enzyme-specific activity. However, when the initial amount of Triton X-100 used was in large excess, i.e. a ratio of detergent to SR protein of 1.5, the enzyme activity was irreversibly depressed, especially in the skeletal SR preparation. In this case, fractional precipitation and washing of the pellet did not reactivate the enzyme. This deactivating amount of Triton X-100 was less than that reported by Prado et al. (27), who observed that at a detergent-to-protein ratio of 6.5, the solubilized fraction of SR retained maximum enzyme activity although the activities of the pellets were totally abolished. The number of Triton X-100 molecules which remain bound to each molecule of the purified skeletal Ca\(^{2+}\)-ATPase agrees with that observed by Chiesi et al. (28) (about 9 mol/mol of Ca\(^{2+}\)-ATPase) and by Dean and Suarez (29) (10–25 mol/mol of Ca\(^{2+}\)-ATPase).

This procedure was designed primarily for the purification of cardiac SR Ca\(^{2+}\)-ATPase. The Ca\(^{2+}\)-ATPase from skeletal SR obtained by this procedure showed a smaller increase in specific activity and a lower yield compared to cardiac Ca\(^{2+}\)-ATPase, suggesting that SR isolated from different muscle types may require different approaches for optimal purification of the Ca\(^{2+}\)-ATPase. This deactivating amount of detergent to SR protein of 6.5, the enzyme activity was irreversibly depressed, especially in the skeletal SR preparation. We caution must be exerted to avoid accidentally discarding this final product.

Acknowledgments—Our sincere thanks go to Dr. John Solaro and Anne Martin for reading and critiquing this manuscript and to Gwen Kraft for the expert art work.

Note Added in Proof—After the last centrifugation in the purification scheme, the purified Ca\(^{2+}\)-ATPase protein, which appears as a "soft, light brown, upper layer" above a small, dense, white pellet, is only about 300 µg in each of the eight tubes (based on 50 mg of starting SR protein). Caution must be exerted to avoid accidentally discarding this final product.

A swinging bucket rotor should not be used. This often causes poor separation of the purified enzyme from the dense, white pellet. We recommend using a fixed angle rotor.

REFERENCES
Properties and characterization of a highly purified sarcoplasmic reticulum Ca2+-ATPase from dog cardiac and rabbit skeletal muscle.
J Nakamura, T Wang, L I Tsai and A Schwartz


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