

# Common Structural Domains in the Sarcoplasmic Reticulum Ca-ATPase and the Transverse Tubule Mg-ATPase

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**Abstract.** Transverse tubule (TT) membranes isolated from chicken skeletal muscle possess a very active magnesium-stimulated ATPase (Mg-ATPase) activity. The Mg-ATPase has been tentatively identified as a 102-kD concanavalin A (Con A)-binding glycoprotein comprising 80% of the integral membrane protein (Okamoto, V. R., 1985, *Arch. Biochem. Biophys.*, 237:43-54). To firmly identify the Mg-ATPase as the 102-kD TT component and to characterize the structural relationship between this protein and the closely related sarcoplasmic reticulum (SR) Ca-ATPase, polyclonal antibodies were raised against the purified SR Ca-ATPase and the TT 102-kD glycoprotein, and the immunological relationship between the two ATPases was studied by means of Western immunoblots and enzyme-linked immunosorbent assays (ELISA). Anti-chicken and anti-rabbit SR Ca-ATPase antibodies were not able to distinguish between the TT 102-kD glycoprotein and the SR Ca-ATPase. The SR Ca-ATPase and the putative 102-kD TT Mg-ATPase also possess common structural elements, as indicated by

amino acid compositional and peptide mapping analyses. The two 102-kD proteins exhibit similar amino acid compositions, especially with regard to the population of charged amino acid residues. Furthermore, one-dimensional peptide maps of the two proteins, and immunoblots thereof, show striking similarities indicating that the two proteins share many common epitopes and peptide domains. Polyclonal antibodies raised against the purified TT 102-kD glycoprotein were localized by indirect immunofluorescence exclusively in the TT-rich I bands of the muscle cell. The antibodies substantially inhibit the Mg-ATPase activity of isolated TT vesicles, and Con A pretreatment could prevent antibody inhibition of TT Mg-ATPase activity. Further, the binding of antibodies to intact TT vesicles could be reduced by prior treatment with Con A. We conclude that the TT 102-kD glycoprotein is the TT Mg-ATPase and that a high degree of structural homology exists between this protein and the SR Ca-ATPase.

THE transverse tubular (TT)<sup>1</sup> membranes of striated muscle cells are invaginations of the cell surface that make periodic contacts with elements of the sarcoplasmic reticulum (SR) membranes. Because of this unique structural arrangement, TT membranes are thought to participate in several important processes that take place during muscle contraction, including conducting the action potential from the sarcolemma (SL) to the interior of the cell and triggering calcium release from the SR (28).

Transverse tubule vesicles have been isolated from rabbit (14, 19, 36), rat (2), and chicken (26, 34, 37, 43) skeletal muscle. Identification of isolated chicken skeletal TT vesicles was carried out by using stereological methods that com-

pared the freeze-fracture protein particle distributions of the isolated TT vesicles (680 particles/ $\mu\text{m}^2$ ) with the distribution of particles in the intact T-tubules (690/ $\mu\text{m}^2$ ), the SR (6020/ $\mu\text{m}^2$ ), and the SL (1630/ $\mu\text{m}^2$ ) membranes (41). To fully separate SR and TT vesicles, it was necessary to pass heterogeneous microsomal suspensions containing both SR and TT vesicles through two consecutive multistep sucrose gradients and an oxalate-loading step (37). The TT membranes were separated from the SR vesicles with the expectation that SR, and not TT, membranes were capable of forming intravesicular calcium-oxalate deposits subsequent to calcium uptake. Through this functional density shift procedure, >99% of the contaminating SR vesicles were removed from the TT fractions (37). Highly purified TT vesicles of chicken skeletal muscle prepared in this way were characterized by their unique freeze-fracture morphology (41), lipid content (43), and distribution of enzymatic activities (37).

While earlier reports indicated that TT membranes possessed significant calcium-stimulated ATPase (Ca-ATPase)

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1. *Abbreviations used in this paper:* SL, sarcolemma; SR, sarcoplasmic reticulum; TT, transverse tubule.

activity with catalytic properties similar to those of the SR Ca-ATPase (6, 19, 26, 36), more recent studies indicate that highly purified TT fractions that are substantially devoid of SR contamination (based on stereological and other criteria) do not contain any ATPase activity that is stimulated by calcium in the micromolar range (14, 16, 34, 37), suggesting that the TT preparations described prior to 1983 were likely to be contaminated by SR. Further, two-dimensional isoelectric focusing gels confirmed that no significant 102-kD Ca-ATPase protein (pI 6.6–6.2) was present in TT fractions correlating with the absence of high affinity Ca-ATPase activity (34).

The most prominent enzymatic activity associated with the highly purified TT vesicles is a magnesium-stimulated ATPase (Mg-ATPase) (EC 3.6.1.3), which displays enzymatic activities as high as 5–10  $\mu\text{mol}/\text{min}$  per mg in the vesiculated form (26, 34, 37) and 17  $\mu\text{mol}/\text{min}$  per mg in the partially purified form (34). Very low values of Mg-ATPase activity are found in highly purified SR membranes (12, 37) and are found at levels 10-fold lower in crude SL fractions (37). The TT Mg-ATPase has unique catalytic properties in that is insensitive to vanadate and FITC at levels that substantially inhibit Ca-ATPase and Na,K-ATPase activities (32). The Mg-ATPase also displays an unusual temperature dependence and pH optimum (37) and is significantly stimulated (five- to eightfold) by Con A at 37°C (31, 38).

In preparations of TT vesicles isolated from chicken skeletal muscle, a Con A- and wheat germ agglutinin-binding glycoprotein of  $\sim 102$  kD was tentatively identified as the Mg-ATPase (34). This was based on the concomitant enrichment of both the 102-kD protein and the Mg-ATPase activity after partial extraction of the TT vesicles with Triton X-100. While possessing the same relative molecular mass in one-dimensional gels as the well-characterized SR Ca-ATPase, the TT 102-kD glycoprotein displayed a more acidic isoelectric point when resolved on two-dimensional gels, most likely as a result of its carbohydrate content.

In this study, we have used immunological evidence to definitively identify the TT 102-kD glycoprotein as the Mg-ATPase. We have also used immunological analyses as well as amino acid compositional and peptide mapping analyses to probe the structure of the TT Mg-ATPase, and we have found that the two ATPases possess substantial structural elements in common.

## Materials and Methods

### Chemicals

Most chemicals were reagent grade and were obtained from Sigma Chemical Co. (St. Louis, MO), Merck A. G. (Darmstadt, Germany), or Carlo Erba (Milano, Italy). *p*-Nitrophenylphosphate, alkaline phosphatase, and avidin-alkaline phosphatase were purchased from Boehringer Mannheim GmbH (Mannheim, Germany). Con A-biotin was purchased from Polysciences, Inc. (Warrington, PA). Ampholines were obtained from LKB Instruments Inc. (Bromma, Sweden). Nitrocellulose paper was purchased from Bio-Rad Laboratories (Richmond, CA). The cationic dye Stains All, (1-ethyl-2-[3-(1-ethylnaphtho[1,2d]-thiazolin-2-ylidene)-2-methyl-propenyl]-naphtho-[1,2d] thiazolium bromide), Nitro Blue Tetrazolium, and 5-bromo-4-chloro-3-indolyl phosphate were obtained from Sigma Chemical Co.

### Preparative Procedures

Mixed microsomal fractions and purified TT were prepared from the breast muscle of 5–8-wk-old chickens, according to Sabbadini and Okamoto (37) and Okamoto et al. (34). Briefly, 150 g of breast muscle were homogenized in 3 vol of 10% sucrose, 10 mM morpholino propane sulfonic acid (MOPS),

pH 6.8, 0.1 mM EDTA, 0.1 mM phenylmethylsulfonyl fluoride (PMSF) for 20 s every 5 min for 1 h, keeping the pH constant at 7.0 by adding 10% NaOH. The homogenate was centrifuged at 15,000 *g* for 20 min. The filtered supernatant was centrifuged at 40,000 *g* for 90 min. The pellets were resuspended in 0.6 M KCl, 10 mM MOPS, pH 6.8, incubated for 40 min and then centrifuged at 15,000 *g* for 20 min. The final supernatant was centrifuged at 40,000 *g* for 90 min, and the pelleted mixed microsomes were resuspended in 30% sucrose, 10 mM MOPS, pH 6.8.

The SR and TT vesicles were separated by two tandem density shifts by incubation of the mixed microsomal fraction with 20 mM MOPS, pH 6.8, 2 mM  $\text{CaCl}_2$ , 2 mM EGTA, 5 mM K-oxalate, 80 mM KCl, 5 mM MgCl<sub>2</sub>, and 5 mM ATP for 5 min at 37°C. The mixture was layered on a 25% sucrose cushion containing 10 mM MOPS, pH 6.8, and then centrifuged in a rotor (SW 40; Beckman Instruments, Palo Alto, CA) at 150,000 *g* for 60 min. Oxalate-loaded SR appeared in the pellet, and the TT vesicles appeared at the buffer–25% sucrose interface. The TT vesicles were washed in 25 mM imidazole, pH 7.0, and pelleted by centrifugation at 150,000 *g* for 1 h. The final TT pellets and the oxalate-loaded SR pellets were resuspended in 20 mM MOPS, pH 7.0. Protein concentration was determined by the method of Lowry et al. (22) with bovine serum albumin as a standard.

### ATPase Assay

ATPase activity was determined with a coupled-enzyme spectrophotometric ADP-release assay (48) by measuring the oxidation of NADH at 340 nm in a medium containing 25 mM imidazole, 0.3 mg/ml NADH, 5 mM MgCl<sub>2</sub>, 0.2 mM EGTA, 450  $\mu\text{M}$  phosphoenolpyruvate (PEP), 1 mM ATP, and 3–5  $\mu\text{g}$  of protein, as previously described (37). Basal magnesium-stimulated ATPase was measured at 25°C and pH 7.3. Extra Ca-ATPase was measured at 37°C and pH 7.0, after adding 0.2 mM  $\text{CaCl}_2$  and 4  $\mu\text{M}$  A23187.

### Gel Electrophoresis and Peptide Mapping

One-dimensional 10% SDS PAGE was carried out according to Laemmli (18). Two-dimensional gel electrophoresis was carried out according to O'Farrell (33) with isoelectric focusing in the first dimension in the presence of 1% Ampholine (LKB Instruments Inc.) (0.8%, pH 5–7; 0.2%, pH 3–10). The pH range of the first dimension tube gel was usually 4.5–7.0. The second dimension slab gel (0.5-mm thick) was a standard Laemmli 10% SDS PAGE.

One-dimensional peptide maps of the SR Ca-ATPase and the TT 102-kD protein (isolated from preparative 10% polyacrylamide slab gels) were performed according to Cleveland et al. (8) after partial digestion with *Staphylococcus aureus* V8 protease or with chymotrypsin. The two 102-kD proteins were isolated by dissection from 10% preparative slab gels, and the protease-treated fragments were resolved on 15–20% polyacrylamide linear gradient slab gels.

Gels were stained with Coomassie Blue or with the silver nitrate method of Merrill et al. (30). The staining with the carbocyanine dye Stains All was carried out as described by Campbell et al. (7). Densitometric scans of the slab gels were carried out under conditions identical to those reported by Volpe et al. (46). Apparent relative molecular masses were calculated from a graph of relative mobilities versus log relative molecular mass of standard proteins (bovine serum albumin, 68 kD; pyruvate kinase, 57 kD; lactate dehydrogenase, 36 kD; carbonic anhydrase, 29 kD).

### Amino Acid Analysis

Amino acid compositions of the purified SR Ca-ATPase and TT Mg-ATPase proteins were determined after hydrolyzing the proteins in 6 N HCl for 24 h in sealed evacuated tubes at 110° followed by analysis in an amino acid analyzer (Carlo Erba).

### Preparation of Antisera and Purification of Antibodies

Antiserum to the chicken SR Ca-ATPase was raised in adult rabbits by giving four weekly intramuscular injections of cholate-purified Ca-ATPase (1 mg per injection) emulsified with an equal volume of Freund's complete adjuvant. The cholate treatment (46) was performed on calcium-oxalate-purified SR vesicles prepared as previously described (37). After 45 d, a booster injection was given, and the animal was bled. Preimmune serum was obtained from the animal before the start of immunization.

Antiserum to chicken TT 102-kD glycoprotein was raised after the same immunization schedule but using 100  $\mu\text{g}$  per injection. The 102-kD TT glycoprotein was purified by electrophoretic elution. Specifically, preparative

10% SDS PAGE slabs of TT membrane proteins were incubated in 0.6 M KCl to visualize the 102-kD protein bands, which were then dissected from the gels and soaked in 40 mM Tris-acetate, pH 8.3, 1 mM EDTA, and 0.1% SDS for 30 min. The slices were then placed in the concentration well of an electrophoretic sample concentrator (Isco, Inc., Lincoln, NE). After adding the buffer to the tank (40 mM Tris-acetate, pH 8.3, 1 mM EDTA, 0.1% SDS) and the sample cup (4 mM Tris-acetate, pH 8.3, 1 mM EDTA, 0.1% SDS), the electrophoretic elution was carried out overnight at constant power (1 W). The purity of the eluted protein was evaluated as described in Results. IgG fractions were obtained from both antisera as described previously (3). The batches of antibodies used in the present study were from single bleedings.

### Immunological Techniques

One-step noncompetitive enzyme-linked immunosorbent assays (ELISAs) were carried out as described previously (3). Intact vesiculated membranes were dispersed in 0.1 M Na<sub>2</sub>CO<sub>3</sub>, pH 9.6, and then 0.2-ml aliquots were adsorbed to microtiter wells by incubating for 1 h at 37°C. After washing with 0.9% NaCl and 0.05% Tween-20, 0.2-ml aliquots of antibodies (dissolved in PBS at various concentrations) were added and incubated for 1 h at 37°C. After washing away excess, unbound antibody, anti-IgG conjugated to alkaline phosphatase (1:1000 dilution in PBS) was then added and incubated for 1 h at 37°C. The alkaline phosphatase activity associated with the antigen immobilized on the microtiter wells was measured after incubating the wells with *p*-nitrophenylphosphate (1.65 mg/ml) in 50 mM Na<sub>2</sub>CO<sub>3</sub>, pH 9.8, 1 mM MgCl<sub>2</sub> for 30 min at 37°C and subsequently measuring the absorbance at 400 nm after the reaction had been stopped with 50 µl of 2 N NaOH. A two-step competitive ELISA was carried out as described by Damiani et al. (9). Briefly, anti-chicken SR Ca-ATPase antibodies were diluted to 10 µg/ml in PBS buffer and were then incubated overnight with an equal volume of membranes diluted to various protein concentrations with PBS. Aliquots (0.2 ml) of the mixture were then transferred to microtiter wells previously precoated with 5 µg/ml of purified SR membranes. All subsequent steps were as described for the one-step noncompetitive ELISA. Western immunoblots of slab gels were carried out under the general conditions of Towbin et al. (45), as described in detail by Biral et al. (5).

### Indirect Immunofluorescence

Thick (1 mm) strips of breast muscle were dissected from freshly killed chickens and then fixed by immersion in PBS with 3% formaldehyde for 1 h at room temperature. The strips were tied to wooden sticks during fixation in order to maintain the myofibrils in an uncontracted state. The fixative was removed from the tissue by three 30-min rinses of PBS with 0.01% glycine, and then the tissue was stored at 4°C. Before ultracyotomy, the tissue was trimmed into 0.5-mm cubes. After infusing the muscle cubes with 2.3 M sucrose for 30 min, the cubes were placed on aluminum stubs, frozen in liquid nitrogen, and mounted on a Sorvall MT2B microtome with a Sorvall FS 1000 cryoattachment (RMC Corp., Tucson, AZ). 1–2-µm frozen sections were cut at –60°C, picked up with drops of 2.3-M sucrose, and placed on glass slides. The sections were rinsed in PBS to remove the sucrose and then labeled for simultaneous localization of the TT Mg-ATPase and myosin.

As a control for the position of the A band, myosin was labeled with a mouse anti-chicken monoclonal antibody (MF20), kindly given by Dr. Donald Fischman of Cornell University, New York. 100 µl of the undiluted (5 µg/ml) hybridoma culture medium were applied to the sections, and then the excess antibody was washed off with two 5-min rinses of PBS. 80–100 µl of fluorescein-conjugated goat anti-mouse IgG (15–20 µg/ml) were applied for 10 min and then rinsed as before.

The 102-kD TT Mg-ATPase was labeled with the rabbit anti-chicken IgG described above. The rabbit anti-chicken Mg-ATPase antibody was applied to sections at concentrations ranging from 50 to 200 µg/ml, and the excess antibody was washed off with PBS. The following successive labels were then applied for 10 min each: goat anti-rabbit IgG, biotin-conjugated swine anti-goat IgG, and then avidin-Texas red (all immunolabeling reagents were obtained from Boehringer Mannheim Biochemicals, Indianapolis, IN). The biotin conjugate was used at 0.1 µg/ml, and the others were used at 15–20 µg/ml. Volumes of 80–100 µl were needed to cover the sections. Each labeling step was followed by two 5-min rinses in PBS. After all labeling reagents had been applied, the sections were then rinsed in distilled water, mounted in 90% glycerol, and stored at 4°C. The slides were viewed on a Zeiss Photomicroscope III equipped for epifluorescence and photographed on Kodak Tri-X Pan 400 ASA film. Controls using rabbit IgG preimmune serum were performed and showed no labeling in the I or A bands of the myofibrils.

### Con A Binding to SR and TT Proteins

We evaluated the binding of Con A to both native TT vesicles and to solubilized TT proteins resolved on Laemmli gels. SR and TT proteins were resolved by SDS PAGE and then transferred to nitrocellulose sheets by electroblotting. After washing with 0.9% NaCl, 0.1% Tween 20 for 60 min at 37°C, the sheets were incubated for 5 h at 37°C with Con A-biotin (1 µg/ml) diluted in PBS. After washing again four times for 5 min, the sheets were incubated for 90 min at 37°C with avidin conjugated to alkaline phosphatase and diluted in PBS (1:3,000). The sheets were then incubated at room temperature in the dark with 0.1 M Tris-HCl, pH 9.5, 0.1 M NaCl, 5 mM MgCl<sub>2</sub> containing Nitro Blue Tetrazolium (0.33 mg/ml) and 5-bromo-4-chloro-3-indolyl phosphate (0.17 mg/ml) according to the methods of Leary et al. (21). Color development was terminated after 60 min by washing the sheets with distilled water.

Con A binding to native TT vesicles was carried out as follows: Con A-biotin was diluted in PBS and was incubated for 60 min at 37°C in microtiter wells previously coated with 5 µg/ml TT vesicles. Unbound Con A-biotin was removed by washing three times with 0.9% NaCl and 0.05% Tween 20. The immobilized vesicles were incubated for 60 min at 37°C with avidin conjugated to alkaline phosphatase (1:3,000 diluted in PBS) and then washed as above. The alkaline phosphatase activity associated with the TT vesicles immobilized on the microtiter wells was determined as described above for one-step noncompetitive ELISA.

### Results

#### Evaluation of Membrane Purity

Several criteria were used to assess the purity of the SR and TT membrane proteins used for subsequent immunological and comparative structural studies. Fig. 1 shows the SDS PAGE profiles of chicken SR and TT proteins. Similar to the rabbit SR (28, 39, 44) the Ca-ATPase was the predominant chicken SR protein (Fig. 1, lane 1) averaging  $77.3 \pm 7.7\%$  ( $n = 4$ ) of total protein, based on densitometric scans of Coomassie Blue-stained gels. These preparations displayed a very low basal Mg-ATPase activity ( $0.41 \pm 0.06$  µmol/min per mg,  $n = 5$ ) compared with the extra, Ca-ATPase ac-

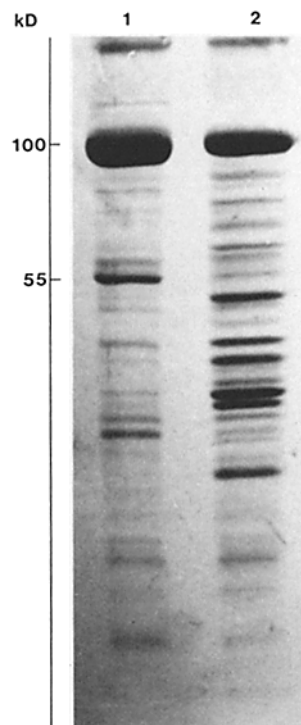


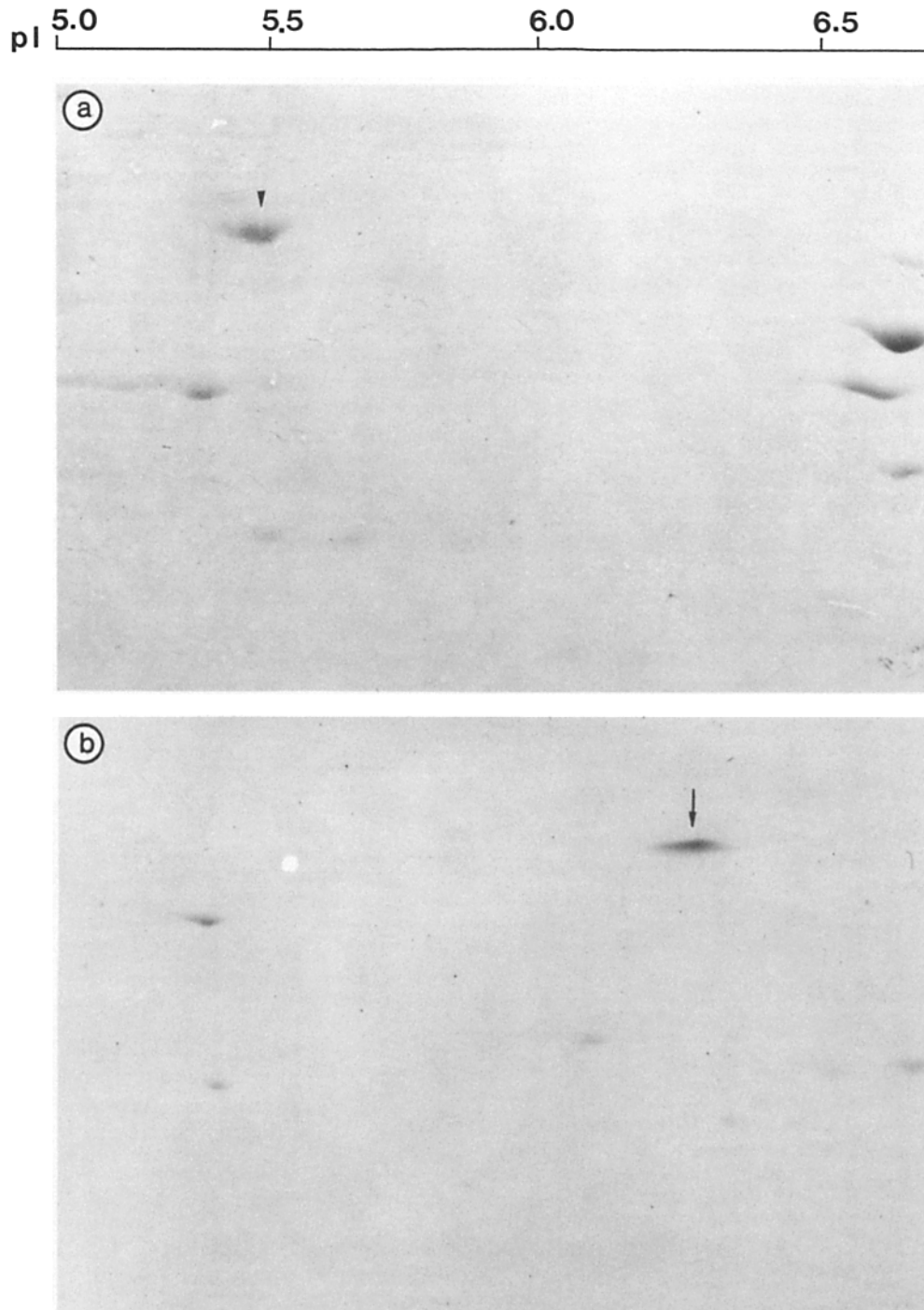
Figure 1. One-dimensional 10% SDS PAGE of SR and TT membranes was carried out as described by Laemmli (18). The slabs were stained with Coomassie Blue. Approximately 20 µg of protein were loaded on each lane. Lane 1, chicken calcium-oxalate-loaded SR; lane 2, chicken TT.

tivity of  $3.53 \pm 0.39 \mu\text{mol}/\text{min}$  per mg ( $n = 4$ ) measured in the presence of the calcium ionophore A23187 at  $37^\circ\text{C}$ .

Chicken TT vesicles possess a more complex protein composition with a prominent protein at  $\sim M_r 102,000$  (Fig. 1, lane 2), a protein which averaged  $\sim 23.1 \pm 3.9\%$  of the total protein ( $n = 4$ ). The Mg-ATPase activity found in TT fraction was  $2.43 \pm 0.26 \mu\text{mol}/\text{min}$  per mg ( $n = 8$ ) under optimal conditions of pH 7.3 and  $25^\circ\text{C}$  (37). No Ca-ATPase activity was observed in any of the TT fractions used in this study; in fact, a characteristically slight inhibition of the Mg-ATPase activity was consistently observed after calcium and A23187 addition (data not shown). The 102-kD TT peptide

has been tentatively identified as a glycoprotein responsible for the Mg-ATPase activity (34).

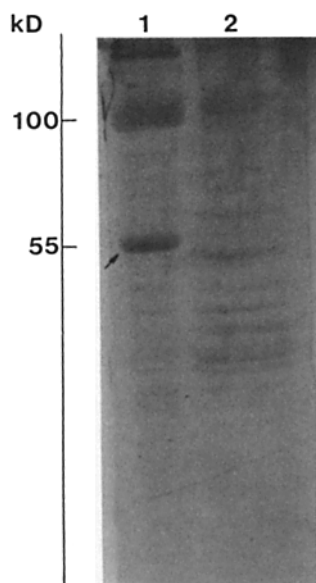
In a previous study, Okamoto et al. (34) demonstrated that even though they possess similar relative molecular masses in Laemmli gels, the SR Ca-ATPase and the TT 102-kD putative Mg-ATPase proteins could easily be differentiated by their distinctly different isoelectric points. To be certain that no detectable Ca-ATPase protein was present in our native TT preparation, we analyzed the content of 102-kD protein in two-dimensional gels overloaded with TT protein (Fig. 2). As previously shown (34), solubilized TT vesicles (Fig. 2 *a*) had only one protein in the 102-kD range possessing a char-



**Figure 2.** Two-dimensional gel electrophoresis of TT (*a*) and SR (*b*) membrane protein according to the method of O'Farrell (33). The first dimension isoelectrofocusing gels were performed in the presence of 1% Ampholines in the pH range 4.5–7.0. The slabs were stained with Coomassie Blue. About 50  $\mu\text{g}$  of TT and 15  $\mu\text{g}$  of SR protein were loaded per gel. Arrow and arrowhead indicate the SR Ca-ATPase (pI 6.3), and the TT Mg-ATPase (pI 5.3), respectively.

acteristic pI of 5.3 (see arrow). On the other hand, the SR Ca-ATPase exhibited a more alkaline pI of 6.3, and there was no evidence of a contaminating protein with the same relative molecular mass in the pI range characteristic of the TT 102-kD protein (Fig. 2 *b*). In control experiments using Coomassie Blue stained SDS PAGE slabs of SR protein where the 102-kD Ca-ATPase represented 80% of the total membrane protein, 400 ng of 102-kD protein could clearly be detected in the electrophoretic conditions used for the two-dimensional gels. In gels such as Fig. 2 *a* that were overloaded with >50  $\mu$ g of protein, we would have been capable of detecting at least a 3.4% SR Ca-ATPase contamination in TT preparations in which the 102-kD component represents 23% of total protein (see above).

To further evaluate the extent of SR contamination in our TT fractions, we investigated the possible presence of another characteristic SR protein, calsequestrin, in gels of electrophoretically resolved TT proteins. Stains All has been extensively used in the identification of calsequestrin in skeletal and cardiac muscle SR (see reference 24 for a recent review). Stains All treatment of slab gels demonstrated that chicken skeletal muscle SR has a metachromatically stained glycoprotein of 55 kD (see arrow, Fig. 3, lane 1) that we have identified as calsequestrin (10). The Stains All pattern of TT proteins resolved by slab gel electrophoresis was routinely examined, and it was found that none of the TT proteins in the 55-kD range exhibited metachromatic staining properties characteristic of calsequestrin (Fig. 3, lane 2). In control experiments using purified calsequestrin, as little as 100 ng calsequestrin was clearly identifiable by metachromatic staining. Considering that the calsequestrin content of SR is ~10% (data not shown), then we could have been able to detect the presence of 3.3% SR contamination in a gel loaded with 30  $\mu$ g of protein. Further, anti-calsequestrin antibodies did not react to any 55-kD component in Western immunoblots of electrophoretically resolved TT vesicle proteins (data not shown), and Con A did not bind to any species in the 55-kD region (see below). From the absence of Ca-ATPase activity, the absence of SR Ca-ATPase and calsequestrin proteins in two-dimensional gels and Stains All-



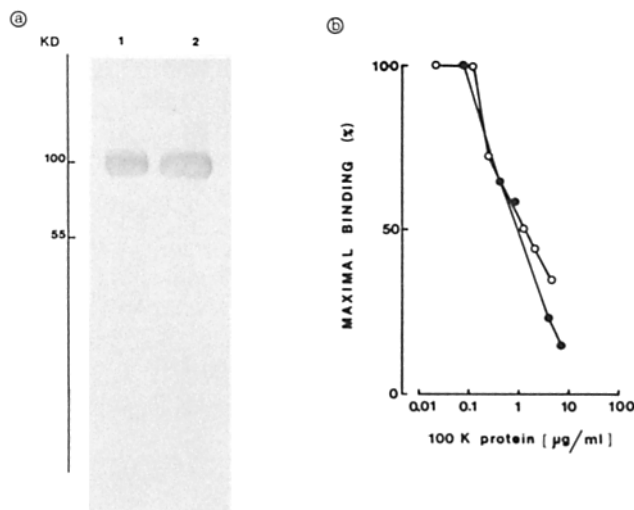
**Figure 3.** Stains All staining of SR and TT proteins resolved in a 12% Laemmli gel as described by Campbell et al. (7). Approximately 30  $\mu$ g of SR (lane 1) and TT (lane 2) protein were loaded on each lane. The SR, but not the TT, membrane shows the presence of the 55-kD calsequestrin that characteristically stains metachromatically with the dye (arrow).

treated Laemmli gels, and from the lack of calsequestrin in immunoblots of TT membrane protein, we conclude that the level of contamination of SR proteins was below the limit of detection of the several techniques used.

#### **Immunological Relationship between the SR Ca-ATPase and the Putative TT Mg-ATPase**

We developed monospecific polyclonal antibodies against the purified chicken SR Ca-ATPase and against the putative TT Mg-ATPase (see Materials and Methods) in order to test the cross-reactivity of the two 102-kD proteins against each antibody.

Western immunoblots were performed on SR and TT proteins resolved by SDS PAGE and subjected to indirect immunoenzymic staining with anti-chicken SR Ca-ATPase antibody (Fig. 4 *a*) using alkaline-phosphatase conjugated to rabbit anti-chicken IgG. As expected, these antibodies reacted specifically with the Ca-ATPase band of SR Laemmli gels (lane 2). Importantly, the TT 102-kD protein cross-reacted with the anti-Ca-ATPase antibody (lane 1). The binding of anti-chicken SR Ca-ATPase antibody to the SR Ca-ATPase did not inhibit the Ca-ATPase activity, as reported previously by others (11, 44). The extent of antibody



**Figure 4.** Immunological cross-reactivity of SR and TT proteins with anti-chicken SR Ca-ATPase antibody. (a) Western immunoblot of TT (lane 1) and SR (lane 2) proteins resolved by 10% slab gel electrophoresis was carried out under the general conditions of Towbin et al. (45) as modified by Biral et al. (5). Membrane proteins were resolved in a 10% Laemmli gel and then transferred to a nitrocellulose sheet. The sheet was incubated with anti-chicken SR Ca-ATPase antibody (5  $\mu$ g/ml) and then with alkaline phosphatase conjugated to rabbit anti-chicken IgG for immunoenzymic staining. Approximately 2.5  $\mu$ g of SR and 12  $\mu$ g of TT protein were loaded on each lane. (b) Two-step competitive ELISA was carried out as described by Damiani et al. (9). In the first step, a solution of anti-chicken SR Ca-ATPase antibody (20  $\mu$ g/ml) was preincubated overnight with an equal volume of either SR or TT vesicles at the concentrations of 102-kD protein indicated on the abscissa. The second step was performed by incubating aliquots (0.2 ml) of the antigen-antibody solutions in microtiter wells coated with intact chicken SR vesicles (5  $\mu$ g/ml). The 102-kD protein content of SR and TT was estimated by densitometry of Coomassie Blue-stained gels. Each point is the average value of duplicate determination. (Solid circle) SR Ca-ATPase; (open circle) TT 102-kD protein.

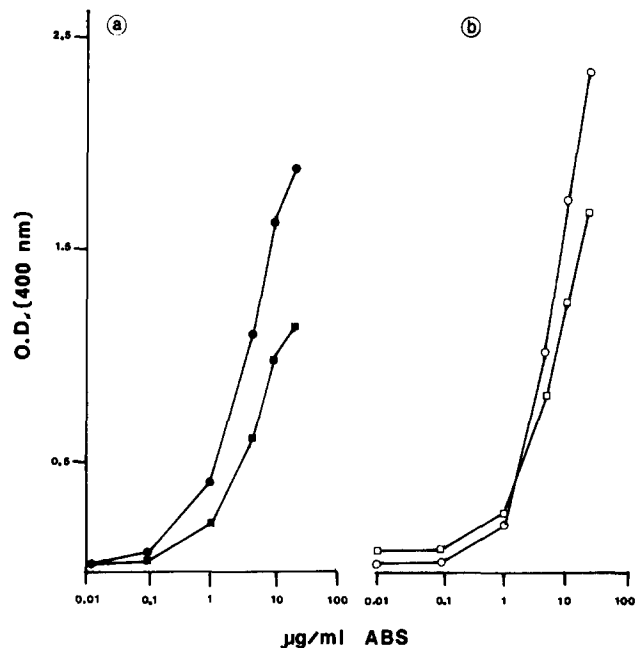
cross-reactivity between the SR Ca-ATPase and the putative 102-kD TT Mg-ATPase was further evaluated by the two-step competitive ELISA (4, 9, 39, 47) in which we quantified the binding of anti-chicken SR Ca-ATPase antibodies to microtiter wells precoated with intact SR vesicles after preincubation with TT vesicles (Fig. 4 *b*, see Materials and Methods for details). The amount of 102-kD TT protein required for 50% inhibition of antibody binding to the adsorbed SR vesicles was virtually identical to the amount of SR 102-kD Ca-ATPase protein required for the same level of inhibition. These results indicated that, quantitatively, the anti Ca-ATPase antibody could not distinguish between the two 102-kD proteins. In fact, in order for the above results to be interpreted on the basis of contaminating SR Ca-ATPase, the level of contamination would have to be far in excess of the 3% estimated as the highest possible level of contamination.

We also tested our chicken SR and TT membranes for cross-reactivity to antibodies developed against the SR Ca-ATPase of rabbit fast-twitch muscle, whose specificity has been previously characterized (3, 4, 9, 39, 40, 47). As shown by the Western immunoblot in Fig. 5, the anti-rabbit SR Ca-ATPase antibody reacted with the chicken SR 102-kD Ca-ATPase (lane 2) and with the TT 102-kD protein (lane 4). In agreement with the finding that both the SDS-denatured SR Ca-ATPase and the TT Mg-ATPase were cross-reactive in Western immunoblots, intact TT vesicles isolated from rabbit (Fig. 6 *a*) as well as chicken (Fig. 6 *b*) skeletal muscle exhibited very high levels of cross-reactivity with anti-rabbit SR Ca-ATPase antibody in the one-step ELISA.

Polyclonal antibodies were also raised against the electroeluted putative TT Mg-ATPase purified from TT preparations that were devoid of SR contamination (i.e., no Ca-ATPase activity and lack of evidence of SR proteins in one- or two-dimensional slab gel electrophoresis, see above). Two-dimensional slab gels of the eluted Mg-ATPase displayed only one protein in the 102-kD region (data not



**Figure 5.** Immunoblot of SR and TT proteins using anti-rabbit SR Ca-ATPase antibody. The immunoenzymic staining was carried out as in Fig. 4 *a*, with anti-rabbit SR Ca-ATPase antibody (10  $\mu\text{g}/\text{ml}$ ). Approximately 20  $\mu\text{g}$  of protein were loaded on each lane. Lane 1, Coomassie Blue-stained chicken SR; lane 2, immunoblot of SR; lane 3, Coomassie Blue-stained chicken TT; lane 4, immunoblot of TT.

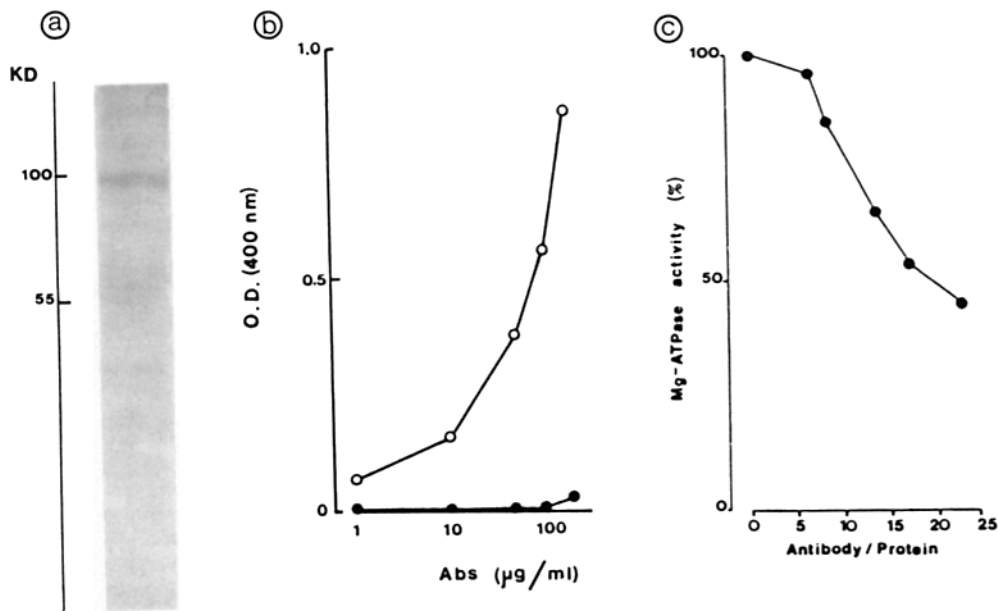


**Figure 6.** Immunological cross-reactivity of rabbit and chicken SR and TT vesicles with anti-rabbit SR Ca-ATPase antibody. One-step ELISAs of rabbit (*a*) and chicken (*b*) SR (solid circle, open circle) and TT (solid square, open square) vesicles were carried out in microtiter wells coated with SR or TT vesicles at a protein concentration of 5  $\mu\text{g}/\text{ml}$  (SR) and of 10  $\mu\text{g}/\text{ml}$  (TT), respectively. The binding of anti-rabbit SR Ca-ATPase antibody (ABS) to microtiter wells precoated with either SR or TT vesicles was used at the concentrations indicated on the abscissa.

shown), confirming the results shown in Fig. 2, which indicated that only the more acidic TT Mg-ATPase was present in the TT fraction. Western immunoblots were performed on electrophoretically resolved TT proteins stained with anti-chicken TT 102-kD protein antibody. This antibody reacted only to the TT 102-kD protein (Fig. 7 *a*). The specificity of the anti-chicken TT 102-kD protein antibody was further demonstrated by the ELISA shown in Fig. 7 *b*. These data show that the antibody was capable of binding to intact TT vesicles and, importantly, that the antibody could not cross-react with vesiculated SR membranes.

This finding was supported by indirect immunofluorescence micrographs of cryostat-sectioned muscle, which showed that anti-chicken TT 102-kD protein antibodies could be localized only in the I bands (where the T tubules are found) and not in the SR-rich A band region (Fig. 8). Further, anti-Mg-ATPase antibody staining above background levels was not observed on the SL.

The specificity of the antibody was further demonstrated by the data shown in Fig. 7 *c*, which indicated that the Mg-ATPase activity of vesiculated TT membranes could be significantly (>55%) inhibited by anti-chicken TT 102-kD protein antibodies. Although inhibition of ATPase activity beyond 55% was seen when higher antibody concentrations were used, higher ratios of antibody to protein (>25  $\mu\text{g}$  antibody/ $\mu\text{g}$  microsomal protein) were not routinely used due to concerns that paradoxical postzonal effects might occur in this range. The TT Mg-ATPase activity was not inhibited by control rabbit IgG or by the PBS used in the antibody solu-



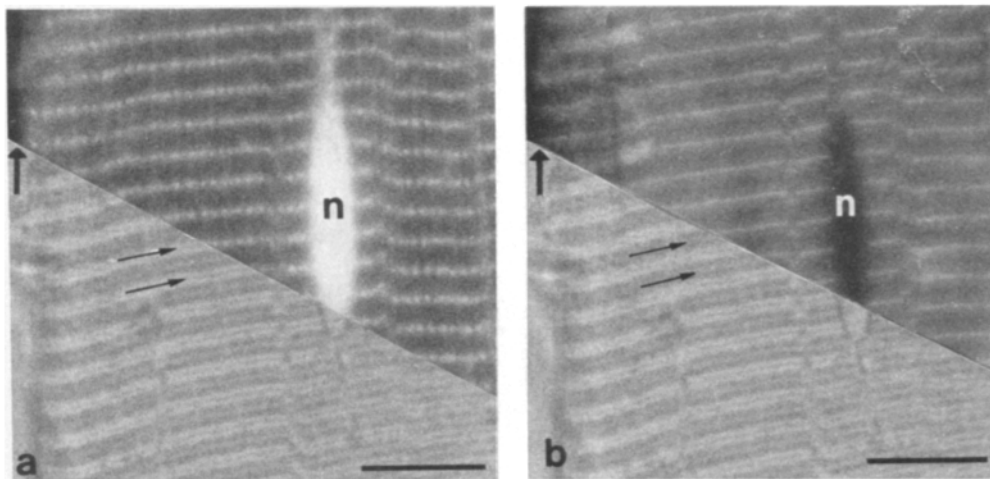
**Figure 7.** Characterization of anti-chicken TT 102-kD protein antibody. (a) Immunoblot of TT protein using anti-chicken TT 102-kD protein antibody (20 µg/ml) as described in Fig. 4 a. Approximately 30 µg of TT protein were used. (b) One-step ELISA of SR (solid circle) and TT (open circle) vesicles was carried out in microtiter wells coated with antigen at a protein concentration of 5 µg/ml, using anti-chicken TT 102-kD protein antibody at the concentrations indicated on the abscissa. (c) Inhibition of the TT Mg-ATPase activity by anti-chicken TT 102-kD antibody. Approximately 8 µg of TT protein were preincubated with anti-chicken TT 102-kD protein antibodies for 10 min

at 37°C. The vesicles were then diluted 40-fold into the ATPase medium (1 ml), and the rate of ATP hydrolysis was measured at 25°C, pH 7.0. The values are expressed as percentages of the Mg-ATPase activities of control samples preincubated with preimmune IgG or PBS under the same conditions.

tion, indicating that the observed antibody inhibition of Mg-ATPase activity was specific.

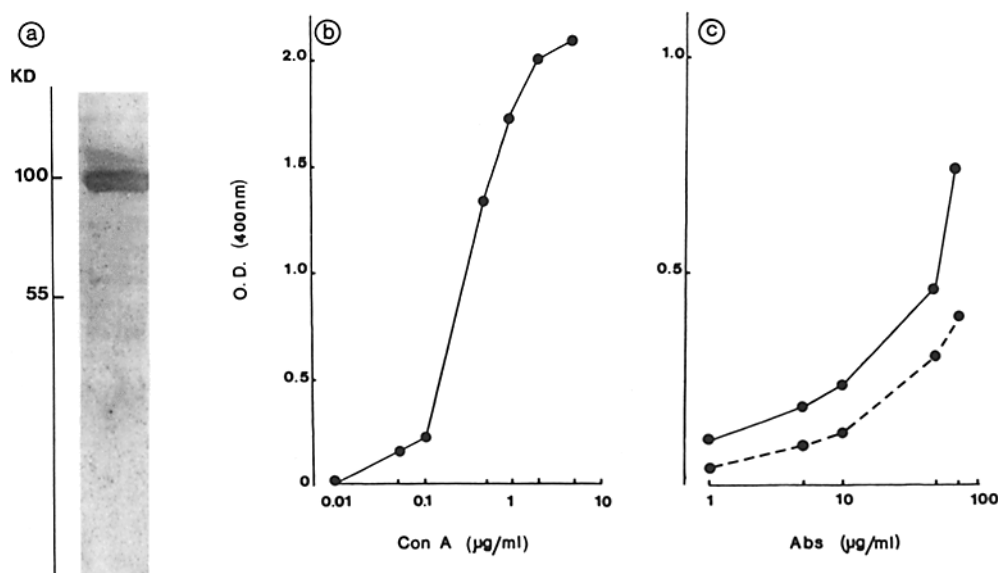
In a previous study (34), we demonstrated that the TT 102-kD protein (used here as the immunogen) was a glycoprotein representing 80% of the integral membrane protein of TT. This was based on the labeling of the 102-kD protein with  $^{125}\text{I}$ -wheat germ agglutinin and  $^{125}\text{I}$ -Con A in autoradiograms of SDS PAGE-resolved proteins. The data in Fig. 9 a confirmed that the 102-kD protein is the major Con A-binding glycoprotein of TT membranes. In addition, no

contaminating 55-kD calsequestrin (which also reacts with Con A, reference 10) was present in our TT fractions, supporting other evidence (see above) indicating that the native TT fractions were not contaminated by SR vesicles. This result raises the possibility that the binding of Con A to native TT vesicles previously absorbed to microtiter wells (Fig. 9 b) was due to Con A binding to glycosylated regions of the TT Mg-ATPase that were likely to be exposed on the surfaces of the TT vesicles. We have previously reported that Con A substantially stimulated the Mg-ATPase activity of chicken



**Figure 8.** Indirect immunofluorescence localization of anti-Mg-ATPase antibody. Composite micrographs of phase contrast and fluorescence images of frozen chicken breast muscle sections (1.0 µm). Micrographs of identical regions of a tissue section were cut in half, and adjacent halves of different micrographs were mounted in register with each half using the nucleus (n) and other structural features as landmarks. Top half of each composite is the fluorescence image, the bottom half is the phase image. (a) Texas red-labeled anti-TT Mg-ATPase IgG. Thin arrows denote Z lines of

the I bands. Note the punctate nature of the label and its registry with the I bands of the phase-contrast image. Thick arrows show the location of the cell surface and the absence of Mg-ATPase localization on the SL. The prominent fluorescence exhibited by the nucleus in a is only a consequence of cross-reactivity to the second antibody, since the nucleus was the only structural feature seen in controls where the first antibody was omitted. (b) Fluorescein-labeled antimyosin (monoclonal antibody MF20). Characteristics of the antimyosin antibody have been described in reference 1. Thin arrows denote the central region of the A bands that react with the antibody. Note that the A band labeling is shifted one-half sarcomere relative to the antigenic sites reactive with the anti-Mg-ATPase. Bar, 10 µm.



**Figure 9** Binding of Con A to TT. (a) TT proteins were resolved by electrophoresis and then transferred to a nitrocellulose sheet. The sheet was incubated with Con A biotin (1.0 µg/ml) followed by avidin-alkaline phosphatase to identify Con A-binding glycoproteins. Approximately 5 µg of protein was loaded on the gel. (b) Binding of biotinylated Con A to TT membranes was carried out in microtiter wells coated with TT vesicles at a protein concentration of 5 µg/ml. Con A biotin was used at the concentrations indicated on the abscissa, followed by staining with avidin-alkaline phosphatase at 1:2000 dilution. (c) Effect

of Con A on anti-chicken TT 102-kD protein antibody binding to TT membranes. Microtiter wells were coated with TT vesicles at a protein concentration of 5 µg/ml, and antibody binding was assessed in the range of concentrations indicated on the abscissa. The dotted line represents an experiment in which microtiter wells were incubated with Con A (10 µg/ml) for 30 min at 37°C followed by 1 mM  $\alpha$ -methylmannoside for 30 min at 37°C before introduction of anti-Mg-ATPase antibody. Controls were incubated in the same conditions with PBS instead of anti-Mg-ATPase antibody.

TT (31, 38). The data in Table I demonstrate that Con A could stimulate (twofold at 25°C) the Mg-ATPase activity of native TT vesicles. Therefore, it is possible that Con A activation of enzymatic activity was exerted through binding of the lectin to the exposed glycosylated regions of the TT Mg-ATPase.

Because it is known that the carbohydrate moieties of proteins can greatly influence their immunogenicity, some of the anti-chicken TT 102-kD protein antibodies were probably elicited by glycosylated epitopes. This possibility was supported in an experiment shown in Fig. 9, which demonstrated that the adding of Con A to microtiter wells preadsorbed with TT membranes resulted in a 50% reduction of maximal antibody binding to the TT (Fig. 9 c). In addition, Table I shows that the inhibitory effect of anti-chicken TT 102-kD protein antibody on the Mg-ATPase activity could be removed by preincubating TT vesicles with Con A, and, reciprocally, the ATPase stimulating effect of Con A could be prevented by preincubating the TT vesicles with anti-chicken TT 102-kD protein antibody.

#### ***Amino Acid Composition and One-dimensional Peptide Mapping of the SR Ca-ATPase and the Putative TT Mg-ATPase***

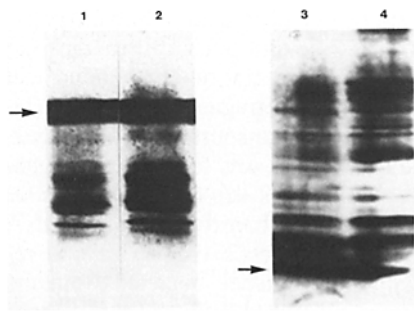
Peptide maps and subsequent immunoblots of the peptide fragments were used to probe common structural elements between the two ATPase proteins. Partial digestion of the SR Ca-ATPase and the TT Mg-ATPase were performed according to the procedures of Cleveland et al. (8) using the proteolytic enzymes *S. aureus* V8 and chymotrypsin. As Fig. 10 shows, the two proteins possessed peptide fragmentation patterns that were very similar, regardless of the protease used. Western immunoblots of the peptide maps stained with anti-chicken SR Ca-ATPase demonstrated that the antigenic determinants responsible for the immunological cross-reactivity of the two proteins were distributed among the same proteolytic fragments (data not shown). In other data not shown, limited (2–5 min) tryptic fragmentation patterns of the SR Ca-ATPase and the TT Mg-ATPase were also identical and reverse-phase HPLC of V8 fragmentation patterns

**Table I. Effects of Concanavalin A and Anti-Chicken TT 102-kD Protein Antibody on the Mg-ATPase Activity of Transverse Tubules**

Order of addition		Mg-ATPase activity <i>µmol/h per mg</i>	Percent of control
First	Second		
None		188	100
Con A		359	191
Anti-TT Mg-ATPase		83	44
Anti-TT Mg-ATPase	Con A	132	70
Con A	Anti-TT Mg-ATPase	372	198

4 µg of TT membranes were incubated for 10 min at 37°C with the listed additions. 50 µg of anti-chicken TT 102-kD antibody and 20 µg of Con A were used where indicated. The samples were 50-fold diluted in the Mg-ATPase activity medium, and the Mg-ATPase activity was measured as described in Materials and Methods.





**Figure 10.** One-dimensional peptide mapping was performed by the procedure of Cleveland et al. (8) by using the 102-kD protein bands excised from 10% polyacrylamide preparative slab gels. Digestions were carried out with 5  $\mu$ g of either *S. aureus* V8 protease (lanes 1 and 2), or chymotrypsin (lanes 3 and 4), and the protein fragments were resolved in a 15–20% polyacrylamide linear gradient gel and then subsequently stained with silver nitrate. Approximately 10  $\mu$ g of protein were loaded on each lane. Half of each slab was stained with silver nitrate. Lanes 1 and 3, SR Ca-ATPase; lanes 2 and 4, TT 102-kD Mg-ATPase protein. Arrows indicate the position of each protease.

showed that the two ATPases share many common peptide fragments.

Amino acid composition analysis was carried out on the SR Ca-ATPase and TT Mg-ATPase proteins electrophoretically purified from chicken skeletal muscle. As shown in Table II, the amino acid compositions of the two ATPases were quite similar, especially with regard to their contents of charged residues. Aspartate and glutamate accounted for 19.6 and 23.9% of the total residues for chicken Ca-ATPase and Mg-ATPase, respectively, while the contents of basic amino acids were 12.2 and 14.7%. The Ca-ATPase contained 33.8% total charged amino acids while the Mg-ATPase contained 38.6%. These values were comparable to the values reported by others (23, 27, 29) for purified rabbit SR Ca-ATPase, but

**Table II. Amino Acid Composition**

	Chicken skeletal muscle		Rabbit skeletal muscle
	Mg-ATPase*	Ca-ATPase*	Ca-ATPase‡
Lys	8.7	7.3	5.8
His	1.7	2.5	1.5
Arg	4.3	4.4	4.8
Asp	11.2	9.8	9.7
Ser	6.3	3.6	5.5
Thr	4.8	6.1	5.5
Glu	12.7	9.8	12.2
Pro	4.8	4.9	5.1
Gly	11.7	14.7	8.5
Ala	9.7	9.5	9.8
Half Cys	nd§	1.3	1.4
Val	7.6	10.6	7.6
Met	1.0	1.1	1.4
Ile	5.3	5.4	5.6
Leu	8.7	8.2	9.8
Tyr	nd§	2.2	2.2
Phe	5.3	5.2	3.9
Total charged	38.6	33.8	34.0

\* Values are percent total amino acids and are averages of two experiments (see Materials and Methods for details).

‡ Reference 27.

§ nd, not detected.

were quite different from the 45.4% value of total charged residues reported previously (10) for the peripheral SR membrane protein, calsequestrin.

## Discussion

The aim of this study was to identify the protein responsible for the TT Mg-ATPase activity of chicken skeletal muscle, and, further, to explore the structural relationship between the TT 102-kD protein and the closely related 102-kD SR Ca-ATPase. The results reported here definitively identify the 102-kD glycoprotein of TT membranes as the Mg-ATPase. The most significant evidence for this is the finding that polyclonal antibodies developed against the purified 102-kD TT glycoprotein not only substantially inhibit the Mg-ATPase activity of TT vesicles but also react with the 102-kD immunogen as seen in immunoblots. Further, the anti-chicken TT 102-kD protein antibodies are able to prevent the characteristic Con A stimulation of the TT Mg-ATPase activity. Con A selectively binds to the 102-kD protein of TT (34, and results reported here); and, conversely, Con A prevents the inhibitory effect of the anti-chicken TT 102-kD protein on the Mg-ATPase activity, apparently by preventing the binding of antibodies to the TT 102-kD protein.

We also provide evidence that the TT Mg-ATPase and the SR Ca-ATPase can be distinguished by their distinct pI properties and by their antigenic differences. The TT 102-kD glycoprotein has a more acidic isoelectric point than the SR Ca-ATPase when resolved on two-dimensional isoelectric focusing gels, probably as a result of the carbohydrate content of the TT protein. In addition, antibodies developed against the purified 102-kD TT glycoprotein do not recognize intact SR vesicles in the ELISA. These data are consistent with our indirect immunofluorescence data, which localize anti-chicken 102-kD protein antibodies exclusively in the I band regions of chicken skeletal muscle where the T tubules are located and not in the SR-rich A band regions. In addition, anti-chicken TT Mg-ATPase antibody does not inhibit the Ca-ATPase activity of SR vesicles (data not shown).

The levels of possible cross-contamination in the SR and TT fractions were assessed to ensure that the two 102-kD proteins used here as antigens are distinct entities. Conventional electrophoretic techniques set the level of SR Ca-ATPase contamination in the TT fraction at <3.4%. However, the lack of a 55-kD calsequestrin protein band reacting after Stains All treatment, the lack of Con A binding in that region, and the inability of anti-calsequestrin antibodies to identify chicken calsequestrin in Western immunoblots of resolved TT membrane proteins suggest an even lower value for SR contamination. The Western immunoblot method is capable of detecting picogram quantities of SR proteins, and the Stains All method is sensitive in the 100-ng range (significantly less than 1% of the total protein in a typical gel). These findings agree with freeze-fracture stereological data, which reveal less than 1% SR contamination in native TT fractions that are subjected to two multistep sucrose gradients and calcium-oxalate loading steps (37). Also, we are unable to detect the presence of Ca-ATPase activity in the TT fraction. All of the above data support the position that the limit of SR Ca-ATPase contamination in the 102-kD region of the TT Mg-ATPase could be ~1% at most.

Our data also rule out the possibility that the 102-kD TT glycoprotein is a calcium-independent form of the SR Ca-ATPase that has been artifactually inactivated or modified during membrane preparation. We have shown that the 102-kD Mg-ATPase is localized exclusively in the TT-rich I band region of the muscle cell and can be isolated in a membrane vesicle fraction that can be physically separated from SR, a fraction that has both distinctly different morphological characteristics than SR (e.g., low density of 9-nm protein particles, see reference 41) and a distinctly different lipid composition than SR (especially with regard to cholesterol levels that are threefold higher than in SL vesicles and fivefold higher than in SR, see reference 43). In order for the 102-kD TT protein to represent an artifactual calcium-independent form of the SR Ca-ATPase, one would most certainly not expect it to be glycosylated or to possess such unique catalytic properties as the prominent ability to be stimulated by Con A, the inability to be inhibited by FITC and vanadate, and a lack of *p*-nitrophenylphosphatase activity (32).

It is unlikely that the TT 102-kD glycoprotein band is substantially contaminated by the alpha subunit of the Na,K-ATPase, since anti-TT 102-kD antibodies do not cross-react with purified Na,K-ATPase in Western immunoblots; further, the Na,K-ATPase does not possess a prominent Con A-reactive glycoprotein in the 102-kD range. Of interest, the anti-chicken TT 102-kD antibodies do not cross-react in the ELISA with beef heart submitochondrial particles (data not shown), and the TT Mg-ATPase is not inhibited significantly by oligomycin and azide (37).

We conclude that the 102-kD glycoprotein band is at least 99% homogeneous and that the band does not contain appreciable contamination from the SR Ca-ATPase, the Na,K-ATPase subunits, or ATPases from other sources. Thus, the TT Mg-ATPase could be a new member of the class of the extramitochondrial cation-ATPases, including the SR Ca-ATPase, the plasma membrane Na,K-ATPase, and the K,H-ATPase of the gastric mucosa. These ATPases share structural properties such as the presence of a polypeptide of ~100 kD (17) and a high degree of structural homology (13, 25, 42). A possible relationship to ATPases in lysosomes, clathrin-coated vesicles, and secretory granules also exists.

We demonstrate that a high degree of structural homology exists between the 102-kD SR Ca-ATPase and the 102-kD TT Mg-ATPase. This conclusion is based on the following. (a) Polyclonal antibodies developed against the SR Ca-ATPases of chicken and rabbit cross-react with the 102-kD TT Mg-ATPase protein band in Western immunoblots. (b) The anti-SR Ca-ATPase antibodies cross-react against antigenic determinants exposed to the surfaces of the intact TT vesicles and cannot distinguish SR from TT membranes in the ELISA. (c) The two ATPases display very similar amino acid compositions and peptide fragmentation patterns, and they share many common immunoreactive peptide fragments on immunoblots. The inability of the anti-chicken TT Mg-ATPase antibody to recognize the SR Ca-ATPase in the membrane-bound form of the ELISA immunoassay is not surprising considering that the antibodies are likely to react with carbohydrate moieties of the Mg-ATPase protein that are not present in the SR Ca-ATPase.

Our finding that the two ATPases share many structural domains is not surprising in view of recent reports demon-

strating that a significant degree of structural homology exists between the SR Ca-ATPase and other cation-transport ATPases. For example, Hesse et al. (13) reported amino acid sequence homology between three fragments of the SR Ca-ATPase and the KdpB potassium transport protein of *Escherichia coli*. One of the homologies was found in the region of the phosphorylated aspartate residue of the Ca-ATPase. Further, the amino acid sequence constructed from cDNA fragments of rabbit SR Ca-ATPase (25) shows several regions of homology with the sequence obtained from the cloned Na,K-ATPase alpha subunit (42). In addition, monoclonal antibodies produced against the SR Ca-ATPase cross-react with common epitopes on several calcium-binding proteins and common epitopes on the 102-kD Ca-ATPases obtained from a wide variety of tissues besides muscle (49).

However, not all cation-transport ATPases and calcium-binding proteins share cross-reactive epitopes. For example, antibodies raised against a putative lysosomal proton pump protein cross-react to the K,H-ATPase of gastric mucosa, but not to the SR Ca-ATPase or the Na,K-ATPase (35). Also, our anti-rabbit SR Ca-ATPase antibody does not cross-react in the Western immunoblot with the Na,K-ATPase isolated from rabbit kidney (data not shown). DeFoor et al. (11) provided immunological evidence that cardiac and skeletal SR Ca-ATPases could be distinguished, and we have shown that the SR Ca-ATPase of fast-twitch and slow-twitch fibers is immunologically distinct in both rabbit (9, 39) and human skeletal muscle (40). Because, in the present study, we have demonstrated that antibodies raised against the SR Ca-ATPase purified from either rabbit or chicken skeletal muscle are both highly cross-reactive to the chicken TT Mg-ATPase and that the anti Ca-ATPase antibodies cannot distinguish between the chicken Ca- and Mg-ATPases in the ELISA, the SR Ca-ATPase may be more structurally related to the TT Mg-ATPase than it is to the Ca-ATPases of other species or of different fiber types within the same species.

The physiological role of the SR Ca-ATPase is well established in its support of ATP-energized calcium transport during muscle relaxation. The mechanism by which calcium is released from storage sites within the SR lumen has not as yet been elucidated, but it is presumed to be dependent upon the intimate association between the SR and the TT membrane system. Thus, structural and functional details of the TT membranes may be prerequisite to understanding excitation-contraction coupling mechanisms. Structural homologies between ATPases may indicate, for example, that the two proteins have similar transport functions, as was suggested for the proton pumping ATPases of gastric mucosa and lysosomes (35).

The most prominent enzymatic activity associated with isolated TT membranes is the Mg-ATPase, which can cleave ATP at a rate approaching 17  $\mu\text{mol}/\text{min}$  per mg in the partially purified form (34). It is not known what role, if any, the TT Mg-ATPase plays in excitation-contraction coupling; however, it is quite interesting not only that the Mg-ATPase is structurally related to the Ca-ATPase, but that the Mg-ATPase displays such significantly different catalytic properties compared with the Ca-ATPase. Although the TT Mg-ATPase is glycosylated, has a more acidic isoelectric point (34), and is embedded in a substantially different lipid environment compared with the SR Ca-ATPase (15, 20, 43), it is possible that the Mg-ATPase and the SR Ca-ATPase are

products of the same gene or gene family, and that, as a result of selective co- or posttranslational processing, the Mg-ATPase is routed into the T tubules rather than into the SR membrane during membrane assembly. Further, as a result of the glycosylation itself, or because of the different lipid environment in which it resides, the Mg-ATPase may exhibit completely different catalytic properties. However, in the absence of amino acid sequence information, our data are equally consistent with the possibility that the two ATPases are different gene products that merely enjoy a high degree of structural homology. Similarities in amino acid composition between the two ATPases should not be taken by itself as indicative of common structural elements. However, because the amino acid composition data is complemented by similarities in peptide maps and immunological cross-reactivity when anti-Ca-ATPase antibodies are used, it is likely that the two ATPases enjoy considerable structural homology.

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