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The Effects of Physical States of Phospholipids on Ca^{2+}-ATPase Activity of Biological Membranes

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ABSTRACT

The Ca^{2+}-ATPase of sarcoplasmic reticulum (SR) was solubilized and reconstituted into a mixture of phosphatidylcholine (PC) and phosphatidylethanolamine (PE) of varying ratios in order to assess the effect of physical states of phospholipids on the incorporation and functions of Ca^{2+}-ATPase. On the basis of the spectral data of Ca-arsenazo III, the Ca^{2+} uptake of SR was increased linearly as the PC content increased in the reconstituted vesicles. The ATP hydrolysis activity also increased as PC content increased up to 25% and then decreased slightly as the PC content further increased. On the other hand the incorporation of Ca^{2+}-ATPase into the reconstituted vesicls occured maximally at 25% PC and 75% PE mixture which is known to have a non-bilayer structure in reconstitution system. From the above results it is clear that preexisting defects in the lipid bilayer promote protein incorporation into the bilayer during reconstitution and lamellar structure of the bilayer facilitates the Ca^{2+}-ATPase function.

Key Words: Ca^{2+}-ATPase, Sarcoplasmic reticulum, Phospholipids, Physical states, Arsenazo III, Reconstitution

INTRODUCTION

The generally accepted fluid-mosaic model of membrane structure proposed by Singer and Nicolson (1972) regards biological membranes essentially as two dimensional solutions of oriented globular proteins in a fluid lipid bilayer phase. It implies that membrane lipid composition and physical properties of the membrane lipids, would be important in determining the rates of lipid and protein motion and the functions of proteins in membranes. Any researches have been centered upon the relationship of lipid composition to membrane structure (Levine, 1972; Chapman, 1975; Cullis et al., 1978; Cullis & Kruijff, 1979; Verkleiz et al., 1979; Hui et al., 1981a; Hah et al., 1983). Although how lipid bilayer structure relates to membrane function remains still unclear, an ample body of evidence in intact cells and membranes is accumulating that lipid composition and architecture of membranes play important roles from growth to cell recognition through changing lipid fluidity and phase state (McElhaney, 1982).

One way to study the nature of lipid-protein interactions at the molecular level can be from reconstitution experiments where specific compositions of lipid can be pre-selected and the physical state of lipids controlled to modify the function of protein and membrane structure.

The most occurring physical structure of the membranes is the lamellar phase. However several other phases coexist in lipid model membranes.

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Alternative configurations include the hexagonal (Daemer et al., 1970), inverted hexagonal (Cullis et al., 1978), inverted Cubic phases (Sen et al., 1981) and the lipidic particles (Hui et al., 1983). A mixture of phospholipids exhibit a complex phase mixing which is determined by the characteristics of mixed lipids (Stewart et al., 1979). For example, in the phase diagram of soybean PE/egg PC, hexagonal phase was seen in pure unsaturated PE at all experimental temperatures whereas bilayer phase seen in pure PC.

In mixtures containing between 5 to 25% of egg PC in PE, the structure of the mixture was seen to gradually transform from stable to a structure that is dotted with non-bilayer defects or lipidic particles (Hui et al., 1981b). Several studies reported that the existence of non-bilayer defects in lipid structure facilitates protein incorporation and affects the functions of the protein incorporated in reconstitution experiments (Wickner, 1977; Pownall et al., 1979; Epand et al., 1981; Hah et al., 1983; Hah, 1986). In our previous study, a putative glucose transporter was reconstituted into liposomes which show non-bilayer defects. It resulted that the incorporation and activities of the proteins were affected by the instability of the lipid structure (Hah, 1986). Therefore another protein might be needed to reconstitute into the same lipid system in order to verify and extend these concept.

Sarcoplasmic reticulum membranes (SR) have been extensively characterized in their structural and functional aspects (Weber et al., 1973; Tada et al., 1978; Hasselbach, 1979). The major protein, the Ca^{2+} ATPase, is intrinsically associated with membrane lipids which greatly influence the enzyme activity (Martonosi et al., 1971; Bennett et al., 1980; Johannson et al., 1981). Lipids in contact with the ATPase enzyme modulate its function through physical interactions (Bennett et al., 1980; Johannson et al., 1981). The lipids consist of essentially two phospholipids, phosphatidylcholine and phosphatidylethanolamine (Korenbrot, 1977).

Thus in this study Ca^{2+}-ATPase was purified from sarcoplasmic reticulum of rabbit hind muscle and reconstituted into various PC/PE mixture vesicles which exhibit polymorphic phase behaviour to see the effect of physical states of the lipids on the incorporation and functions of the Ca^{2+}ATPase.

MATERIALS AND METHODS

Materials

Phosphatidylcholine (PC) and phosphatidylethanolamine were purchased from Avanti Polar-Lipids Inc., Birmingham, Alabama. $Ca^{45}Cl_2$ was from the Radiochemical Centre, Amersham, England. Triton X-100 and sodium deoxycholate were from J. T. Baker chemical, phillipsburg, New Jersey. Octylglucoside was synthesized in the laboratory. ATP, arsenazo III and EDTA were purchased from Sigma Chemical Co., Saint Louis, Missouri. Bio-Beads (SM -2) was from Bio Rad Laboratories, Richmond, California. All other chemicals used were of reagent grade.

Preparation of sarcoplasmic reticulum

Sarcoplasmic reticulum vesicles were prepared from rabbit skeletal muscle by a modification of published procedures (Fiehn & Peter, 1971; McFarland & Inesi, 1971; Meissner et al., 1973). All procedures were conducted at 4℃ or with ice-cold buffers. Briefly, freshly dissected white muscle from rabbit hind leg was minced with scissors, placed in buffer I (1 mM EDTA, pH 7.0) until all muscle is excised and then transferred to Waring blender with two volumes of buffer II (1 mM EDTA, 10 mM histidine and 10% Sucrose, pH 7.0).

Muscle fragments were homogenized for 12 times with 15 second periods allowing 3 minutes between homogenizations for cooling. The homogenate was centrifuged at 5,000 g for 20 minutes, and the supernatants were filtered through 4~8 layers of cheese-

cloth. The filtered supernatant was centrifuged at 30, 000 g for 90 minutes. The pellets were resuspended in buffer III (1 mM EDTA, 10 mM histidine and 0.6 M KCl, pH 7.0) and incubated for 40 minutes at 4℃. This preparation was centrifuged at 8,500 g for 20 minutes and decanted supernatant (and any loose pellet which followed) into a clean centrifuge tube. This was recentrifuged at 30,000 g for 90 minutes, the pellets were taken up in a minimum of buffer IV (1 mM EDTA, 10 mM histidine, and 30% sucrose, pH 7.0) and homogenized with teflon pestle homogenizer.

The resulting sarcoplasmic reticulum vesicles were placed in a cryotube and stored at a liquid nitrogen tank (−80℃) until use.

Solubilization of Ca^{2+}-ATPase

Ca^{2+}-ATPase was solubilized basically according to the method of Hah et al (1983) by using octylglucoside, a mild detergent. The ratio of protein to octylglucoside was 1:6(W/W) keeping the concentration of octylglucoside to be 46 mM with 50 mM Tris-HCl(pH 7.4) and 1 mM DTT. The preparation was kept on ice for 20 minutes with stirring and centrifuged at 100,000 g for 30 minutes. The supernatant was centrifuged again with the same centrifugal force. The ATPase protein of sarcoplasmic reticulum was solubilized about 80% of the total protein as estimated by measuring protein amount of the remained pellet.

Reconstitution of membrane vesicles containing Ca^{2+}-ATPase

A mixture of phosphatidylcholine and phosphatidylethanolamine in chloroform at varying ratios containing 0, 15, 25, 50 and 75% by weight of PC with the total 40 mg of phospholipids was dried under nitrogen stream with a constant rotation to form a thin film in a 40 ml pyrex glass test tube. An aliquots of 10 ml octylglucoside solubilized Ca^{2+}-ATPase containing 5∼10 mg protein was added to each of these phospholipid mixtures and vigorously shaken with a vortex shaker for 2 minutes, and incubated for 30 minutes with gentle stirring at 4℃. The mixture was dialyzed for 2 hours to remove a bulk of detergent against a buffer containing 100 mM KCl and 50 mM Tris-HCl (pH 7.4) and then subjected to a successive dialysis overnight against a buffer containing 100 mM KCl, 50 mM Tris-HCl and 0.3 M sucrose. The dialyzed suspensions were centrifuged at 100,000 g for 60 minutes and the pellets were resuspended in the same buffer containing 100 mM KCl 50 mM Tris-HCl and 0.3 M sucrose.

The reconstituted Ca^{2+}-ATPase with exogenous lipid mixtures were transferred to cryotubes and stored at liquid nitrogen tank.

Ca^{2+} uptake by the reconstituted vesicles

Ca^{2+} uptake rate was measured by a dual wavelength, dual beam spectrophotometer (Hitachi Model 557) with arsenazo III as a metalochromic indicator, which gives good signal to noise characteristics, appropriate affinity for Ca^{2+}, high selectivity, and fast response (Scarpa et al., 1978; Scarpa, 1979). Extravesicular free Ca^{2+} concentration was monitored by the changes in absorbance of 50 μM arsenazo III at 655 nm with a time-sharing manner. Calibration was carried out under the same condition as those used for the measurement of Ca^{2+} uptake except for the absence of the membrane vesicles. The absorbance changes were a linear function of Ca^{2+} concentration up to 50 μM.

Unless otherwise specified, Ca^{2+} uptake was started by the addition of 2 mM ATP in a reaction mixture containing 50 μg/ml Ca^{2+}-ATPase protein, 100 μM $CaCl_2$, 5 mM $MgSO_4$, 10 mM Potassium oxalate, 90 mM KCl, 100 mM triethanolamine and 50 μM arsenazo III.

Ca^{2+} uptake was also measured by means of an isotope technique as described previously (Hah,

1976). Briefly, approximately 0.1~1 mg protein/ml was incubated at 37℃ in 1 ml medium containing 20 mM MOPS-Tris (pH 7.2), 100 mM KCl, 1 mM $MgCl_2$, 1 mM ATP, 0.2 mM EGTA, 0.05 μC $Ca^{45}Cl_2$/ml and 10^{-5}M $CaCl_2$. At the required intervals, 0.1 ml aliquots were removed and filtered rapidly through 0.45 μm HAWP Millipore filters. The radioactivities of filtrate and incubation mixture were counted and the Ca^{2+} uptake rate was calculated by the equation,

$$\text{uptake rate}(\%) = \left(\frac{A_{total} - A_{filtrate}}{A_{totel}} \times 100\right)$$

A_{total}: CPM of 20 μl of incubation mixture

$A_{filtrate}$: CPM of 20 μl of filtrate

Ca^{2+}-ATPase activity

The ATPase activities of reconstituted membranes were determined by measurement of Pi release from ATP at a temperature of 37℃. The incubation mixture for total ATPase contained 100 mM KCl, 5 mM $MgCl_2$, 10 mM Histidine (pH 7.0), 1 μM $CaCl_2$ and 10 μg of the reconstituted membrane protein in a total volume of 5 ml.

Following preincubation at 37℃ for 3 minutes, the reaction was started by the addition of 2 mM ATP. After 30 minutes the reaction was stopped by addition of 1 ml of 30% TCA. Centrifugation for 2 minutes at 15,000× g removed the precipitated protein and Pi was measured on 2.5 ml sample of the supernatant by the method of Fiske and Subba-Row (1925). The Ca^{2+}-dependent ATPase activity was calculated by subtracting the ATPase activity measured in the presence of 2 mM EGTA. The results obtained are expressed as micromoles of Pi liberated per mg of protein per assay time.

Lipid/protein analysis

In order to determine the lipid composition, and aliquot (0.2 ml) of the membrane suspension was applied to a thin-layer chromatograph. The phosphatidylcholine and phosphatidylethanolamine spots were indicated by charring at 180℃ for 10 minutes after spraying with a sulfuric acid/ethanol mixture (1 : 1). The charred spots were scraped out, and the phosphate composition was determined by the method of Bartlett (1959). Total phospholipid was measured separately by the same method protein was assayed by the method of Lowry et al. (1951) with bovine serum albumin as the standard.

Electron microscopy

Both the sarcoplasmic reticulum and the reconstituted vesicles were studied by negative staining electron microscopy. For staining, membrane vesicles were allowed to settle on carbon-coated grids and stained with 2% ammonium molybdate. The negative-stained specimens were observed through a Siemen 101 electron microscope.

RESULTS

Properties and spectral characteristics of arsenazo III

Figure 1 shows the absorbance spectra of arsenazo III in the presence of 1 μM $CaCl_2$ with various pHs in the buffer. The spectra have two peaks of absorbance at 595 and 650 nm in which pH does not affect the spectral characteristics.

Figure 2 shows the differential spectra of arsenazo III versus arsenazo III plus various Ca^{2+} concentrations. It is clear from the spectra that at Ca^{2+} concentrations greater than 20 μM the absorbance changes undergone by arsenazo III are not a linear function of Ca^{2+} concentration, since the indicator has become saturated with Ca^{2+}. At low ionic strength this effect is even more dramatic. The differential spectrum shows that an increase in Ca^{2+} concentration produces increases in arsenazo III absorbance at both wavelengths.

Figure 3 shows the differential spectra of arsenazo III versus arsenazo III plus various concentrations of Mg^{2+}. The presence of Mg^{2+} produces a single

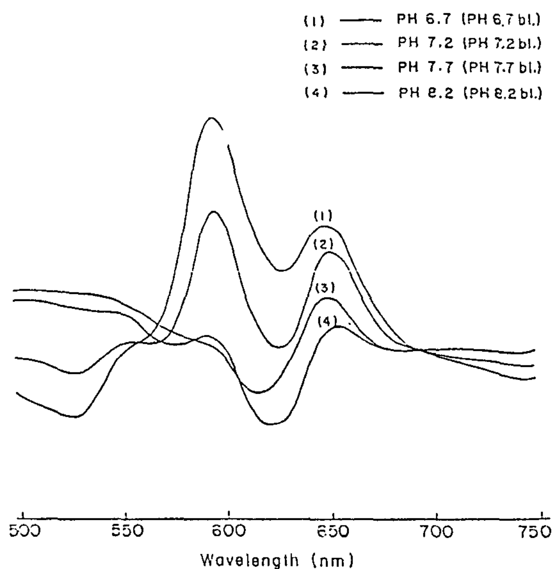

Fig. 1. Effect of pH on the spectra of AIII. The sectra were obtained by changing pH of the reaction mixture as indicated in the figure.

broader absorbance change with a maximum peak of absorbance at 615 nm.

Ca^{2+} transport in sarcoplasmic reticulum

The kinetics of ATP dependent Ca^{2+} transport by sarcoplasmic reticulum can be successfully measured using arsenazo III as a calcium indicator. These measurements can be performed in the presence of concentrations of Ca^{2+} in the reaction mixture ranging from 1 μM to 20 μM, which are close to the concentrations of Ca^{2+} under physiological conditions. Figure 4 shows an example of these measurements.

In order to calibrate the changes in absorbance of arsenazo III with the concentration of Ca^{2+} in the reaction mixture, a known concentration of EGTA was added to the reaction mixture in the absence of

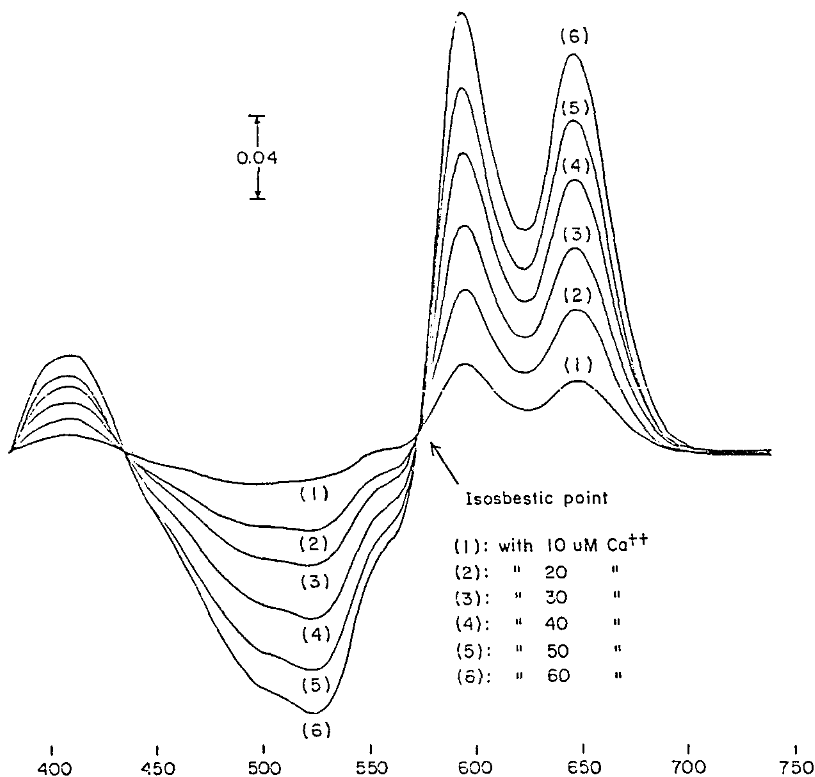

Fig. 2. Effect of a various concentration of Ca^{2+} on the sp .ra of AIII. The spectra were obtained by adding Ca^{2+} indicated in figure to the measuring cuvet.

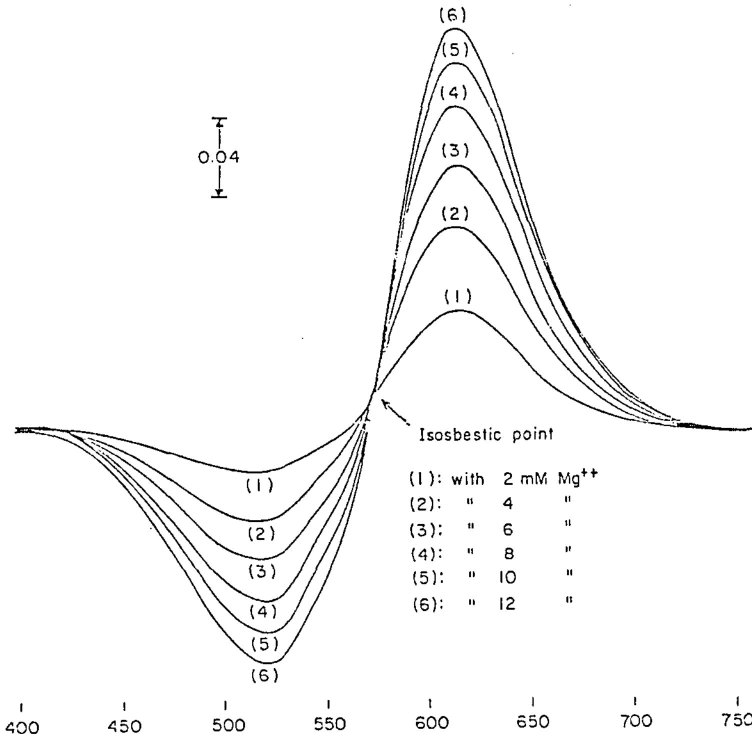

Fig 3. Effect of various concentration of Mg^{2+} on the spectra of AIII. The spectra were obtained by adding Mg^{2+} indicated in figure to the measuring cuvet.

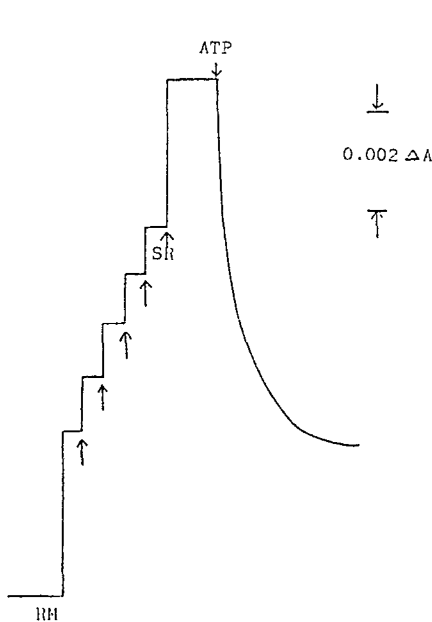

Fig. 4. Absorbance changes of Ca^{2+}+AIII complex by adding 2 mM ATP showing Ca^{2+} uptake by SR. Arrows indicate addition of Ca^{2+} into the cell. RM represents reaction mixture.

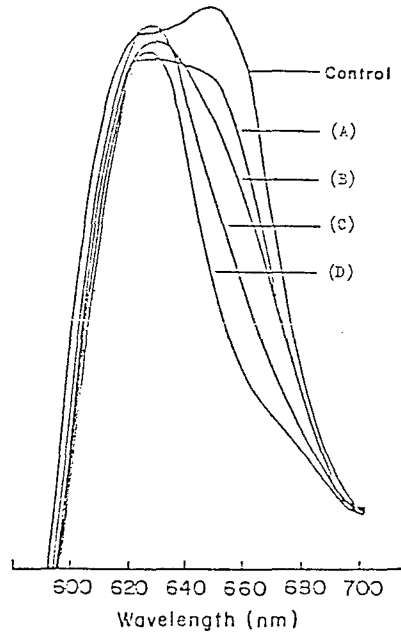

Fig. 5. Spectral changes of Ca^{2+}+AIII complex by adding ATP.
(A) with 0.5 mM ATP (B) with 1 mM ATP
(C) with 1.5 mM ATP (D) with 2 mM ATP

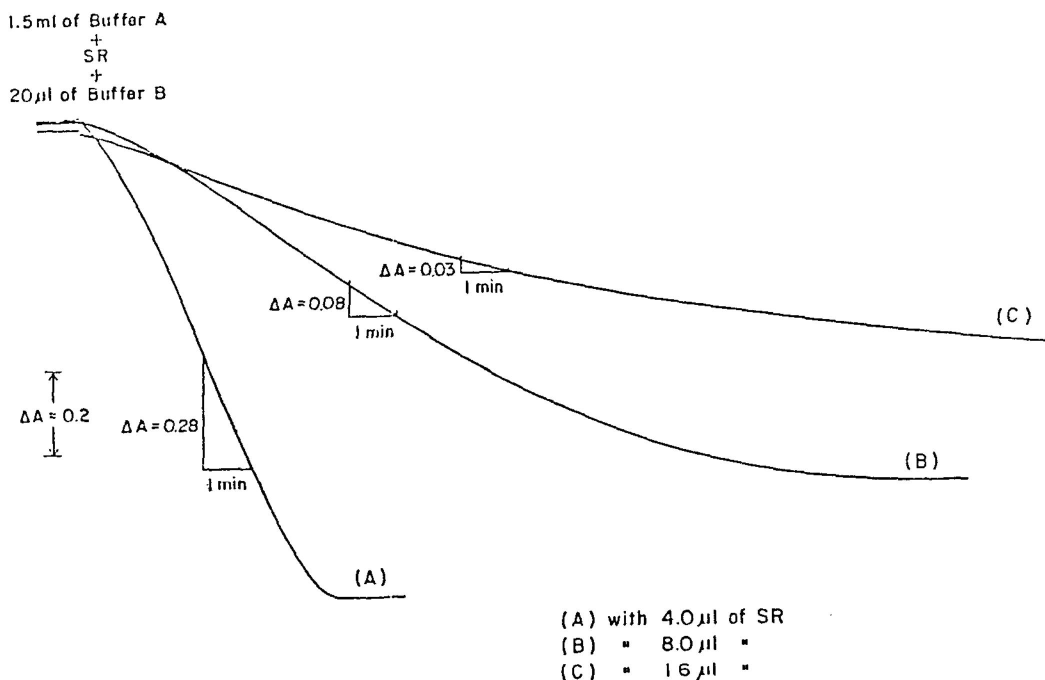

Fig 6. Protein dependent Ca^{2+} uptake rate.
(A) with 4.0 μl of SR (B) with 8.0 μl of SR
(C) with 16 μl of SR For details see the text

sarcoplasmic reticulum. Figure 5 shows that the addition of ATP to the reaction mixture causes a fast decrease in absorbance, which is due to the decrease in Ca^{2+} concentration in the reaction mixture consequent to uptake by the sarcoplasmic reticulum. Control experiments show that the addition of ATP does not produce significant changes in arsenazo III absorbance, since the formation of ATP-Ca^{2+} complex is prevented by the presence of 5 mM $MgCl_2$. There are many evidences which support that the decrease of absorbance reflects the Ca^{2+} uptake by SR. Figure 6 shows that Ca^{2+} uptake is dependent on the amount protein added to the reaction mixture.

The Ca^{2+} uptake rate increased linearly as the portein amount increased. The uptake rates of 4 μl, 8 μl and 16 μl of sarcoplasmic reticulum suspension were 1.5, 4.0 and 14.0 μmoles/min, respectively. Figure 7 shows that Ca^{2+} uptake was also dependent on ATP concentration of the reaction mixture. The maximal uptake amounts of Ca^{2+} of sarcoplasmic reticulum was 30.5 μmoles when 1 mM ATP was added. When 2 mM ATP was added the maximal uptake amount of Ca^{2+} by sarcoplasmic reticulum was increased to 34 μmoles. Figure 8 shows the Ca^{2+} chelating effect of EGTA with various concentrations in the reaction mixture.

The maximal uptake amounts of Ca^{2+} by SR decreased to 83.9%, 61.2% and 0% as the concentrations of EGTA increased to 1 mM, 2 mM and 4 mM resectively in the reaction mixture. Both ATP and EGTA affected the maximal uptake amounts but did not affect the uptake rate of Ca^{2+} by SR.

Other earth ions like La^{3+} also affected Ca^{2+} uptake by sarcoplasmic reticulum. Figure 9 shows that the Ca^{2+} uptake rate and maximal Ca^{2+} uptake amount decreased to 58.3% and 55.4% in the presence of 33 μM lanthanum chloride. Figure 10 shows that the Ca^{2+} uptake rate was affected by addition of

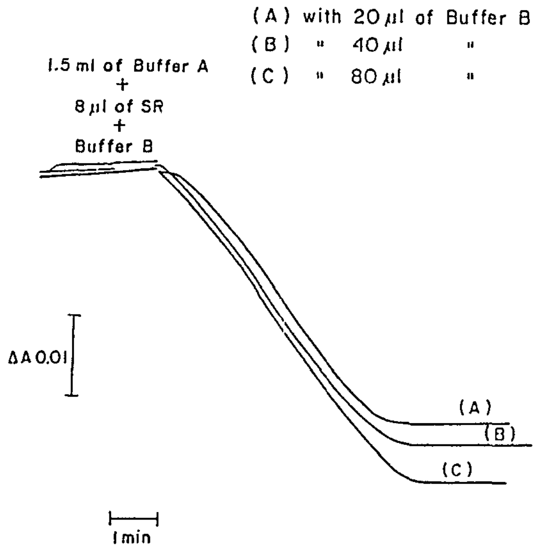

Fig. 7. ATP dependent Ca^{2+} uptake rate.
(A) with 1 mM ATP (B) with 2 mM ATP
(C) with 4 mM ATP

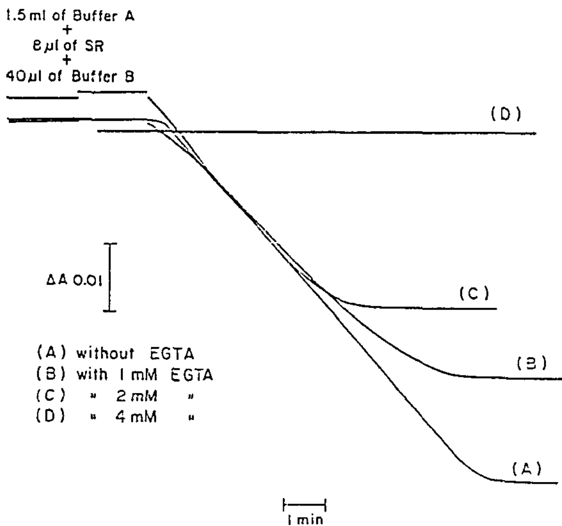

Fig. 8. Effect of EGTA on Ca^{2+} uptake rate.
(A) without EGTA (B) with 1 mM EGTA
(C) with 2 mM EGTA(D) with 4 mM EGTA

Ca^{2+} to the reaction mixture.

The Ca^{2+} uptake rate of sarcoplasmic reticulum was 1.94 μmoles/min/mg protein in the presence of 50 μM of calcium chloride. When 50 μM Ca^{2+} were added once and twice to the reaction mixture the uptake rate decreased to 1.10 and 0.3 μmoles/min/mg protein, respectively. In the steady state, the addition of Ca^{2+} to the reaction medium induced a rapid Ca^{2+} release which was followed by the complete reuptake by the sarcoplasmic reticulum.

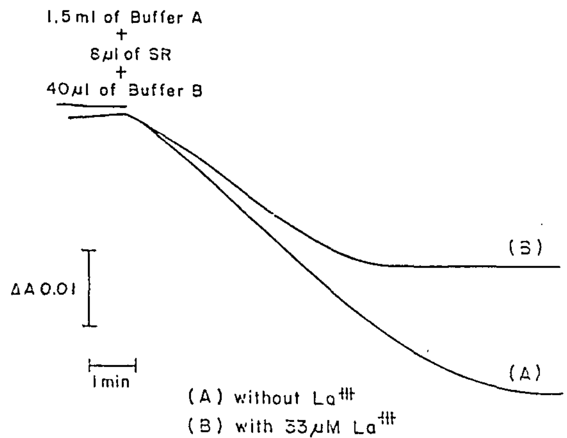

Fig. 9. Effect of lanthanum on Ca^{2+} uptake rate.
(A) without La^{3+} (B) with 33 μM La^{3+}

Ca^{2+} ATPase in sarcoplasmic reticulum

Fugure 11 shows the Ca^{2+}-ATPase activities measured as ATP hydrolysis functions with various temperature and pHs. The optimal pH of the Ca^{2+}-ATPase was 7.0, of which activity reduced rapidly below 6.0 and above 8.0. The Ca^{2+}-ATPase activity was affected by temperature of incubation medium. The Ca^{2+}-ATPase activity increased as temperature increased at range of 17°~37℃.

Effect of various PC/PE mixtures on the reconstituted Ca^{2+}-ATPase

Ca^{2+} uptake

Previous studies have showed that a lipid structure of membrane may be an important regulator of a glucose transport function in reconstituted vesicles. To examine this possibility in other membranes, the Ca^{2+}-ATPase of sarcoplasmic reticulum was iso-

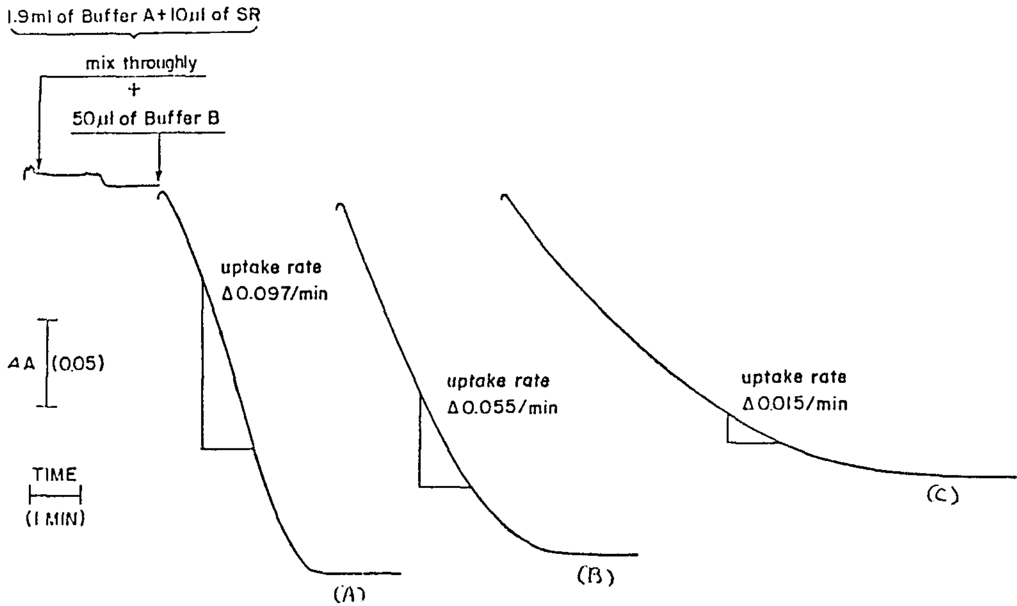

Fig 10. Effect of additive Ca^{2+} on the Ca^{2+} uptake rate.
(A) with 50 μM Ca^{2+} (B) with 100 μM Ca^{2+}
(C) with 150 μM Ca^{2+}

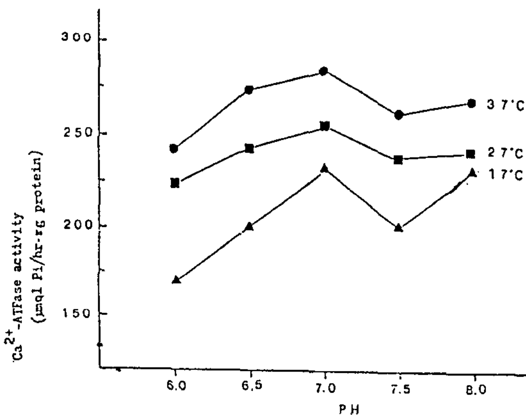

Fig. 11. Effect of pH and temperature on Ca^{2+}-ATPase of SR. Each point represents an average of results of two independent experiments.

lated, purified, and reconstituted into vesicles of known phospholipid composition.

The activity of Ca^{2+}-ATPase was examined by measurement of Ca^{2+} uptake rate using arsenazo III as Ca^{2+} indicator dye. Table 1 A shows the results of experiments. As shown in this table, Ca^{2+} uptake rates of reconstituted Ca^{2+}-ATPase vesicles were increased linearly as the PC content increased in reconstitution system. The percent increments of Ca^{2+} uptake rate of Ca^{2+}-ATPase vesicles reconstituted from 15%, 25%, 50% and 75% PC in PE were 10%, 30%, 50% and 65% respectively of the uptake rate of the vesicle with no exogenously added lipids as control group.

On the other hand, Ca^{2+} uptake rate measured by filtration technique showed the same result as above (data not shown).

ATP hydrolysis

In order to examine whether the Ca^{2+} uptake was coupled with ATP hydrolysis, the activities of Ca^{2+} -ATPase reconstituted with various PC/PE mixtures were determined by inorganic phosphate measurement. Table 1 B shows that the ATP hydro-

Table 1. Ca^{2+} -ATPase Pump activities of reconstituted vesicles

	(A) Ca^{2+} uptake rate (μ moles Ca^{2+}/mg/hr)	(B) Ca^{2+} -ATPase activities (μ moles Pi/mg/hr)
Without exogenous lipids	0.20±0.01	4.03±0.84
With 15% PC+85% PE	0.22±0.01	4.50±0.80
With 25% PC+75% PE	0.26±0.02	10.78±3.59
With 50% PC+50% PE	0.30±0.03	8.31±3.40
With 75% PC+25% PE	0.33±0.03	9.41±4.38

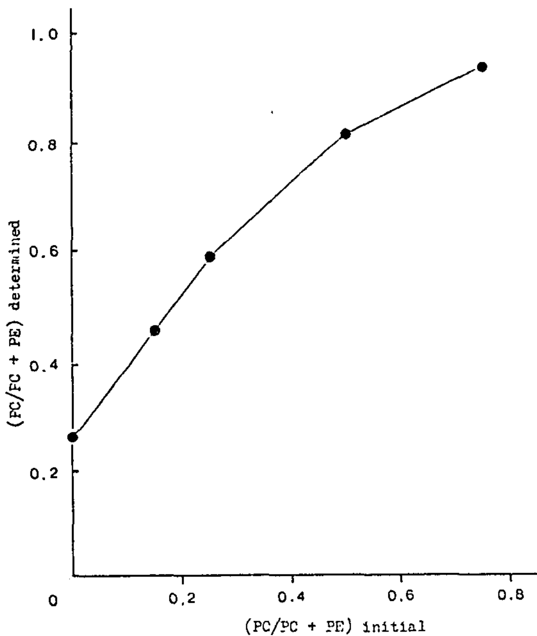

Fig. 12. The ratio PC/(PC+PE) in reconstituted Ca^{2+}-ATPase as function of that in exogenously added lipid. Each point represents an average of results of two independent experiments.

lysis function of Ca^{2+} ATPase is dependent on phospholipid composition of membranes. For egg PC/soybean PE mixtures, the ATP hydrolysis activities increased as PC content increased, showing about 2.7 fold increase at 25% PC, and then decreased slightly as the PC content further increased. As shown in this table, Ca^{2+} ATPase activities of vesicles reconstituted with exogenous lipids were not correspond exactly to the Ca^{2+} uptake rate.

Composition of the reconstituted Ca^{2+}-ATPase

The post freeze-and-thaw yield of reconstituted Ca^{2+} -ATPase in terms of lipid is almost indepéndent of the ratio of exogenous PC to PE. The relative ratio of PC to PE in these reconstituted Ca^{2+} -ATPase shows almost a linear relationship with that of exogenously added PC and PE, but indicates that the incorporation of PC into purified Ca^{2+} -ATPase is less than that of PE as shown in Figure 12.

Protein incorporation in these reconstituted Ca^{2+} -ATPase, however, shows an interesting pattern according to the PC content in the exogenously added lipid mixture. For egg PC/soybean PE mixtures, the protein/lipid ratio increases as the PC content increases, showing about 50% more incorporation at 25% PC, and then decreases gradually as the PC content further increases as shown in Figure 13. The protein incorporation showed a gradual increase as the PC in the mixture increased up to 50 % and then leveled off when the PC content further increased.

DISCUSSION

The results obtained in this study can be divided

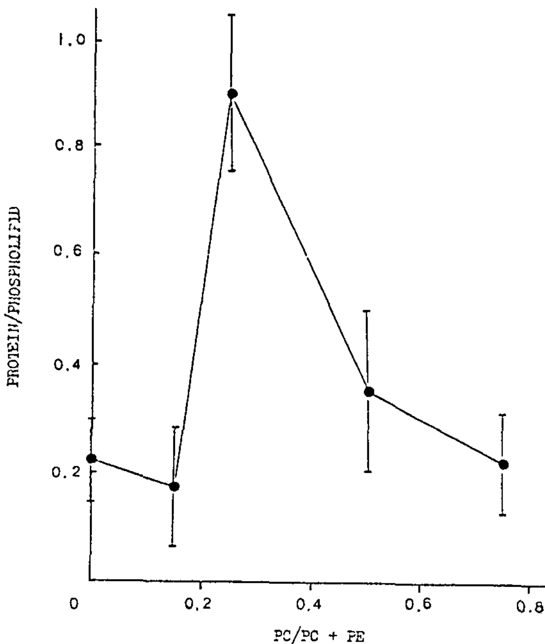

Fig. 13. The ratio of protein to phospholipid in reconstituted Ca^{2+}-ATPase as function of "PC/PC+PE" ratio of exogenously added lipids. Each point represents an average of results of three independent experiments with bars representing standard errors.

into two parts. In the first part, attempts were made to confirm the availability of arsenazo III for measuring Ca^{2+} uptake rate of sarcoplasmic reticulum under various conditions. Arsenazo III was known to have high affinty for Ca^{2+} (Smaal et al., 1985), has a very intense absorbance at 650 nm when it is bound to Ca^{2+} (Smaal et al., 1985) and is available in chemically pure from. These properties made arsenazo III a useful tool for Ca^{2+} flux studies. Our results show that arsenazo III has two peaks of absorbance at 595 nm and 650 nm (Fig. 1), an increase in Ca^{2+} concentration produces increases in arsenazo III absorbance at both wavelength (Fig. 2), and the addition of ATP to the reaction mixture containing sarcoplasmic reticulum in the presence of Ca^{2+} causes a fast decrease in absorbance, which is due to the decrease in Ca^{2+} concentration in the reaction mixture consequent to uptake by the sarcoplasmic reticulum (Fig. 5).

These are consistent with other reports (Herbette et al., 1977; Scarpa, 1979; Salama & Scarpa, 1985). Arsenazo III was also suitable to measure the Ca^{2+} uptake rate selectively and quantitatively at both sarcoplasmic reticulum and Ca^{2+}-ATPase vesicles reconstituted with phospholipids. The results show that the Ca^{2+} uptake rate depend on the amount of sarcoplasmic reticulum (Fig. 6), ATP concentration (Fig. 7), EDTA concentration (Fig. 8) and the ionic composition of the medium (Fig. 9 & 10).

Several investigators reported the same results in sarcoplasmic reticulum (Scarpa, 1979; Smaal et al., 1985; Weissmann et al., 1980; Beeler & Gable, 1985), mitochondria (Crompton et al., 1976), red blood cells (Yingst & Hoffman, 1983 & 1984) and microsomes (Muto & Nozawa, 1985). The main purpose of this study showing the effects of lipid structures on the Ca^{2+} -ATPase of sarcoplasmic reticulum during reconstitiution is seen in the second part.

In the preceding papers (Hah et al., 1983; Hah, 1986) we reported that proteoliposomes reconstituted from both a Triton extract and a band 4.5 protein of human erythrocyte membrane with a mixture of phosphatidylcholine and phosphatidylethanolamine of varying ratios showed a interesting phenomenon. With mixtures of egg PC and soybean PE, the protein/lipid ratio of the reconstituted vesicles was maximal at 25% phosphatidylcholine and 75% phosphatidylethanolamine, the composition is known to have a maximum bilayer disruption (highest occurrence of lipidic particles seen by freeze-fracture electron microscopy).

The specific activity of the cytochalasin B binding protein in the reconstituted vesicles, on the other hand, was increased monotonically up to several fold as the phosphatidylcholine content was increased in the egg phosphatidylcholine/soybean phosphatidylethanolamine mixture. In this study Ca^{2+} -ATPase of skeletal sarcoplasmic reticulum was chosen as a

model protein in order to examine the roles of the same lipid structure on the protein incorporation and Ca^{2+} ATPase function regulating the coupling between Ca^{2+} transport and ATP hydrolysis in reconstituted vesicles. The results show that Ca^{2+} uptake rates of the reconstituted Ca^{2+} ATPase vesicles were increased linearly as the phosphatidylcholine content increased in reconstitution system (Table 1 A).

The measurement of Ca^{2+} uptake rate by filtration method showed the same result. This is consistent with the previous result. An ample body of literature exists on the effects of SR membrane lipid composition on the transport and enzymatic activities of the Ca^{2+} ATPase (reviewed by Bennett et al., 1980; Hidalgo, 1985). Hidalgo et al.(1982) and Racker's group (Knowles & Racker, 1975; Knowles et al.,1975) proposed that PE & PC are essential for coupling of the Ca^{2+} -ATPase.

Navarro et al. (1984) proposed that Ca^{2+} transport/ATP hydrolysis coupling is regulated by the phospholipids capable of adopting non-bilayer structures. Cheng & Hui (1986) also reported that destabilization of phospholipid bilayer enhances the activity of membrane Ca^{2+} pump.

However, in the latter case it is not due to actual formation of nonbilayer structure, but rather the tendency to destabilize bilayer. According to partial phase diagram of the lipid mixtures of egg phosphatidylcholine and soybean phosphatidylethanolamine (Hui et al., 1981b) a range between 15% and 35% of egg PC in soybean PE shows a non bilayer structure. In our results the effects of lipid structures on Ca^{2+} ATPase activities are not fully coincide with a destabilization of bilayer. The ATP hydrolysis function of Ca^{2+} ATPase was highest at 25% PC in 75% PE (Table 1B).

A plausible explanation why the ATP hydrolysis activity is not correspond to the Ca^{2+} pumping activity at the reconstituted Ca^{2+} ATPase is not available at this time. The Ca^{2+} uptake measurement however reflects the net rate of Ca^{2+} accumulation inside the proteoliposomes containing Ca^{2+}-ATPase and is given by the difference between the influx of calcium by active transport and the efflux due to leakage. Therefore, uncoupling of Ca^{2+} transport/ATP hydrolysis of Ca^{2+}-ATPase may be attributed to either a head group preference or a preference for more saturated lipids of the protein. In our reconstitution system both phospholipid (Soybean PE and egg PC) are unsaturated. At 23℃, the temperature at which the assays were performed, the lipids of the proteoliposomes are in the liquid crystalline phase.

Therefore, membrane fluidity may be an important regulator of the Ca^{2+}-ATPase function even though the lack of correlation between the measured order parameter of an ESR probe 5-deoxylstearic acid and the ATPase activity of Ca^{2+}-ATPase reconstituted with different lipid composition was shown by East et al. (1984). In contrast, Cheng et al. (1986) proposed that the Ca^{2+} transport function of the Ca^{2+}-ATPase depend on some other intrinsic properties of membrane lipids rather than the state of membrane order.

On the other hand, the amount of Ca^{2+}-ATPase protein reconstituted into phospholipid vesicles appeared as function of the lipid composition. As shown in Figure 13, the maximum incorporation occurred at a region between 15% and 35% of egg PC in soybean PE. It shows that the maximal incorporation efficiency region coincides with the highest occurrence of lipidic particles or interbilayer connections in the egg PC/soybean PE phase diagram (Hui et al., 1981). This indicates that preexisting defects in the lipid bilayer promote protein incorporation into the bilayer during reconstitution.

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═ 국문초록 ═

지질의 물리학적 성상이 Ca^{2+}-ATPase 활성도에 미치는 영향

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세포막을 구성하고 있는 지질의 물리학적 성상이 단백질의 세포막 속으로의 삽입과정 및 단백질의 기능에 미치는 영향을 관찰하기 위하여 골격근의 근세망(SR)으로부터 Ca^{2+}-ATPase 단백질을 분리한 후 이를 세포막의 주 구성성분인 포스파티딜콜린(PC)과 포스파티딜에타노라민(PE)의 혼합지질과 재조합(reconstitution)시켰다. 이와같이 인공적으로 재조합된 구조물에서 Ca^{2+}-ATPase의 기능을 측정하기 위하여 칼슘지시색소인 아르세나죠III(AIII)를 이용한 분광방법과 방사선동위원소를 이용한 여과법으로 칼슘흡수율을 측정하였고 또한 ATP 가수분해 능력을 측정하였다. 실험결과 칼슘의 흡수율은 포스파티딜코린의 함량이 많은 혼합지질과 재조합시킬 때에 증가하였고, ATP 가수분해 능력은 포스파티딜함량이 25%까지는 포스파티딜코린의 양에 비례하여 증가하였으나 50% 이상에서는 약간 감소하는 경향을 보였다. 한편 지질세포막속으로 단백질이 삽입되는 양은 포스파티딜 함량이 25%일 때 최고의 값을 보였으며 함량이 그 이하 또는 이상일 때는 감소하였다. 이상의 실험결과로보아 단백질의 기능은 세포막이 "bilayer" 구조를 갖출때에 증가하고 세포막속으로 단백질이 삽입되는 양은 세포막이 "non-bilayer" 구조를 형성할 때에 증가함을 알 수 있다.