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Evidence That Platelet and Skeletal Sarcoplasmic Reticulum Ca^{2+}-ATPase Are Structurally Distinct*

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Proteolytic digestion and indirect immunostaining were used to compare the platelet and sarcoplasmic reticulum Ca^{2+}-ATPase proteins. When the platelet and sarcoplasmic reticulum Ca^{2+}-ATPase proteins were digested in the native state with trypsin, the platelet Ca^{2+}-ATPase, which had an apparent undigested molecular mass of 103 kDa, yielded 78-kDa and 25-kDa fragments. Calcium transport activity depended on the integrity of the 103-kDa protein, while the digested protein had residual ATPase activity. Tryptic digestion of the sarcoplasmic reticulum pump protein, which also had an undigested molecular mass of 103 kDa, yielded products with apparent molecular masses of 55 kDa, 36 kDa, and 26 kDa. Distinct patterns were also observed when the platelet and sarcoplasmic reticulum calcium pump proteins were digested with chymotrypsin and *Staphylococcus aureus* protease in the presence of sodium dodecyl sulfate. Chymotrypsin digestion of the platelet protein resulted in the appearance of products with apparent molecular masses of 70 kDa, 39 kDa, and 31 kDa, while a similar digestion of the sarcoplasmic reticulum calcium pump protein yielded 54-kDa, 52.5-kDa, 46-kDa, 41-kDa, and 36-kDa fragments. Exposure of the sarcoplasmic reticulum and platelet Ca^{2+}-ATPase proteins to *S. aureus* protease also yielded dissimilar fragmentation patterns. These results indicate that the Ca^{2+}-ATPases from platelets and sarcoplasmic reticulum are distinct proteins.

Eukaryotic cells have evolved precise mechanisms for regulating intracellular levels of calcium. These mechanisms help cells maintain high gradients of calcium across their plasma membranes with calcium concentrations inside the cell several thousandfold less than calcium concentrations in the extracellular fluid. At the same time, they provide methods, especially in excitable cells, whereby levels of intracellular calcium can be increased rapidly but in graded fashion. This typically involves release of calcium from various subcellular stores so that the cell is not exposed to the high levels of extracellular calcium. The mechanisms involved in these processes are unique from cell type to cell type and, to a certain extent, reflect the specialized function of the cell.

Blood platelets are excitable cells which appear to utilize calcium as a second messenger. Calcium levels inside the cell rise from 10^{-7} to 2×10^{-6} M during cell activation (1), triggering a number of calcium-dependent processes (2, 3). The major mechanism for calcium regulation in platelets appears to be through a subcellular membrane system called the dense tubular system. Histochemical studies have localized a putative Ca^{2+}-ATPase to the dense tubular system (4) and microsomal fractions from sonicated platelets demonstrate calcium transport and Ca^{2+}-ATPase activities (5). In many respects, the dense tubular system resembles the sarcoplasmic reticulum of muscle. Both sequester calcium in an energy-dependent fashion, contain a Ca^{2+}-ATPase (4, 6), and can be induced to release calcium by appropriate stimuli (3, 6). The platelet Ca^{2+}-ATPase is stimulated by cAMP-dependent protein kinase activity, while the skeletal sarcoplasmic reticulum pump protein seems to be regulated principally by substrate and product concentrations (7, 8).

These and other differences in the platelet and skeletal muscle systems suggest that a better understanding of calcium movements in these cells can be obtained by comparative studies. In this report, we compare the structural and functional properties of the platelet microsome and skeletal muscle sarcoplasmic reticulum Ca^{2+}-ATPases. The results demonstrate important structural and functional differences between the two proteins.

MATERIALS AND METHODS

All chemicals were reagent grade. Sodium dodecyl sulfate was obtained from BDH Chemicals Ltd. (United Kingdom). Nitrocellulose paper and goat anti-rabbit IgG-horseradish peroxidase conjugate were purchased from Bio-Rad. Soybean trypsin inhibitor, tosylphenylalanyl chloromethyl ketone-treated-trypsin, and α-chymotrypsin were obtained from Worthington Biochemicals, while *S. aureus* protease was from Sigma. $^{45}Ca^{2+}$ (chloride salt, 0.43 Ci/mmol) was purchased from ICN.

Antisera to the sarcoplasmic reticulum Ca^{2+}-ATPase was prepared and characterized as follows. Rat skeletal muscle Ca^{2+}-ATPase was prepared (9, 10) and then subjected to preparative gel electrophoresis according to the procedure of Laemmli (11) to remove contaminating proteins. After a brief staining with Coomassie Blue, the 103-kDa Ca^{2+}-ATPase band was excised and then extracted from the gel with 0.1% sodium dodecyl sulfate and 10 mM sodium bicarbonate. Antisera were raised in a rabbit by a series of intramuscular injections with 0.5 mg of electrophoretically purified Ca^{2+}-ATPase in Freund's complete adjuvant initially and then in Freund's incomplete adjuvant. The ability of the antiserum to specifically stain the Ca^{2+}-ATPase was demonstrated by indirect immunostaining of nitrocellulose blots of rabbit or rat sarcoplasmic reticulum protein separated by gel electrophoresis.

Preparation of Membrane Fractions—The isolation of platelet microsomes was described in detail elsewhere (12). Briefly, washed platelets, obtained from fresh human blood, were subjected to sonication, followed by centrifugation to isolate a 14,000 to 40,000 $\times$ *g* microsomal fraction. The final microsomal pellet was suspended in

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"incubation medium" (94 mM KCl, 5 mM $MgCl_2$, 50 μM $CaCl_2$, 20 mM Tris/Tris-HCl, pH = 7.0) at a protein concentration of 3–5 mg/ml. The method of Peterson was utilized to measure protein concentration (13). Platelet microsomes were used immediately for tryptic digestion experiments or frozen at −80 °C for Cleveland digestions.

Sarcoplasmic reticulum vesicles were prepared from rabbit skeletal muscle according to the method of MacLennan (9) as modified by Campbell and MacLennan (14) in the presence of the following protease inhibitors: pepstatin A (10 units/ml), antipain (10 units/ml), leupeptin (10 units/ml), chymostatin (10 units/ml), benzamidine (100 units/ml), kallikrein inactivator (500 units/ml), and phenylmethylsulfonyl fluoride (1.0 mM). Sarcoplasmic reticulum membrane preparations were stored frozen at −70 °C in 0.25 M sucrose, 10 mM Tris, pH = 8.0, and 1.0 mM histidine.

Functional Characterization—Ca^{2+}-ATPase activity was measured spectrophotometrically with a coupled enzyme system in which ATP hydrolysis was coupled to NADH oxidation through the action of pyruvate kinase and lactate dehydrogenase (15). The ATPase assays were conducted at 24 °C in "incubation medium" that contained 2 mM ATP, 100 μM NADH, 4.2 mM phospho(enol)pyruvate, 10 units/ml of pyruvate kinase, and 10 units/ml of lactate dehydrogenase. Microsomal protein concentration during the ATPase assay was 0.03–0.05 mg/ml. NADH oxidation was followed spectrophotometrically at 340 nm. Ca^{2+}-ATPase activity was defined as the difference in ATPase activity in the presence and absence of 1 mM EGTA.[1]

Calcium uptake assays were performed by diluting platelet microsomes 10-fold into "incubation medium" that contained 2 mM ATP and a trace amount of ^{45}Ca. Internalized calcium was measured as a function of time by placing 100-μl portions of the uptake mixture on micropore filters (Millipore type HA, 0.45-μm pore size) attached to a vacuum filtration apparatus. Filters were subsequently washed with two 5-ml portions of ice-cold 150 mM NaCl, then placed in 5 ml of Aquasol-2 and counted by liquid scintillation counting.

Proteolytic Digestion Studies—Trypsin digestion experiments were performed by diluting stock suspensions of tosylphenylalanyl chloromethyl ketone-treated trypsin (prepared immediately before use in H_2O) 100-fold into sarcoplasmic reticulum or platelet microsomal suspensions (in incubation medium) with respective protein concentrations of 0.15 or 3–5 mg/ml. The proteolysis reaction was terminated by the addition of soybean trypsin inhibitor at a soybean trypsin inhibitor/trypsin ratio of 3:1 (w/w). Digestions were carried out at 24 °C. Digested samples were then used immediately for Ca^{2+}-ATPase and Ca^{2+} uptake assays or frozen at −80 °C for subsequent electrophoresis.

For Cleveland digestion experiments, sarcoplasmic reticulum and platelet microsomes were solubilized with 5% (w/v) SDS, then dialyzed at 24 °C for 2 days against "Cleveland digestion medium" (0.5% (w/v) SDS, 10% (w/v) glycerol, and 125 mM Tris/Tris-HCl, pH = 6.8). Solubilized samples were stored at −80 °C, then incubated at 100 °C for 3 min, and returned to room temperature before initiation of digestion. The Cleveland digestion reaction was initiated by diluting stock chymotrypsin or *S. aureus* protease (prepared in H_2O immediately before use) 100-fold into the "Cleveland digestion medium" at 24 °C. The digestion reaction was terminated by elevating the sample temperature to 100 °C for 3 min.

Electrophoresis of trypsin and Cleveland digest samples employed the system of Laemmli (11). Samples were mixed with an equal volume of "sample buffer" (125 mM Tris/Tris-HCl, 4.6% (w/v) SDS, 20% (w/v) glycerol, 10% (v/v) β-mercaptoethanol), incubated at 100 °C for 1 min, and then applied to 1-mm-thick slab gels prepared with a 10% polyacrylamide content. Gels were stained with Coomassie Brilliant Blue or subjected to an immunodetection procedure. A molecular mass standard mixture consisting of phosphorylase, bovine serum albumin, ovalbumin, carbonic anhydrase, and soybean trypsin inhibitor was used.

Since the platelet Ca^{2+}-ATPase is a trace protein in the microsomes and has a similar electrophoretic mobility as glycoprotein IIIa (16), detection of the platelet Ca^{2+}-ATPase and its proteolytic fragments was achieved with an immunodetection procedure after protein transfer from polyacrylamide gels to nitrocellulose paper. After electrophoresis, the protein was electrotransferred to nitrocellulose paper in a Bio-Rad trans-blot cell for 18 h at 150 mA using a solvent system that contained 25 mM Tris/Tris-HCl, 192 mM glycine, and 20% (v/v) methanol. After electrotransfer, nitrocellulose was washed twice with "Tris-buffered saline" (150 mM NaCl, 10 mM Tris/Tris-HCl, pH = 7.4), then incubated at 37 °C for 1 h with Tris-buffered saline that contained 3% (w/v) bovine serum albumin. Upon cooling to room temperature, the nitrocellulose paper was covered with "first antibody solution" (Tris-buffered saline with 3% bovine serum albumin and rabbit anti-rat sarcoplasmic reticulum Ca^{2+}-ATPase antisera in 1:500 (v/v) dilution) for 16 h. The paper was then washed five times with "Tris-buffered saline" and incubated for 2 h at room temperature with goat anti-rabbit IgG horseradish peroxidase conjugate (1:1000 (v/v) dilution) in "Tris-buffered saline" with 3% bovine serum albumin. The paper was washed five more times with "Tris-buffered saline" and the immune complexes were detected using "Tris-buffered saline":methanol = 4:1 containing 2 mM 4-chloro-1-naphthanol and 1.8×10^{-4} % (w/v) H_2O_2.

After staining of gels with Coomassie Brilliant Blue or color development of nitrocellulose paper, inverse negatives were prepared and densitometrically scanned to quantitate peak position and height.

RESULTS

Electrophoresis and immunostaining of undigested platelet microsomes yielded a prominent band with an apparent molecular mass of 103 kDa, as demonstrated in *lane 1* of Fig. 1*A*. A similar band was observed when undigested sarcoplasmic reticulum microsomes were electrophoresed and stained with Coomassie Blue (see *lane 1* of Fig. 1*B*) or subjected to the same immunodetection procedure as the platelet material (data not shown). The 103-kDa bands in platelet and sarcoplasmic reticulum samples is the Ca^{2+}-ATPase (6, 17). A faint platelet 50-kDa band was also noted upon immunostaining

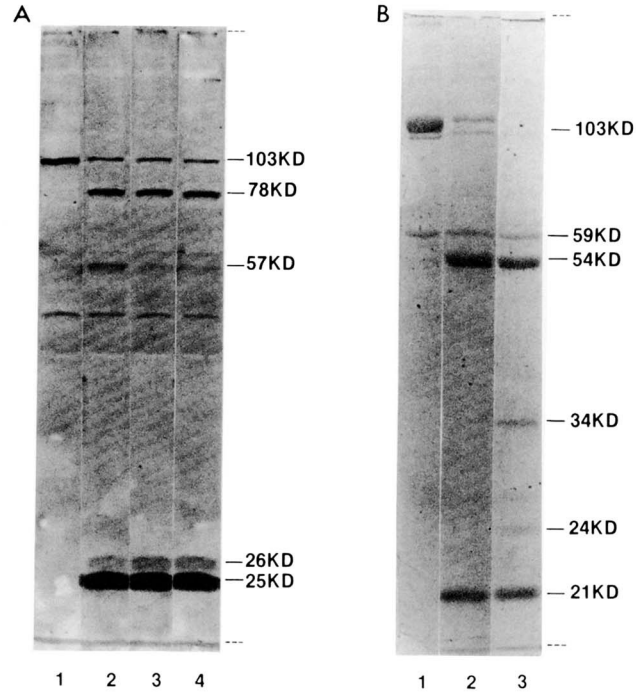

FIG. 1. *A*, digestion of platelet microsomes with trypsin for 0 min (*lane 1*), 10 min (*lane 2*), 30 min (*lane 3*), and 60 min (*lane 4*) at a microsomal protein/trypsin ratio (w/w) of 32:1. Digestion reactions were carried out in medium containing 94 mM KCl, 5 mM $MgCl_2$, 50 μM $CaCl_2$, 20 mM Tris/Tris-HCl, pH = 7.0, 1.83 mg/ml microsomal protein, at 23 °C, then terminated with the addition of soybean trypsin inhibitor. Proteins (46 μg of microsomal protein/lane) were then electrophoresed on 10% polyacrylamide gels, transferred to nitrocellulose, and immunodetected as described under "Materials and Methods." *B*, digestion of sarcoplasmic reticulum microsomes with trypsin for 0 s (*lane 1*), 20 s (*lane 2*) and 40 min (*lane 3*) at a microsomal protein/trypsin ratio (w/w) of 100:1. Digestion reaction conditions were the same as in *A*, with a microsomal protein concentration of 0.15 mg/ml. Proteins (3 μg/lane) were electrophoresed on 10% polyacrylamide gels, then stained with Coomassie Brilliant Blue.

[1] The abbreviations used are: EGTA, ethylene glycol bis(β-aminoethyl ether)-*N,N,N',N'*-tetraacetic acid; SDS, sodium dodecyl sulfate.

(*lane 1*, of Fig. 1*A*) and represented a protein in the platelet microsomes that weakly cross-reacted with the antibody. This band was present in undigested samples and did not degrade in the presence of trypsin. The broad 45-kDa platelet band (*lanes 1–4* of Fig. 1*A*) represented nonspecific antibody binding to actin.

In the subsequent experiments, the platelet Ca^{2+}-ATPase and its proteolytic products were detected with the immunodetection procedure, while the sarcoplasmic reticulum calcium pump protein and related proteolytic fragments were detected with Coomassie Brilliant Blue stain of gels. To assess the applicability of the immunodetection procedure for detecting proteolytic fragments, sarcoplasmic reticulum samples digested with each protease utilized in this study were subjected to the immunodetection procedure. All Coomassie Brilliant Blue-stainable bands correlated with immunodetected bands with each protease utilized in the following experiments (data not shown). The sarcoplasmic reticulum Ca^{2+}-ATPase and its proteolytic fragments were thus routinely detected with Coomassie Blue staining to conserve antibody.

Effect of Trypsin on the Calcium Pump Proteins in Intact Membranes—The results of a trypsin digestion of native platelet microsomes is presented in Fig. 1*A*. There was the progressive cleavage of the intact platelet Ca^{2+}-ATPase, with an apparent molecular mass of 103 kDa, with the appearance of 78-kDa and 25-kDa fragments. The small digestion fragments were immunodetected with a much higher efficiency than the larger fragments. This effect is probably due to differences in immunogenicity and/or electrotransfer efficiency. The rapid appearance of a minor 57-kDa band (*lanes 2–4* of Fig. 1*A*) was also noted. This protein appeared during the initial few seconds of the digestion, then was progressively degraded. The degradation of the 57-kDa band correlated with the appearance of a 26-kDa product. The appearance of the 57-kDa band did not correlate with a loss of intensity of the 103-kDa band.

As demonstrated in Fig. 1*B*, tryptic digestion of sarcoplasmic reticulum material, with the same conditions used in the digestion of platelet material, resulted in the hydrolysis of the calcium pump protein (103-kDa band) into 55-kDa (A + B) products. With prolonged digestion, the 55-kDa A fragment (18, 19) was further degraded into 34-kDa (A_1) and 24-kDa (A_2) products. The sarcoplasmic reticulum 59-kDa and 21-kDa bands were, respectively, calsequestrin (20) and the soybean trypsin inhibitor that was used to quench the digestion.

The effect of trypsin hydrolysis on calcium transport and Ca^{2+}-ATPase activities of the platelet microsomes is presented in Fig. 2, where the time course of activity loss in the presence of trypsin is presented. The temporal dependence of Ca^{2+}-uptake and Ca^{2+}-ATPase activity loss was biphasic; a rapid initial loss in activity being followed by a slower, more linear decrease. The loss of calcium transport activity was closely correlated with the loss of the intact 103-kDa protein. This correlation is demonstrated in Fig. 3, in which loss of the 103-kDa band is plotted against loss of Ca^{2+}-uptake activity. In contrast to the close correlation demonstrated in Fig. 3, Ca^{2+}-ATPase activity did not correlate well with loss of the 103-kDa protein, as demonstrated in Fig. 4. The best correlation was obtained when a proteolytic product was assumed to have a non-zero, but reduced (45%), activity:

$$V_{total} = V_{103} \times f_{103} + V_p \times f_p = 100\% \times f_{103} + 45\% \times f_p \quad (1)$$

where V_{total}, V_{103}, V_p, f_{103}, and f_p, respectively, refer to the total activity, the relative activity of the undigested Ca^{2+}-ATPase, the relative activity of the fragment which has the residual Ca^{2+}-ATPase activity, the mole fraction of the undigested Ca^{2+}-ATPase, and the mole fraction of the product with activity. f_p was calculated from the loss of the 103-kDa fragment ($f_p = 1 - f_{103}$) and assumed to be either the 78-kDa or 25-kDa fragment (which are presumably formed with a 1:1 stoichiometry).

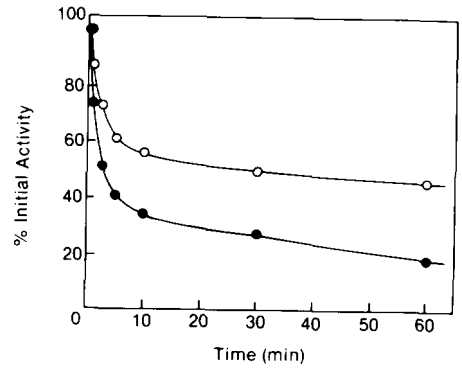

FIG. 2. **The loss of Ca^{2+}-ATPase activity (○) and Ca^{2+}-uptake activity (●) as a function of trypsin digestion time.** Digestion conditions were the same as in Fig. 1*A*. Activities were expressed as a percentage of the activity of the undigested sample.

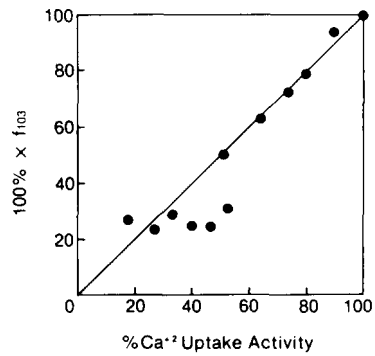

FIG. 3. **The correlation between the per cent of undigested platelet Ca^{2+}-ATPase (100% × f_{103}) and calcium uptake activity loss due to the action of trypsin.** The percentage of undigested Ca^{2+}-ATPase was quantitated by densitometrically scanning inverse negatives of color-developed nitrocellulose paper. Data are from the results of two independent experiments.

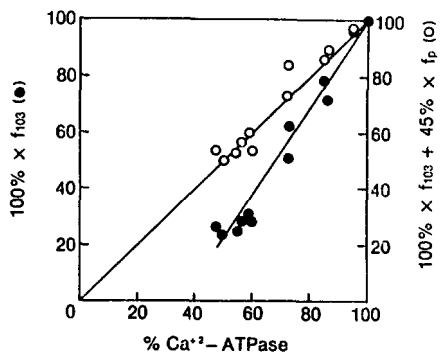

FIG. 4. **The correlation between platelet Ca^{2+}-ATPase activity loss and (*a*) the per cent of undigested pump protein (●, *left verticle axis*) and (*b*) the percentage of undigested pump protein corrected for an activity contribution from a proteolytic fragment (○, *right verticle axis*).** The best correlation being obtained when the activity contribution of a fragment with reduced (45%) Ca^{2+}-ATPase activity was added to the activity of the undigested protein. The percentage of undigested Ca^{2+}-ATPase was quantitated as in Fig. 3. Data are from the results of two independent experiments.

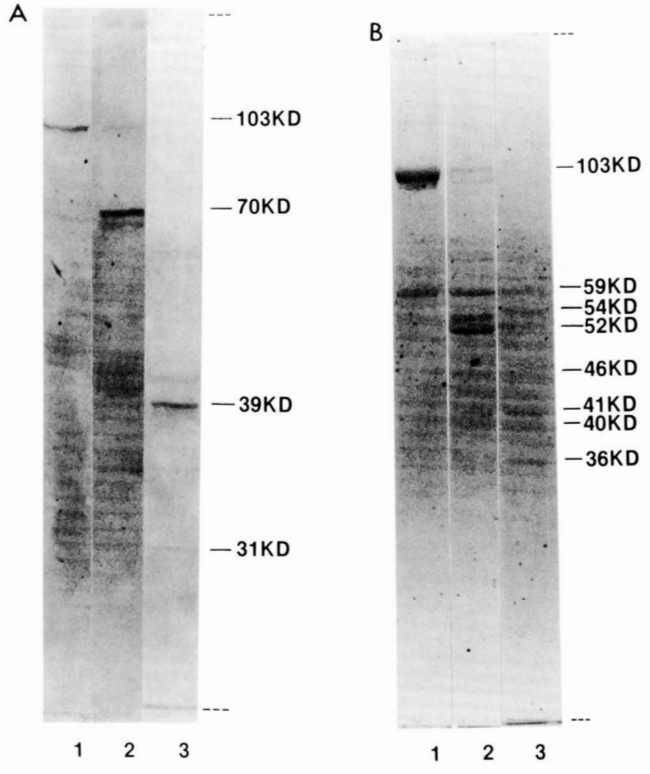

FIG. 5. *A*, the digestion of platelet microsomes with chymotrypsin for 0 min (*lane 1*) and 20 min (*lane 2*) with a microsomal protein/chymotrypsin ratio (w/w) of 100:1 and 60 min (*lane 3*) with a protein/chymotrypsin ratio (w/w) of 10:1. Digestions were carried out at room temperature in medium that contained 125 mM Tris/Tris-HCl, pH = 6.8, 10% (w/w) glycerol, 0.5% (w/v) sodium lauryl sulfate, and 1.6 mg/ml microsomal protein. Digestion reactions were terminated by elevating the temperature to 100 °C for 3 min, then electrophoresed, and subjected to immunodetection as in Fig. 1*A*. *B*, the digestion of sarcoplasmic reticulum microsomes with chymotrypsin for 0 s (*lane 1*), 20 s (*lane 2*), and 15 min (*lane 3*) with a microsomal protein/chymotrypsin ratio (w/w) of 20:1. The microsomal protein concentration was 0.15 mg/ml. Other conditions were as in *A*. Samples were electrophoresed and stained with Coomassie Brilliant Blue as in Fig. 1*B*.

Effect of Calcium Pump Conformation on Tryptic Cleavage—Experiments were also performed to analyze the effect of protein conformation and membrane sidedness on the trypsin digestion pattern. The effect of conformation was investigated by conducting digestion experiments in the presence and absence of 2 mM ATP, 550 mM sucrose, 1 mM EGTA, or 7.5 mM EDTA. Digestion with these four medium conditions did not change the proteolytic pattern depicted in Fig. 1*A*. Also, thermal denaturation, by elevating microsomes to 70 °C for 5 min, which eliminated Ca^{2+}-ATPase activity, did not change the fragmentation pattern. The platelet calcium pump molecules are oriented in the membrane so that the site of ATP hydrolysis is exposed to the external medium, resulting in ATP-dependent calcium transport into the microsome interior. This was ascertained by noting that solubilization of the microsomes with a variety of detergents (which effectively removes the sidedness of the membrane) did not expose additional hydrolytic sites. If the microsomes contained a substantial fraction of calcium pump proteins with the hydrolytic site on the inside of the membrane, and thus occluded from ATP, fragmentation of microsomes with detergents would expose the sites, giving an increase in Ca^{2+}-ATPase activity that would be proportional to the fraction of exposed hydrolytic site. The finding that detergent exposure did not increase Ca^{2+}-ATPase activity shows that the calcium pump protein containing microsomes retain the same orientation as in the dense tubular system. The retention of a native orientation after microsome isolation has been observed in other systems (21). When platelet microsomes were digested with trypsin in the presence of 2 mM deoxycholate, a detergent concentration which is high enough to fragment the membrane but not reduce Ca^{2+}-ATPase activity, no changes in the digestion pattern were observed.

Cleavage of Sodium Lauryl Sulfate-solubilized Calcium Pump Proteins—The platelet and sarcoplasmic reticulum Ca^{2+}-ATPase proteins were also compared by performing Cleveland digestions with chymotrypsin and *S. aureus* protease. In contrast to the experiments performed with trypsin, in which digestion was carried out with native state proteins, Cleveland digestions were performed with thermally denatured, SDS-solubilized samples. Cleveland digestions of platelet and sarcoplasmic reticulum pump proteins with chymotrypsin are shown in Fig. 5, *A* and *B*. Digestion of platelet material with chymotrypsin first resulted in a 70-kDa product. With more extensive proteolysis, 39-kDa and 31-kDa fragments appeared. In contrast, digestion of the sarcoplasmic reticulum Ca^{2+}-ATPase with chymotrypsin yielded 54- and 52.5-kDa fragments, which were further degraded into numerous fragments between 46 kDa and 36 kDa. The Cleveland digestion of platelet and sarcoplasmic reticulum Ca^{2+}-ATPase with *S. aureus* protease are presented in Fig. 6, *A* and *B*. *S. aureus* protease digestion of platelet Ca^{2+}-ATPase yielded a

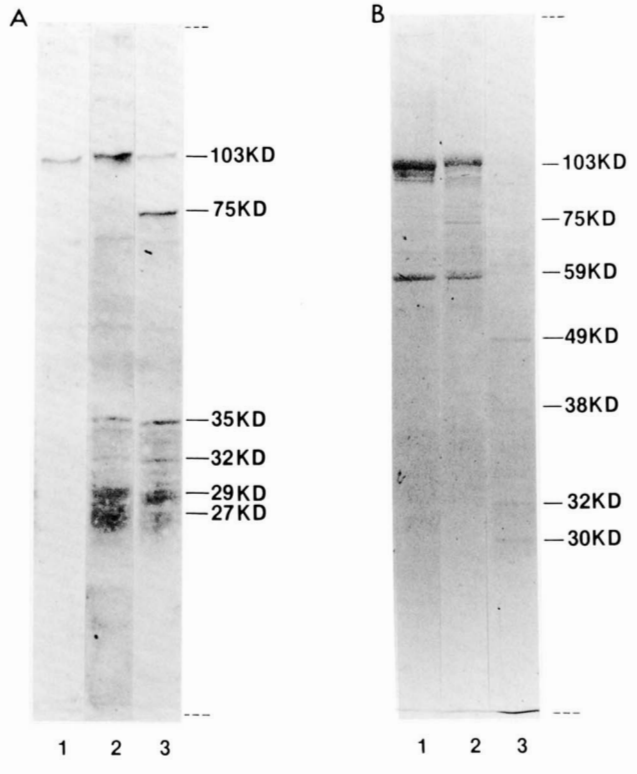

FIG. 6. *A*, the digestion of platelet microsomes with *S. aureus* protease for 0 s (*lane 1*), 20 s (*lane 2*), and 20 min (*lane 3*) with a microsomal protein/*S. aureus* protease ratio (w/w) of 100:1. Other conditions were as in Fig. 5*A*. Samples were electrophoresed and subjected to immunodetection as in Fig. 1*A*. *B*, the digestion of sarcoplasmic reticulum microsomes with *S. aureus* protease for 0 min (*lane 1*), 5 min (*lane 2*), and 60 min (*lane 3*) with a microsomal protein/*S. aureus* protease ratio (w/w) of 10:1. Other conditions were as in Fig. 5*B*. Samples were electrophoresed and stained with Coomassie Brilliant Blue as in Fig. 1*B*.

virtual continuum of fragments with apparent molecular mass values ranging from 25 kDa to 36 kDa. Prominent bands at 35 kDa, 32 kDa, 29 kDa, and 27 kDa were noted. A 75-kDa fragment appeared later in the time course of *S. aureus* protease digestion. Digestion of the sarcoplasmic reticulum Ca^{2+}-ATPase resulted in the appearance of a 75-kDa fragment, followed by the formation of 49-kDa, 38-kDa, 32-kDa, and 30-kDa proteolysis fragments.

DISCUSSION

The results of this study demonstrate that, while the sarcoplasmic reticulum and platelet calcium pump proteins catalyze a similar reaction (5, 6), immunologically cross-react (17), and have approximately equivalent molecular weights (6, 17), the platelet protein is not simply a copy of the sarcoplasmic reticulum protein. Three principle differences between the sarcoplasmic reticulum and platelet calcium pump proteins were demonstrated by this study. First, contrasts between trypsin digestion patterns of native state platelet and sarcoplasmic reticulum calcium pump proteins demonstrate a structural difference in the disposition of tryptically reactive Lys and/or Arg residues (22) on the cytoplasmic face of the proteins. Tryptic digestion of the platelet pump protein yields 78-kDa and 26-kDa fragments, while a similar digestion of the sarcoplasmic reticulum protein gives unresolved 55-kDa fragments. The sarcoplasmic reticulum 55-kDa fragments correspond to the 55-kDa and 45-kDa fragments (A and B fragments) obtained with the Weber and Osborn (23) electrophoretic systems. The consistency of the platelet Ca^{2+}-ATPase tryptic digestion pattern in the presence and absence of molecules which modulate the conformation of the protein (6) shows that the tryptically reactive peptide residues are on a part of the protein that is not occluded by conformational changes. Also, this consistency demonstrates that conformational changes do not expose additional hydrolysis sites. The minor 57-kDa platelet band probably arose from hydrolysis of a protein other than the calcium pump, resulting in the exposure of a cross-reactive peptide sequence, since its appearance does not correlate with a loss of the 103-kDa band. The hypothesis that the 57-kDa band results from cleavage of a fraction of the Ca^{2+}-ATPase molecules that exist in an alternate conformation, is denatured, or is inverted with respect with the sidedness of the membrane, is unlikely given the consistency of the trypsin digestion pattern under a variety of conditions.

A second point of contrast is the correlation between Ca^{2+}-ATPase activity loss and fragment appearance. In the platelet system, cleavage of the parent protein into 78-kDa and 25-kDa fragments resulted in a reduction of Ca^{2+}-ATPase activity, with the retention of a reduced ATPase activity by one of the fragments. In contrast, the sarcoplasmic reticulum A and B fragments retained full or stimulated Ca^{2+}-ATPase activities, depending on the medium composition during hydrolysis (20). The sarcoplasmic reticulum Ca^{2+}-ATPase activity was reduced only upon degradation of the A fragment. A single tryptic cleavage of the platelet and sarcoplasmic reticulum Ca^{2+}-ATPase results in a reduction of calcium transport capability. The platelet 78-kDa and 25-kDa fragments have no calcium transport activity, while it is not known if the sarcoplasmic reticulum A and B Ca^{2+}-ATPase fragments have a zero or a non-zero, but reduced, transport capability. The tryptically reactive platelet Ca^{2+}-ATPase residue that yields 78-kDa and 25-kDa fragments is evidently more important in maintaining partial functionality of the protein than the tryptically reactive residue on the sarcoplasmic reticulum calcium pump protein that yields A and B fragments.

Proteolysis of denatured calcium pump proteins in the presence of SDS revealed a third point of contrast between the platelet and sarcoplasmic reticulum Ca^{2+}-ATPase. Cleveland digestions with chymotrypsin demonstrates that there are differences in the positions of bulky hydrophobic amino acid residues such as Phe, Trp, and Tyr (24). Cleveland experiments with *S. aureus* protease revealed similar differences in the positioning of Asp and Glu residues (25). The production of 75-kDa fragments with both platelet and sarcoplasmic reticulum calcium pump proteins can be coincidental or represent a structural similarity. The different time course of appearance of the 75-kDa fragments suggest that this similarity is coincidental.

A potential difficulty with the use of the immunodetection system for following the proteolysis of the platelet Ca^{2+}-ATPase is that certain fragments are not recognized immunologically. This would result in a failure to detect additional fragments that are different from the sarcoplasmic reticulum Ca^{2+}-ATPase proteolytic products, since a one to one correspondence is obtained with the sarcoplasmic reticulum pump protein between Coomassie Brilliant Blue and immunodetected fragments with all proteolytic enzymes used in this study.

The structural differences pointed out by this study correlate with functional differences in the two pump proteins; the platelet Ca^{2+}-ATPase being modulated by cAMP-dependent protein kinase activity (8), while the activity of the sarcoplasmic reticulum pump protein is regulated principally by substrate and product concentrations (6). The structural and functional differences demonstrated by this study and the functional contrasts with respect to response to protein kinase activity potentially correlate with differences in the function of the source cells. The skeletal muscle sarcoplasmic reticulum calcium pump protein, which is present in the sarcoplasmic reticulum membrane at a high concentration (6), is responsible for causing fast, repetitive, cellular relaxation, through a rapid sequestering of intracellular calcium. In contrast, the platelet Ca^{2+}-ATPase, which is a trace protein, is responsible for maintaining the platelet in a resting state in the absence of stimulation. Platelet response to stimulation by a variety of chemical agents is essentially irreversible; the Ca^{2+}-ATPase activity being too small to clear the cytoplasm of calcium after full activation.

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