Reconstitution of an Active Calcium Pump in Sarcoplasmic Reticulum*

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Recovery of calcium transport and calcium-activated ATPase activity was studied in relation to the retention of protein components in sarcoplasmic reticulum reconstituted after solubilization with deoxycholate and centrifugation, followed by removal of the detergent from the supernatant by dialysis. Control sarcoplasmic reticulum was similarly treated except for omission of deoxycholate. Maximum capacity for oxalate- and phosphate-supported calcium uptake was increased 2- to 3-fold in reconstituted sarcoplasmic reticulum compared to original and control. Calcium uptake velocity of the reconstituted sarcoplasmic reticulum was approximately 80% that of original and 90% of control sarcoplasmic reticulum. Calcium uptake/ATP hydrolysis ratio was approximately 2 in the original sarcoplasmic reticulum and decreased to approximately 1 in the control and reconstituted sarcoplasmic reticulum.

Calcium storage in the absence of calcium-precipitating anions was approximately 85% in control and 70% in reconstituted sarcoplasmic reticulum, compared to the original sarcoplasmic reticulum. Ethylene glycol bis(β-aminoethyl ether)-N,N'-tetraacetic acid-induced calcium release after phosphate-supported calcium uptake was slower in reconstituted sarcoplasmic reticulum than in original or control sarcoplasmic reticulum. Polyacrylamide gel electrophoresis of original and control sarcoplasmic reticulum showed similar amounts of protein components of approximately 93,000, 59,000, 50,000, 30,000 to 37,000, and 20,000 to 26,000 daltons. Reconstituted sarcoplasmic reticulum, however, lost over 85% of the 50,000- and 20,000- to 26,000-dalton proteins while retaining most of its calcium transport functions.

The sarcoplasmic reticulum of skeletal muscle is a highly specialized membrane with the primary function of regulating calcium fluxes during the contraction-relaxation cycle (2-4). During relaxation, calcium is transported into the sarcoplasmic reticulum by a tightly coupled energy-dependent process in which 2 mol of calcium are transported for each mole of ATP hydrolyzed (4, 5). The major component of the sarcoplasmic reticulum is an ATPase protein which has a molecular weight of approximately 100,000 (6). In addition to the ATPase protein, several smaller protein components with molecular weights ranging from 20,000 to 26,000 daltons while retaining most of its calcium transport functions.

The ATPase protein and possibly other protein components of the sarcoplasmic reticulum are imbedded in a lipid bilayer, in the absence of which the calcium transport system is not functional. These lipoprotein membranes can be dissociated, for example by detergents, under conditions where removal of the detergent allows subsequent reassembly of vesicles that possess activities associated with the original sarcoplasmic reticulum (6-11). In such preparations, generally designated "reconstituted" sarcoplasmic reticulum or calcium pump, ATPase activity is usually greater than in nontreated membranes, whereas calcium transport activity is low (6, 7). Recovery of calcium transport is enhanced when phospholipids are added during reconstitution of these vesicles (9, 10). These procedures offer a promising approach toward defining the role of the various components of the sarcoplasmic reticulum in the mechanism of calcium transport into these vesicles.

The interactions between calcium and vesicles prepared from the sarcoplasmic reticulum can be examined in several ways. Calcium transport is commonly measured in the presence of calcium-precipitating anions, most commonly oxalate and phosphate. These anions, which pass freely into these vesicles (12), cause insoluble calcium salts to be precipitated when calcium is transported into the vesicles. When calcium transport is measured in the absence of the calcium-precipitating anions, the final amount of calcium retained by the vesicles (sometimes referred to as "calcium binding") reflects the ability of the sarcoplasmic reticulum to store calcium and thus
provides an index for the amount of calcium-binding material attached to the vesicles. In the present report conditions are described which allow the sarcoplasmic reticulum to be solubilized and reconstituted with a high recovery of these calcium transport and storage functions. Evidence is presented that these high recoveries are achieved in the presence of the ATPase protein and some, but not all, of the smaller protein components described above.

MATERIALS AND METHODS

Isolation of Sarcoplasmic Reticulum—Sarcoplasmic reticulum was prepared from rabbit white skeletal muscle by the method of Haragava and Schwartz (13), modified slightly (14). The pellets obtained after final centrifugation were suspended in 0.4 M sucrose and 20 mM Tris-HCl at pH 7.25 and stored on ice. These preparations, which will be designated original sarcoplasmic reticulum, ordinarily retained full calcium transport activity for at least 4 days.

Preparation of Control and Reconstituted Sarcoplasmic Reticulum—Sarcoplasmic reticulum was solubilized at 0°C with 4 mg per ml of protein and 5.1 mM deoxycholate (0.55 mg per mg of protein) in the presence of 0.15 M KCl/20 mM Tris-HCl at pH 7.25/2 mM MgCl₂/1 mM EDTA/1 mM diethylenetriol/0.4 M sucrose. The solubilized mixture was centrifuged immediately for 1 hour at 176,000 × g. Control sarcoplasmic reticulum was incubated in a similar reaction mixture except for the omission of deoxycholate and the step of centrifugation. The control and deoxycholate-treated sarcoplasmic reticulum preparations were dialyzed separately in 1-cm diameter dialysis bags for 22 hours at 21–23°C against several changes of a medium containing 0.4 M KCl/20 mM Tris-HCl at pH 7.25/2 mM MgCl₂/1 mM EDTA/1 mM diethylenetriol/0.4 M sucrose. After removal of deoxycholate by dialysis, both the control and reconstituted sarcoplasmic reticulum preparations were centrifuged for 1 hour at 176,000 × g. The pellets were suspended in 0.4 M sucrose and 20 mM Tris-HCl at pH 7.25 and used immediately for assays. The amount of protein solubilized was calculated from the amount of insoluble protein recovered in the pellet after the reaction mixture containing deoxycholate was centrifuged.

Assays—Calcium uptake was measured at 25°C in 2.5 mM Tris-oxalate or 50 mM potassium phosphate/0.12 M KCl/40 mM histidine at pH 6.8/0.2 mM MgATP/52.6 μM ⁴⁵CaCl₂/10 μM ionized calcium, see Ref. 15). Protein concentrations were 5 to 15 μg per ml. Calcium storage in the absence of calcium-precipitating anions was measured in a similar reaction mixture except that the protein concentration was increased to 250 μg per ml. Reactions were started in most cases with MgATP, and after a 5-min incubation ⁴⁵CaCl₂ was added. The initial rate of calcium uptake was taken as the linear part of the slope of the calcium uptake plot based on five measurements taken between 15 and 2 min. The maximum amount of calcium stored in the absence of calcium-precipitating anions was determined from a sample taken 4 min after the addition of ⁴⁵CaCl₂ (16). Calcium release was measured after sarcoplasmic reticulum was loaded with calcium to a determined steady state level in the presence of 50 mM phosphate. Calcium release was induced by transferring 5 ml of the reaction mixture to a tube containing 50 μl of 550 mM EGTA1 (final concentration, 2.5 mM). All samples were filtered through Millipore Swinnex adapters, and 50 μl aliquots of the filtrate were added to 5 ml of Bray’s solution and counted in a Packard liquid scintillation spectrophotometer as described previously (16).

ATPase activity was determined as P₂ liberation measured by the method of Taussky and Shorr (17). All reactions were started by the addition of protein. The Ca⁺⁺-activated ATPase activity associated with calcium uptake was calculated by subtracting the "basic" ATPase activity measured in 2.5 mM EGTA, from that measured in the presence of Ca⁺⁺. Determinations of Ca⁺⁺-activated ATPase activity were carried out concurrently with calcium uptake measurements made under identical conditions. Gel electrophoresis was carried out in sodium dodecyl sulfate on 7.5% polyacrylamide gels according to Weber and Osborn (18). Gels were stained with Coomassie brilliant blue for 3 hours and destained for 24 hours in a mixture of 40% methanol and 7% acetic acid. For the estimation of molecular weights of sarcoplasmic reticular proteins, six standard proteins with known molecular weights were run simultaneously under the same conditions. Densitometry tracings of gels were recorded with a Gilford linear absorbance recorder as the optical density of the stained protein bands at 550 nm.

Protein concentrations were determined by the biuret method (19), bovine serum albumin being used as a standard.

Deoxycholate (Sigma Chemical Co., St. Louis, Mo.) was treated with charcoal and recrystallized twice from ethanol. Disodium-ATP (Sigma) was deionized on a Dowex AG 50W-X8 column and neutralized with Tris and MgCl₂. Calcium-45 was purchased from ICN Corporation, Chemical and Radioisotope Division, Irvine, Calif. Dialysis tubing (Union Carbide Corporation, Films and Packaging Division, Chicago, Ill.) was boiled in 0.1 M EDTA for 1 hour and then reboiled in several changes of glass-distilled water. Immediately before use, the dialysis tubing was rinsed thoroughly with dialysis solution.

RESULTS

Solubilization and Reconstitution of Sarcoplasmic Reticulum—The extent of solubilization by deoxycholate and the properties of the reconstituted sarcoplasmic reticulum depended on the KCl concentration during solubilization. Only 60% of the protein was dissolved by 0.5 mg of deoxycholate per mg of protein in the absence of KCl. The amount of protein solubilized increased with increasing KCl concentration, more than 90% of the protein being solubilized in the presence of 0.40 M KCl (Table I). Calcium uptake by reconstituted sarcoplasmic reticulum first increased and then decreased as KCl concentration during solubilization was raised. Maximal uptake was seen with approximately 0.20 M KCl.

The properties of reconstituted sarcoplasmic reticulum and the extent of solubilization depended on both deoxycholate concentration and the ratio of deoxycholate to protein during solubilization. When the concentrations of deoxycholate and protein were increased together at a constant ratio of 0.5 mg of deoxycholate per mg of protein, increasing amounts of protein were solubilized. This effect is not due to the increasing protein concentration as increasing protein concentration at a constant deoxycholate concentration reduced the amount of protein solubilized (data not shown). Recovery of calcium uptake activity in the sarcoplasmic reticulum reconstituted in the experiment shown in Table II decreased sharply when deoxycholate concentration exceeded 5.8 mM (2.5 mg of deoxycholate per ml). Recovery of calcium uptake in reconstituted sarcoplas-

<table>
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<tr>
<th>KCl (M)</th>
<th>Calcium uptake (μmol Ca⁺⁺/mg protein)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>0.87</td>
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<tr>
<td>0.10</td>
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<tr>
<td>0.15</td>
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<tr>
<td>0.40</td>
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</table>

TABLE I

Effect of KCl concentration on solubilization and properties of reconstituted sarcoplasmic reticulum

Sarcoplasmic reticulum (4 mg per ml) was solubilized with 0.5 mg of deoxycholate per mg of protein in the presence of various KCl concentrations with other conditions as described under "Materials and Methods." Calcium uptake, measured with 7.8 μg of protein per ml, is expressed as the maximum amount of calcium taken up in the presence of 2.5 mM oxalate.

1 The abbreviation used is: EGTA, ethylene glycol bis[(β-aminoethyl ether)-N,N'-tetraacetic acid].
plasmic reticulum was highest after 4 mg of protein per ml were treated with 5.1 mM deoxycholate (0.55 mg of deoxycholate per mg of protein). Under the latter conditions, 80 to 90% of the sarcoplasmic reticulum was solubilized.

Omission of the 0.4 M sucrose during solubilization increased the amount of protein recovered in the pellet obtained when the mixture containing deoxycholate was centrifuged. As long as this concentration of sucrose was added to the solubilized sarcoplasmic reticulum immediately prior to and during dialysis, both calcium uptake rate and the coupling ratio between calcium transport and ATP hydrolysis were increased (see below). Neither the protein composition nor the recovery of calcium transport in the reconstituted sarcoplasmic reticulum was influenced significantly when CaCl₂, in the concentration range 0 to 1 mM, was included during solubilization and reconstitution of the membranes.

The recovery of calcium transport activities depended on the conditions under which the vesicles were reconstituted after solubilization, as well as on the conditions used for solubilization. Recovery of activity was considerably greater when deoxycholate was removed from the supernatant by dialysis at room temperature (21–23°) than by dialysis at 4°. Frequent changes of the solution outside the dialysis bags for the first 2 hours of dialysis improved the quality of the reconstituted sarcoplasmic reticulum.

Oxalate- and Phosphate-supported Calcium Uptake—The total capacity for oxalate-supported calcium uptake by reconstituted sarcoplasmic reticulum was usually 2 to 3 times greater than that of the original or control sarcoplasmic reticulum (Fig. 1). However, the initial rate of calcium uptake was slightly less than that of the original and control sarcoplasmic reticulum. In the presence of either oxalate or phosphate, 80% of the initial rate of calcium uptake was seen in the reconstituted sarcoplasmic reticulum compared to original, and 90% compared to control sarcoplasmic reticulum (Fig. 2). When sarcoplasmic reticulum was solubilized in the absence of sucrose, the recovery of calcium uptake was approximately 15% higher than that seen when sarcoplasmic reticulum was solubilized in the presence of 0.4 M sucrose.

Electron micrographs of the vesicles in original and reconstituted sarcoplasmic reticulum were obtained in an attempt to explain the increased calcium uptake capacity of reconstituted sarcoplasmic reticulum. The vesicles of reconstituted sarcoplasmic reticulum were several-fold larger than those of the original sarcoplasmic reticulum (Fig. 3), indicating that the surface to volume ratio was less after reconstitution.

Calcium Uptake/ATP Hydrolysis Ratio—The tightness of coupling of the calcium pump can be expressed as the ratio between calcium transport and ATP hydrolyzed. This ratio was examined in reconstituted sarcoplasmic reticulum and compared to original and control sarcoplasmic reticulum. Initial rates of calcium uptake and Ca²⁺-activated ATPase activity were determined simultaneously and under identical conditions. As already shown (Fig. 2), the initial rate of calcium uptake in control sarcoplasmic reticulum was slightly lower than that of the original sarcoplasmic reticulum; however, Ca²⁺-activated ATPase of the former was increased almost 50% (Table III). As a result, the calcium uptake/ATP hydrolysis ratio fell in the control sarcoplasmic reticulum from approximately 2 to slightly more than 1 (Table III).

When sarcoplasmic reticulum was reconstituted after solubilization in 0.4 M sucrose, the calcium uptake rate was less than that of the control sarcoplasmic reticulum, while the Ca²⁺-activated ATPase activity was approximately 1½ times greater. The calcium uptake/ATP hydrolysis ratio of this reconstituted sarcoplasmic reticulum was, therefore, approximately 0.5, less than one-half that of the control sarcoplasmic reticulum.

### Table II

**Effects of deoxycholate and protein concentrations on solubilization and properties of reconstituted sarcoplasmic reticulum**

Solubilization was carried out in 0.15 M KCl with other conditions as described under “Materials and Methods.” Calcium uptake, measured with 6.3 μg of protein per ml, is expressed as the maximum amount of calcium taken up in the presence of 2.5 mM oxalate.

<table>
<thead>
<tr>
<th>Protein</th>
<th>Deoxycholate</th>
<th>Protein solubilized</th>
<th>Calcium uptake mg/ml</th>
<th>Deoxycholate mg/mg protein</th>
<th>mM</th>
<th>%</th>
<th>μmol Ca²⁺/mg protein</th>
</tr>
</thead>
<tbody>
<tr>
<td>3.0</td>
<td>0.5</td>
<td>3.5</td>
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<td>3.08</td>
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<td>99</td>
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<td>5.8</td>
<td>99</td>
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<td>10.0</td>
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<td>99</td>
<td>0.18</td>
<td>11.6</td>
<td>99</td>
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<tr>
<td>13.5</td>
<td>0.5</td>
<td>15.7</td>
<td>96</td>
<td>0.06</td>
<td>15.7</td>
<td>96</td>
<td>0.06</td>
</tr>
</tbody>
</table>

*Original sarcoplasmic reticulum.*

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**Reconstituted Sarcoplasmic Reticulum**

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**FIG. 1.** Time course of calcium uptake of original (●), control (△), and reconstituted (■) sarcoplasmic reticulum. Calcium uptake was measured with 0.5 μg of protein per ml and 2.5 mM oxalate as described under “Materials and Methods.”

**FIG. 2.** Initial rates of calcium uptake of original (●), control (△), and reconstituted (■) sarcoplasmic reticulum in the presence of oxalate and phosphate. Results from eight different preparations are presented as mean ± S.E. Calcium uptake was measured with protein concentrations from 5.6 to 6.2 μg per ml as described under “Materials and Methods.”
reticulum (Table III). When, however, sarcoplasmic reticulum was reconstituted after solubilization in the absence of sucrose, calcium uptake was greater, and Ca\(^{2+}\)-activated ATPase was considerably less (Table III). As a result, the calcium uptake/ATP hydrolysis ratio was greater than 1, a value similar to that seen in the control sarcoplasmic reticulum.

**Calcium Release Induced by EGTA**—To determine the effects of reconstitution on the extent of the back reaction during calcium uptake, EGTA-induced calcium release was measured after these preparations had been preloaded with calcium in the presence of phosphate. Calcium release from reconstituted sarcoplasmic reticulum was considerably slower than that of control and original sarcoplasmic reticulum (Fig. 4).

**Calcium Storage in Absence of Calcium-precipitating Anions**—Calcium transport in the absence of oxalate and phosphate was measured to determine the effects of reconstitution on the capacity to store calcium. Calcium storage by the reconstituted sarcoplasmic reticulum that had been solubilized in 0.4 M sucrose was approximately 70% that of the original sarcoplasmic reticulum and 85% that of the control sarcoplasmic reticulum (Fig. 5). When the sarcoplasmic reticulum was solubilized in the absence of sucrose, calcium storage by the reconstituted sarcoplasmic reticulum approached 90% that seen in the original sarcoplasmic reticulum.

**Protein Composition of Reconstituted Sarcoplasmic Reticulum**—When sarcoplasmic reticulum was solubilized in sodium dodecyl sulfate and fractionated by polyacrylamide gel electrophoresis, protein components with apparent molecular weights of approximately 93,000, 59,000, 50,000, 30,000 to 37,000, and 20,000 to 26,000 were seen both in original and control sarcoplasmic reticulum (Figs. 6 and 7). The 50,000-dalton protein band occasionally appeared in two forms with apparent molecular weights of approximately 48,000 and

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**TABLE III**

**Comparison of calcium uptake/ATP hydrolysis ratio of various types of sarcoplasmic reticulum**

<table>
<thead>
<tr>
<th>Sarcoplasmic reticulum</th>
<th>Calcium uptake (µmol/mg/min)</th>
<th>Ca(^{2+})-activated ATPase (µmol/mg/min)</th>
<th>Calcium uptake/ATP hydrolysis ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>Original</td>
<td>1.20</td>
<td>0.62</td>
<td>1.95</td>
</tr>
<tr>
<td>Control</td>
<td>1.06</td>
<td>0.92</td>
<td>1.15</td>
</tr>
<tr>
<td>Solubilized in sucrose and reconstituted</td>
<td>0.65</td>
<td>1.42</td>
<td>0.46</td>
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<tr>
<td>Solubilized without sucrose and reconstituted</td>
<td>0.81</td>
<td>0.73</td>
<td>1.11</td>
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</table>

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**Fig. 3.** Electron micrographs of original (left) and reconstituted (right) sarcoplasmic reticulum. The preparations were negatively stained with alcoholic uranyl acetate. Magnification x 117,000.
Reconstituted Sarcoplasmic Reticulum

One or two protein bands were seen in the 30,000 to 37,000 and in the 20,000 to 26,000 molecular weight range. Reconstituted sarcoplasmic reticulum invariably lost more than 85% of the 50,000 and the 20,000 to 26,000 molecular weight protein components (Figs. 6 and 7).

Densitometry measurement of the stained protein bands on these gels showed that the 93,000-dalton protein increased by approximately 20% in the reconstituted sarcoplasmic reticulum, compared to original and control sarcoplasmic reticulum where approximately 70% of the total protein consisted of the 93,000-dalton protein. Original and control sarcoplasmic reticulum contained approximately 15% more of the 59,000 molecular weight protein component than did reconstituted sarcoplasmic reticulum. In contrast, only approximately 15% of the 50,000-dalton protein was recovered in reconstituted sarcoplasmic reticulum. Proteins of the 30,000 to 37,000 molecular weight group were almost completely recovered in reconstituted sarcoplasmic reticulum, whereas the proteins of the 20,000- to 26,000-dalton group were virtually absent from reconstituted sarcoplasmic membranes. No significant difference in protein patterns could be found when reconstituted sarcoplasmic reticulum solubilized in the presence and absence of sucrose were compared.

DISCUSSION

The present findings demonstrate that an active and tightly coupled calcium pump can be reconstituted from the supernatant obtained after centrifugation of sarcoplasmic reticulum solubilized in deoxycholate. The vesicles which form after removal of the deoxycholate by dialysis at room temperature are larger than those present originally, as has been previously reported (20). Recovery of calcium uptake capacity is 2- to 3-fold greater than in original or control sarcoplasmic reticulum, probably reflecting the larger size of the reconstituted vesicles in which calcium oxalate can be precipitated (Fig. 3).
and possibly a reduced rate of calcium efflux (Fig. 4). Approximately 80% of the calcium uptake velocity is recovered in the reconstituted membranes (Fig. 2), in contrast to findings of Meissner and Fleischer (20), who recovered approximately 20% of this activity. More recently, Knowles and Racker (21) reported calcium uptake velocities of 0.2 to 0.6 μmol per mg of protein per min in vesicles reconstituted in 200 mM oxalate from purified ATPase protein plus phospholipids. These reported rates, measured in 100 μM CaCl₂, are considerably less than the calcium uptake velocity of approximately 1.9 μmol per mg of protein per min seen for the reconstituted sarcoplasmic reticulum in the present study, where measurements are made in 10 μM Ca²⁺ and 2.5 mM oxalate or 50 mM phosphate (Fig. 2).

A reasonably well coupled calcium pump can be recovered in reconstituted sarcoplasmic reticulum, as evidenced by a calcium uptake/ATP hydrolysis ratio of approximately 1 (Table III). This ratio is approximately one-half of that seen in original sarcoplasmic reticulum (Refs. 4 and 5 and Table III), but is comparable to that of the control sarcoplasmic reticulum. The loss of normal coupling in the reconstituted sarcoplasmic reticulum is most likely due to prolonged dialysis at room temperature, rather than to reconstitution itself. The tightness of coupling in these preparations of reconstituted sarcoplasmic reticulum is reflected in low Ca²⁺-activated ATPase activity. In preparations reconstituted after solubilization in the absence of sucrose, ATPase rates are 0.6 to 0.9 μmol per mg of protein per min, in contrast to rates higher than 1.9 μmol per mg of protein per min reported by Meissner and Fleischer (20). In membrane vesicles prepared from purified ATPase protein and phospholipids from various sources, ATPase rates of 1.9 (21), 8.3 (10), and over 30 (22) μmol per mg of protein per min have been reported.

Recovery of a tightly coupled calcium pump in reconstituted sarcoplasmic reticulum depends on the conditions during both solubilization and reconstitution. Deoxycholate concentrations greater than 5 mM solubilize virtually all of the protein but yield less active reconstituted vesicles. Lower deoxycholate concentrations solubilize less material, though the reconstituted vesicles retain high activity (Table II). The absolute concentration of deoxycholate as well as the ratio of deoxycholate to protein influences the properties of reconstituted sarcoplasmic reticulum. Inclusion of KCl during deoxycholate treatment increases the amount of protein solubilized, although higher KCl concentrations (Table II), like higher detergent concentrations (Table II), reduce calcium transport activity of the reconstituted vesicles. Optimal calcium transport was recovered with 0.15 mM KCl and 5.1 mM deoxycholate (0.55 mg per mg of protein), concentrations that are considerably lower than those used by Meissner and Fleischer (20). In the absence of sucrose, 0.15 mM KCl and 5.1 mM deoxycholate cause slightly more protein to appear in the pellet after the solubilized membranes are centrifuged, and the sarcoplasmic reticulum reconstituted from that supernatant is more tightly coupled (Table III).

Reconstitution of sarcoplasmic reticulum following removal of deoxycholate by dialysis is optimal when 0.45 mM KCl and 3 M sucrose were present during dialysis. Similar conditions have been reported by Meissner and Fleischer (20), who included, in addition, 0.1 mM CaCl₂. Although Ikemoto et al. (23) have reported that calcium precipitates the calcium-binding proteins, no effect of CaCl₂ (up to 1 mM) has been found in the present study.

The eletrophoretograms of original and control sarcoplasmic reticulum are similar to those reported by MacLennan (22) and Meissner et al. (8). A protein reported to have a molecular weight of 54,000 (7, 24) or 60,000 to 65,000 (8) corresponds to the 59,000-dalton protein seen in the present study. The 50,000-dalton protein found here corresponds to the 44,000-dalton protein found by Sarzala et al. (24) and MacLennan and Wong (25), and to the 55,000-dalton protein by Meissner et al. (8). The occasional doubling of this protein band has been reported previously (7). Lower molecular weight proteins, 20,000 to 37,000 daltons, have also been reported (7). More than 85% of the 59,000- and 30,000- to 37,000-dalton proteins are recovered in reconstituted sarcoplasmic reticulum which contain less than 15% of the 50,000- and 20,000- to 26,000-dalton proteins (Figs. 6 and 7). The finding that the 59,000-dalton protein remains associated with the reconstituted membranes is not in accord with reports that this protein remains water-soluble after the removal of the detergent used to solubilize the sarcoplasmic reticulum (8, 24, 26). Loss of the 20,000- to 26,000-dalton proteins and retention of the 30,000- to 37,000-dalton proteins may mean that the former are degradation products of the 50,000-dalton protein and the latter of the 59,000-dalton protein, though the 30,000- to 37,000-dalton proteins may be degradation products of the ATPase protein, as has been suggested by Martinosi and Haldin (27) and by Sarzala et al. (24). No information now exists as to a possible relationship between the 20,000- to 26,000-dalton proteins and the 22,000-dalton protein found to regulate calcium transport in cardiac sarcoplasmic reticulum (28), nor do the present studies exclude a possible role for the 12,000-dalton proteolipid described by Racker and Eytan (29).

Reconstitution of an active and tightly coupled calcium pump in vesicles containing the ATPase protein and the larger of the two "calcium-binding proteins" may clarify the role of the latter in the calcium transport by sarcoplasmic reticulum. The methods which were found in the present study to yield active reconstituted sarcoplasmic reticulum were also found to cause retention of more than 85% of the 59,000-dalton protein, whereas the smaller of these proteins, the 50,000-dalton protein, is reduced to approximately 15% of its initial content. Up to 90% of the calcium storage capacity, measured in the absence of calcium-precipitating anions, is retained. In contrast, Meissner and Fleischer (20) recovered only 25% of the calcium storage capacity in reconstituted sarcoplasmic reticulum. Loss of this capacity coincided with loss of most of the larger of the calcium-binding proteins (20). The high recovery of calcium storage capacity, in the present study, like that of a tightly coupled calcium pump, may, however, reflect the low Ca²⁺ permeability of the reconstituted sarcoplasmic reticulum (Fig. 4).

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