Phosphorylation of a 22,000-Dalton Component of the Cardiac Sarcoplasmic Reticulum by Adenosine 3':5'-Monophosphate-dependent Protein Kinase*

(Received for publication, July 23, 1974)

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SUMMARY

Cardiac microsomes were incubated with [γ-32P]ATP and a cardiac adenine 3':5'-monophosphate (cyclic AMP)-dependent protein kinase in the presence of ethylene glycol bis(β-aminoethyl ether)-N, N'-tetraacetic acid. After solubilization in sodium dodecyl sulfate and fractionation by polyacrylamide gel electrophoresis, a single microsomal protein component of approximately 22,000 daltons was found to bind most of the 32P label. The 32P labeling of this component increased several fold when NaF was included in the incubation medium. No other component of cardiac microsomes, including sarcoplasmic reticulum ATPase protein, contained significant amounts of 32P label. This 22,000-dalton phosphoprotein formed by cyclic AMP-dependent protein kinase had stability characteristics of a phosphoester rather than an acyl phosphate. Washing of microsomes with buffered KCl did not decrease the amount of 32P labeling to the 22,000-dalton protein, suggesting that this protein is associated with the membranes of sarcoplasmic reticulum rather than being a contaminant from other soluble proteins. The 22,000-dalton protein was susceptible to trypsin. Brief digestion with trypsin in the presence of 1 M sucrose did not significantly affect microsomal calcium transport activity, but prevented both subsequent phosphorylation of the 22,000-dalton protein and stimulation of calcium uptake by cyclic AMP-dependent protein kinase, suggesting that this protein is a modulator of the calcium pump.

These results are consistent with previous findings (KIRCHBERGER, M. A., TADA, M., AND KATZ, A. M. (1974) J. Biol. Chem. 249, 6166–6173; TADA, M., KIRCHBERGER, M. A., REPKE, D. L., AND KATZ, A. M. (1974) J. Biol. Chem. 249, 6174–6180) that cyclic AMP-dependent protein kinase-catalyzed phosphorylation is associated with stimulation of calcium transport in the cardiac sarcoplasmic reticulum, and further indicate that this phosphorylation occurs at a component of low mass (22,000 daltons) of the cardiac sarcoplasmic reticulum which, while separable from the calcium transport ATPase protein (100,000 daltons) by sodium dodecyl sulfate-polyacrylamide gel electrophoresis, has the ability to regulate calcium transport by the cardiac sarcoplasmic reticulum.
mately 22,000 daltons, which can be separated from ATPase protein by sodium dodecyl sulfate-polyacrylamide gel electrophoresis. This 22,000-dalton protein appears to have the ability to modulate calcium transport by the cardiac sarcoplasmic reticulum.

**Experimental Procedure**

**Materials**

Cardiac microsomes were prepared from dog heart ventricle according to the procedures of Harigaya and Schwartz (6) with minor modifications (7). Cyclic AMP-dependent protein kinase was purified through the DEAE-cellulose chromatography step from bovine hearts, according to the method of Miyamoto et al. (14). New England Nuclear Co. Disodium ATP, sodium cyclic AMP, and hydroxylamine-HCl (grade I) were obtained from Sigma Chemical Co. Disodium ATP was freed of metal ions by cation exchange chromatography on Dowex 50 and neutralized with Tris HCl and 0.1 M histidine buffer (pH 6.8), respectively, immediately after electrophoresis. Each slice was counted in 5 ml of toluene-base scintillation fluid (4 g of 2,5-diphenyloxazole and 0.2 g of 1,4-bis{2-(4-methyl-5-phenyloxazolyl)}benzene in 1 liter of toluene) by liquid scintillation spectrometry. Digestion of sliced gels with H2O2 did not increase significantly the efficiency of counting. Mobility of proteins bands and radioactive peaks were determined as retardation factor, Rs, relative to the position of the tracking dye. For determination of molecular weight, albumin (bovine serum), ovalbumin, chymotrypsinogen A (bovine pancreas), myoglobin (spiral whale), and cytochrome c (horse heart) were used as markers.

**Methods**

**Phosphorylation of Microsomes by Cyclic AMP-dependent Protein Kinase**

Microsomal protein was phosphorylated at 25° for 10 min in one of the following reaction mixtures in a total volume of 0.2 ml unless otherwise stated.

**Reaction Mixture A**—Mixture A consisted of 40 mM histidine buffer (pH 6.8), 0.12 M KCl, 5 mM MgCl2, 5 mM [Y-32P]ATP (10 μCi per μmol), and 0.5 mM cyclic AMP and various concentrations of protein kinase. The equations used to calculate Ca2+ concentration were those of Kornberg (10). Albumin (bovine serum), ovalbumin, chymotrypsinogen A (bovine pancreas), myoglobin (spiral whale), and cytochrome c (horse heart) were obtained from Schwarz-Mann as part of a molecular weight marker kit and dissolved in 10 mM sodium phosphate buffer, pH 7.2, prior to use. Trypsin (Code TRL, from bovine pancreas), twice crystallized, and trypsin inhibitor (Code SI, from soybean) were obtained from Worthington Biochemical Corp. They were dissolved in 0.001 N HCl and 10 mM histidine buffer (pH 6.8), respectively, immediately prior to use.

**Methods**

**Stability of Phosphoprotein Formed by Protein Kinase**

Microsomes were phosphorylated in Reaction Mixture A, described above, and the reaction was terminated with 2 ml of 10% trichloroacetic acid containing 0.1 mM KH2PO4. The samples were centrifuged at 1500 X g for 5 min, and the pellet was treated under several conditions as described below, after which it was solubilized and subjected to sodium dodecyl sulfate-polyacrylamide gel electrophoresis. Microsomes incubated in Reaction Mixture A were subjected to assay for ATPase and calcium uptake as described below.

**Determination of Phosphoprotein by Sodium Dodecyl Sulfate-Polyacrylamide Gel Electrophoresis**

Reactions A and B were terminated and microsomal protein was solubilized by the procedures described by Pucel1 and Martonosi (17) modified as described below.

a. **Termination with Sodium Dodecyl Sulfate (Procedure 1)**—To the reaction mixture (0.2 ml) was added 0.1 ml of a solution containing 0.1 M sodium dodecyl sulfate, EDTA, and β-mercaptoethanol, to give final concentrations of 2%, 0.1 mM, and 1%, respectively. After standing several minutes on ice, this mixture was incubated for approximately 10 min at 37° to solubilize the microsomal protein and a 0.3-ml aliquot was added to the 0.2 ml of a solution containing 20 mM sodium phosphate buffer (pH 7.2), 0.1 mM EDTA, 1% β-mercaptoethanol, 50% glycerol, and 0.005% bromphenol blue. An aliquot (90 to 100 μl) of this solution, containing 40 to 150 μg of microsomal protein was applied to the gel for electrophoresis.

b. **Termination with Trichloroacetic Acid (Procedure II)**—The reaction was terminated by the addition of 2 ml of ice-cold 10% trichloroacetic acid containing 0.1 mM KH2PO4. After centrifugation at 1500 X g for 5 min at 4°, the precipitated protein was suspended in 2 ml of cold distilled water and centrifuged again. To the precipitate were added 50 ml of a solution containing 2% sodium dodecyl sulfate, 0.1 mM EDTA, 1% β-mercaptoethanol, and 20 mM sodium phosphate buffer (pH 7.2). This mixture was incubated at 37° for approximately 10 min to solubilize the microsomal protein, after which 50 μl of a solution which contained 20 mM sodium phosphate buffer (pH 7.2), 0.1 mM EDTA, 1% β-mercaptoethanol, 50% glycerol, and 0.005% bromphenol blue were added. This mixture, to which was added 0.1 ml of a solution containing approximately 120 μl, was added to the gel for electrophoresis.

Procedures for sodium dodecyl sulfate-polyacrylamide gel electrophoresis were essentially those of Weber and Osborn (18). After electrophoresis, duplicate gels were analyzed for the distribution of protein radioactivity. For determination of protein distribution the gel was stained with Coomassie brilliant blue for 2 hours and then destained (18) either by electrophoresis (Canalco model 180 destainer), by diffusion (Hoefer Scientific Instruments, Inc., model DJ-100 destainer), or by a combination of both (electrophoresis followed by diffusion). Glyceroprotein was stained by the periodic acid-Schiff method (19). For determination of radioactivity, the gels were sliced at intervals of 1 mm with a gel slicer immediately after electrophoresis. Each slice was counted for radioactivity. The equations used to calculate Ca2+ concentration were those of Kornberg (10). Mobility of proteins bands and radioactive peaks were determined as retardation factor, Rs, relative to the position of the tracking dye. For determination of molecular weight, albumin (bovine serum), ovalbumin, chymotrypsinogen A (bovine pancreas), myoglobin (spiral whale), and cytochrome c (horse heart) were used as markers.
taken at various time intervals were added to tubes containing trypsin inhibitor that gave a trypsin to trypsin inhibitor ratio of 1:2 by weight. For zero time, a mixture of trypsin and trypsin inhibitor was added. Phosphoprotein formation in trypsin-treated microsomes was determined by incubation in Reaction Mixture A or B and electrophoresis on sodium dodecyl sulfate-polyacrylamide gels as described above. Calcium uptake and Ca\(^{2+}\)-activated ATPase activity of trypsin-treated microsomes were measured by the procedures described previously (5).

In the experiments in which phosphorylated microsomes were treated with trypsin, microsomes were phosphorylated in Reaction Mixture A or C for 10 min at 25\(^\circ\)C, after which trypsin was added at a microsomal protein to trypsin ratio of 20:1. In some experiments, phosphorylated microsomes were washed with buffer solution before treatment with trypsin. At time intervals after the addition of trypsin, trypsin inhibitor was added (trypsin to trypsin inhibitor = 1:2). The amount of phosphoprotein formed in these microsomes (Reaction Mixture A) was determined by gel electrophoresis as described above, and calcium uptake and Ca\(^{2+}\)-activated ATPase activity (Reaction Mixture C) were measured as described previously (5).

**Determination of RNA**

Cardiac microsomes were digested in KOH and RNA content was determined spectrophotometrically by the method of Fleck and Munro (20).

**RESULTS**

**Sodium Dodecyl Sulfate-Polyacrylamide Gel Electrophoresis of Microsomal Proteins**—Canine cardiac microsomes that were solubilized in sodium dodecyl sulfate showed several distinct protein bands after sodium dodecyl sulfate-polyacrylamide gel electrophoresis. A major protein band, a, five secondary bands, b to f, and a diffuse band, z, were found when small amounts (42 \(\mu\)g) of microsomal protein were applied to the gel (A, Fig. 1). Washing of the microsomes with 0.6 M KCl caused no obvious change in this pattern, and no low molecular weight components were found in the supernatant after KCl washing. When larger amounts of canine microsomal protein were applied to the gels, less distinct tertiary bands, including Bands w, x, and y, became visible (Fig. 2B). When the periodic acid-Schiff method was applied, only Band z was stained. Bovine cardiac protein kinase also produced several protein bands when examined by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (B, Fig. 1). Electrophoresis of a mixture of cardiac microsomal protein and...
protein kinase gave the pattern expected from the combination of both proteins (Fig. 2B).

**Phosphorylation of 22,000-dalton Component by Protein Kinase**—Cardiac microsomes phosphorylated by cyclic AMP and protein kinase showed a single significant peak of radioactivity (Peak II, Fig. 2C) when 32P labeling was determined by measurement of the radioactivity of sliced gels. When protein kinase and cyclic AMP were omitted from the incubation medium, no clear peaks were seen at this area or any other area of the gel. A peak of much higher radioactivity was found at this same location (Peak II, Fig. 2C) when 25 mM NaF was included in the reaction medium in order to inhibit phosphoprotein phosphatase activity present in microsomal preparations. The increase of 32P labeling due to NaF was approximately 5-fold when the amounts of 32P were estimated from the area of Peak II. In addition to the major peak, two peaks of much less radioactivity were noted in the presence of NaF (Peaks I and III, Fig. 2C). Peak I may represent 32P labeling of one of the subunits of protein kinase since 32P labeling of the same extent at the same location as Peak I was found when protein kinase was incubated in the absence of cardiac microsomes under identical conditions. This peak of radioactivity corresponded to the protein Band 3 of approximately 55,000 daltons seen in Fig. 1B.

The major peak of radioactivity (Peak II, Fig. 2C) corresponded to one of the minor components (w, Fig. 2B) of microsomes. Among 12 determinations, using 5 different microsomal preparations, Peak II was seen at RF 0.62 ± 0.02 (S.D.), whereas the minor protein band w was found at RF 0.63 ± 0.01 (S.D.). Based on the calibration curve (Fig. 2A), the apparent molecular weight of this phosphoprotein component was estimated from the latter RF value to be 22,000 ± 1,000. An additional minor peak of radioactivity (Peak III, Fig. 2C) was seen when microsomes were phosphorylated in the presence of NaF. The distribution of radioactivity was similar when the specific activity of the [γ-32P]ATP was increased 100-fold. When the total [32P]-phosphophosphate found in Peak II in the presence of NaF (0.035 nmol) is compared with the total microsomal protein applied (0.056 mg) in Fig. 2C, approximately 0.63 nmol of phosphate were found to be incorporated per mg of microsomal protein. This value varied among a number of microsomal preparations within the range of 0.5 to 1.0 nmol of phosphate per mg of microsomal protein, which is in good agreement with the value obtained under similar conditions by the trichloroacetic acid precipitation procedure (7).

**Stability of Phosphoprotein**—In order to study the nature of phosphate-binding to the 22,000-dalton component, microsomes phosphorylated in the presence of protein kinase and [γ-32P]ATP were treated under various conditions, after which they were subjected to sodium dodecyl sulfate-polyacrylamide gel electrophoresis and the amounts of 32P bound to this component were estimated from the area of Peak II. The amount of radioactivity found in Peak II when the phosphorylation was terminated by sodium dodecyl sulfate (Procedure I, see "Methods") was virtually the same as that when the phosphorylation was terminated by trichloroacetic acid and the mixture allowed to stand at 0° for 10 min (Procedure II). Incubation in 0.5 x NaOH for 15 min at 0°, 37°, and 90° demonstrated relative alkali stability at the lower temperatures (Table I). The phosphoprotein was stable to 10 min of treatment in 0.8 M hydroxylamine (pH 5.4) at 30°, and also stable in chloroform-methanol and acetone (Table I). Treatment with 10% trichloroacetic acid for 15 min at 90° caused the peak of radioactivity to become more broad although loss of recovery of total radioactivity was only approximately 10%.

**Ribosomal Contamination**—To evaluate the possibility that the 22,000-dalton component was derived from a ribosomal contaminant, three preparations of cardiac microsomes were digested with KOH and analyzed spectrophotometrically for RNA. The average content of ribosomal protein, estimated from RNA content with the assumption that cardiac ribosomes contain approximately equal weights of protein and RNA, was less than 0.4% of the total microsomal protein.

**Washing of Microsomes with KCl**—Microsomes were washed repeatedly with 0.6 M KCl to remove soluble contaminants and myofibrillar proteins. The amount of 32P labeling in Peak II did not change significantly after two washings with 0.6 M KCl. The specific activity of the phosphoprotein increased slightly (approximately 10%) after the first KCl wash and remained unchanged when microsomes were washed further.

**Treatment of Microsomes with Trypsin**—Incubation of microsomes with trypsin for 20 min at 25° in the presence of 0.8 M hydroxylamine (pH 5.4) to trypsin ratio of 20:1 (w/w) prevented subsequent phosphorylation of the 22,000-dalton component by protein kinase (Fig. 3). Under these conditions trypsin unoccupied the calcium transport system in that calcium uptake was markedly decreased while Ca2+-activated ATPase activity was slightly increased (Fig. 4). When trypsin digestion was carried out in the presence of 1.0 M sucrose, however, calcium uptake was only slightly affected (Fig. 4) as shown previously in skeletal microsomes by Ikemoto et al. (21). In the presence of 1.0 M sucrose, where trypsin causes no significant change in calcium transport activity, brief trypsin treatment caused a marked decrease in formation of the phosphoprotein by protein kinase, and less than 5% of control phosphorylation was found after 20 min of digestion (Fig. 5). Addition of the trypsin-trypsin inhibitor complex did not modify the phosphorylation of the 22,000-dalton component.

The relationship between protein kinase-induced stimulation

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**Table I**

<table>
<thead>
<tr>
<th>Treatment</th>
<th>32P bound to 22,000-dalton component</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (10% trichloroacetic acid, 0°)</td>
<td>100</td>
</tr>
<tr>
<td>0.5 x NaOH, 0°</td>
<td>30</td>
</tr>
<tr>
<td>0.5 x NaOH, 37°</td>
<td>&lt;1</td>
</tr>
<tr>
<td>Control (0.8 M NaCl)</td>
<td>100</td>
</tr>
<tr>
<td>0.8 M hydroxylamine</td>
<td>96</td>
</tr>
<tr>
<td>Control (10% trichloroacetic acid, 0°)</td>
<td>100</td>
</tr>
<tr>
<td>Chloroform-methanol, 0°</td>
<td>99</td>
</tr>
<tr>
<td>Acetone, 0°</td>
<td>88</td>
</tr>
</tbody>
</table>

3 M. Tada, M. A. Kirchberger, and H. C. Li, manuscript in preparation. These results were presented at the Second International Conference on Cyclic AMP (1974) in Vancouver, British Columbia, Canada.
of microsomal calcium transport and protein kinase-catalyzed phosphorylation of the 22,000-dalton component of cardiac microsomes was investigated by determining whether protein kinase and cyclic AMP could stimulate calcium uptake by microsomes which, after 20 min of trypsin treatment in 1 M sucrose, had retained calcium uptake activity but lost most of the ability to be phosphorylated by protein kinase (Fig. 6). In contrast to control microsomes, where the rate of calcium uptake was greatly stimulated by treatment with protein kinase and cyclic AMP, calcium uptake by microsomes that had been treated with trypsin was not stimulated after incubation with protein kinase and cyclic AMP.

Trypsin Treatment of Phosphorylated Microsomes—The 22,000-dalton component could be shown to be resistant to trypsin after it had been phosphorylated by protein kinase prior to the addition of trypsin. Microsomes were phosphorylated under standard conditions, and then they were incubated with trypsin in the presence of 1 M sucrose. It was found that the rate of calcium uptake was greatly stimulated by treatment with protein kinase and cyclic AMP, calcium uptake by microsomes that had been treated with trypsin was not stimulated after incubation with protein kinase and cyclic AMP.
kinase induce marked stimulation of calcium uptake and Ca\(^{2+}\) transport ATPase in several aspects. Unlike the ATPase intermediate, this phosphoprotein has the stability characteristics of a 22,000-dalton protein which is electrophoretically distinct from the calcium transport ATPase protein of approximately 100,000 daltons and provides support for the view that this 22,000-dalton phosphorylase has a regulatory role in calcium transport by cardiac sarcoplasmic reticulum.

Cardiac microsomes that have been solubilized in sodium dodecyl sulfate can be fractionated into several components by polyacrylamide gel electrophoresis (Fig. 1A). The major component of 90,000 to 100,000 daltons (Band a) contains the ATPase protein which can undergo phosphorylation to form an acyl phosphoprotein, an intermediate of the ATPase, in that its mobility is similar to that of the previously described ATPase of cardiac (10) and skeletal (17, 24) sarcoplasmic reticulum. Band z may represent proteolipid (24). Some of the other bands of cardiac microsomes shown in Fig. 1 may be analogous to the calcium-binding proteins of skeletal sarcoplasmic reticulum (25, 26). However, none of these previously described components served as a substrate for protein kinase-catalyzed phosphorylation (Fig. 2).

The phosphoprotein formed in the presence of protein kinase differs from the phosphoprotein intermediate of the calcium transport ATPase in several aspects. Unlike the ATPase intermediate, this phosphoprotein has the stability characteristics of a phosphoprotein (Table I) and its formation does not require Ca\(^{2+}\) (7). The possibility that it is primarily phospholipid can be excluded because of its trypsin sensitivity and because neither chloroform-methanol nor acetone extracted the phosphate (Table I). The 22,000-dalton component was not stained by the periodic acid-Schiff method, indicating that it is not a glycoprotein.

Two peaks of low radioactivity were seen in addition to the main peak of radioactivity that is associated with the 22,000-dalton phosphoprotein (Fig. 2C). Peak 1, which was seen when protein kinase was incubated in the absence of microsomes,

<table>
<thead>
<tr>
<th>Digestion time</th>
<th>Rate of calcium uptake</th>
</tr>
</thead>
<tbody>
<tr>
<td>min</td>
<td>umol Ca/min/mg protein</td>
</tr>
<tr>
<td>0</td>
<td>0.130</td>
</tr>
<tr>
<td>5</td>
<td>0.128</td>
</tr>
<tr>
<td>10</td>
<td>0.120</td>
</tr>
<tr>
<td>20</td>
<td>0.118</td>
</tr>
<tr>
<td>40</td>
<td>0.114</td>
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We have tentatively named this phosphoprotein "phospholamban" (1-3).
may represent the phosphorylation of one component of the protein kinase preparation (Band 3 in Fig. 1B) whose molecular weight is approximately 50,000 to 60,000. This finding is consistent with the results of Erlichman et al. (27) who reported autophosphorylation of the cyclic AMP-binding subunit of bovine cardiac protein kinase (55,000 daltons). Peak III which was seen only in the presence of NaF may correspond to one or more components of low molecular weight. However, the low radioactivity and the diffuse nature of this peak precluded further analysis.

The 22,000-dalton component of cardiac sarcoplasmic reticulum appears to be similar to a protein of less than 30,000 daltons reported by Andrew et al. (28) in rat skeletal muscle microsomes to be phosphorylated by a protein kinase that was minimally stimulated by cyclic AMP, and to a 20,000- to 25,000-dalton protein in ammonium sulfate-precipitated protein fractions of bovine cardiac and skeletal muscle found by Sands and Meyer (29) to be phosphorylated by a cyclic AMP-dependent protein kinase. The recent observation that a ribosomal protein of approximately 27,000 daltons is phosphorylated in the reticulocyte by a cyclic AMP-dependent protein kinase (30) raises the possibility that the 22,000-dalton phosphoprotein of cardiac microsomes, which is present in low quantities, represents a contaminant derived from ribosomes. This seems unlikely, however, because the average content of ribosomal protein, estimated from RNA content, is less than 0.4% of the total microsomal protein, whereas the minimal content of the 22,000-dalton phosphoprotein in cardiac microsomes is 4%, if one assumes each mole of the latter to incorporate a single mole of phosphate. The possibility that the 22,000-dalton component might be a polyvalent phosphoprotein of ribosomal origin can also be excluded by the finding that the 32P-labeled phosphoprotein, after treatment with LiCl and urea (31, 32) did not migrate to the cathode in polyacrylamide gels that contained 8 M urea at pH 4.5, in contrast to the behavior of known ribosomal proteins. Furthermore, a single major peak of radioactivity in phosphorylated cardiac microsomes would not be expected if the phosphoprotein were derived from a ribosomal contaminant because mammalian ribosomes incorporate the γ-32P of ATP into a number of phosphoproteins when phosphorylation is carried out in vitro (33–36). This is in contrast to the single phosphoprotein band seen when phosphorylation is carried out in undisrupted cells (30, 34). The present findings, in which phosphorylation of a disrupted cell fraction was examined, do not, therefore, indicate that the 22,000-dalton phosphoprotein is part of a ribosomal contaminant.

The phosphoprotein of cardiac sarcoplasmic reticulum has a molecular weight similar to that of troponin I (37), the component of troponin which inhibits actomyosin ATPase. Troponin I from skeletal muscle has been reported to be phosphorylated by phosphorylase kinase (38) and canine cardiac troponin was recently reported to be phosphorylated by protein kinase (39). It is unlikely, however, that troponin I, which might be introduced as a contaminant from the contractile proteins, accounts for the phosphorylation documented in the present report because phosphorylation of cardiac microsomes did not decrease after washing with 0.6 M KCl, a step that reduces contamination from the contractile proteins. The present results thus suggest that the phosphoprotein is associated with the membranes of the sarcoplasmic reticulum rather than being a contaminant from either ribosomal or soluble proteins. The trypsin sensitivity of the phosphoprotein is greater than that of calcium transport system (Fig. 5), suggesting that the phosphoprotein may be related to the outer surface of the membrane of the sarcoplasmic reticulum.

The present study indicates that the previously suggested regulatory function of protein kinase-catalyzed phosphoprotein formation in the stimulation of calcium transport by cardiac sarcoplasmic reticulum (4, 5, 7) is mediated by phosphorylation of a 22,000-dalton protein. In microsomes treated with trypsin under conditions where calcium transport activity is preserved but in which the 22,000-dalton protein loses its ability to be phosphorylated, calcium uptake could not be stimulated by incubation with cyclic AMP and protein kinase (Fig. 6). Furthermore, in phosphorylated microsomes, whose 22,000-dalton component is resistant to subsequent trypsin treatment (Fig. 7), the stimulated calcium uptake is not lost following exposure to trypsin (Table 11). Regulation of calcium transport by phosphorylation of this 22,000-dalton protein appears to be reasonable from a stoichiometric standpoint as similar amounts of phosphate are incorporated into this protein and into the acyl phosphoprotein intermediate of the calcium transport ATPase (7). Further studies on the interaction between purified 22,000-dalton phosphoprotein and the calcium pump will be necessary to define their precise relationship.

Acknowledgments—We are grateful to Professor Yuji Tonomura, Osaka University, Osaka, Japan, for critical discussion and helpful suggestions. We thank Ms. Jo-Anna M. Iorio for expert technical assistance.

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