Nelson, N., Nelson, H., Naim, V., & Neumann, J. (1971) *Arch. Biochem. Biophys. 145*, 263–267.
Ort, D. R., Dilley, R. A., & Good, N. E. (1976) *Biochim. Biophys. Acta 449*, 108–124.
Petrack, B., Cranston, A., Sheppy, F., & Farron, F. (1965) *J. Biol. Chem. 250*, 906–914.
Saha, S., & Good, N. E. (1970) *J. Biol. Chem. 245*, 5017–5021.
Trebst, A., & Pistorious, E. (1965) *Z. Naturforsch. C: Biochem., Biophys., Biol., Virol. 20C*, 143–147.
Venturoli, G., & Melandri, B. A. (1982) *Biochim. Biophys. Acta 680*, 8–16.

Effect of Lipid Composition on the Calcium/Adenosine 5′-Triphosphate Coupling Ratio of the Ca^{2+}-ATPase of Sarcoplasmic Reticulum†

Javier Navarro, Maria Toivio-Kinnucan, and Efraim Racker*

ABSTRACT: The Ca^{2+}-ATPase of sarcoplasmic reticulum was purified and depleted of proteolipids by solubilization in Triton X-100 and by fractionation on a DE-52 column. The protein reconstituted by deoxycholate–cholate dialysis at low lipid to protein ratios (2–5 mg of lipid/mg of protein), with either dioleoylphosphatidylethanolamine or monogalactosyldiglyceride, exhibited high initial rates of ATP-dependent Ca^{2+} uptake [300–900 nmol min^{-1} (mg of protein)$^{-1}$] and coupling ratios (Ca^{2+} transported/ATP hydrolyzed) up to 1.2. Ca^{2+}-ATPase reconstituted with lipids of increasing degrees of methylation (dioleoylphosphatidylethanolamine, dioleoylmonomethylphosphatidylethanolamine, dioleoyldimethylphosphatidylethanolamine and dioleoylphosphatidylcholine) or increasing degrees of glycosylation (monogalactosyldiglyceride and digalactosyldiglyceride) revealed a progressive decrease in both ATP-dependent Ca^{2+}-uptake and coupling ratios. The rate and extent of Ca^{2+} uptake decreased as the dioleoylphosphatidylethanolamine/dioleoylphosphatidylcholine or monogalactosyldiglyceride/dioleoylphosphatidylcholine molar ratios in the reconstituted vesicles were reduced. Vesicles reconstituted with high molar ratios of dioleoylphosphatidylethanolamine/dioleoylphosphatidylcholine or monogalactosyldiglyceride/dioleoylphosphatidylcholine and at a high lipid to protein ratio became leaky and released the Ca^{2+} accumulated inside the vesicles when the temperature of the incubation mixture was increased (e.g., from 20 to 37 °C). Freeze–fracture electron microscopy of reconstituted vesicles incubated at 37 °C demonstrated fusion of vesicles and formation of hexagonal II structures. Reconstitution of the Ca^{2+}-ATPase with other phospholipids such as dioleoylphosphatidylcholine, dioleoylphosphatidylglycerol, cardiolipin, bovine brain phosphatidylserine, phosphatidylinositol, and mixtures of dioleoylphosphatidylcholine and cholesterol catalyzed Ca^{2+}-dependent ATP hydrolysis [0.5–2 μmol of P_i min^{-1} (mg of protein)$^{-1}$] but low rates of Ca^{2+} uptake [5–10 nmol min^{-1} (mg of protein)$^{-1}$]. Our results suggest that the "coupling state" of the Ca^{2+}-ATPase as numerically expressed as the Ca^{2+}/ATP ratio is stabilized by cone-shaped lipid molecules (e.g., dioleoylphosphatidylethanolamine and monogalactosyldiglyceride).

Ca^{2+}-ATPase[1] of sarcoplasmic reticulum membranes couples the hydrolysis of ATP to Ca^{2+} transport. This protein has been purified and reconstituted into phospholipid vesicles by several methods (Racker, 1979). Reconstitution experiments with the Ca^{2+}-ATPase by deoxycholate–cholate dialysis demonstrated that phosphatidylethanolamine is required for Ca^{2+} uptake. Reconstitution of the enzyme with acetyl-PE yielded vesicles lacking both ATP-hydrolysis and Ca^{2+}-transport activities, which were restored by addition of suitable amounts of stearoylamine or oleoylamine (Knowles et al., 1975). Recently, Hidalgo et al. (1982) demonstrated that blockage of the amino group of PE in sarcoplasmic reticulum vesicles with fluorescamine resulted in low coupling of the Ca^{2+}-ATPase. Ca^{2+} transport was inhibited, but Ca^{2+}-dependent ATP hydrolysis was unaffected. These observations suggest that the amino group of PE is essential for coupling in the Ca^{2+}-ATPase of sarcoplasmic reticulum. However, reconstitution by freeze–thaw sonication yielded different results (Zimniak & Racker, 1979; Caffrey & Feigenson, 1981). Phosphatidylcholine vesicles transported Ca^{2+} though not nearly as well as phosphatidylcholine–phosphatidylethanolamine vesicles. In order to explore the role of phosphatidylethanolamine, we considered the possibility that coupling of the Ca^{2+}-ATPase depends on the presence of lipids capable of adopting nonbilayer structures (e.g., PE's tend to assemble into hexagonal II structures) as described by Cullis et al. (1982). We have therefore reconstituted the Ca^{2+}-ATPase with several lipids and examined Ca^{2+} transport and their tendency to form hexagonal structures. Our results support

† From the Section of Biochemistry, Molecular and Cell Biology, Division of Biological Sciences, Cornell University, Ithaca, New York 14853. *Received June 24, 1983.* This investigation was supported by PHS Grant CA-08964, awarded by the National Cancer Institute, DHHS, and the American Heart Association (Southern New York Chapter, Inc., Northern New York Chapter, Inc., and Dutchess County Chapter, Inc.). J.N. was a postdoctoral fellow of the Muscular Dystrophy Association.

[1] Abbreviations: ATPase, adenosinetriphosphatase; PE, phosphatidylethanolamine from natural sources; DOPE, dioleoylphosphatidylethanolamine; DOPC, dioleoylphosphatidylcholine; MGDG, monogalactosyldiglyceride; PI, phosphatidylinositol; PS, phosphatidylserine; CL, cardiolipin; DOPG, dioleoylphosphatidylglycerol; DGDG, digalactosyldiglyceride; DO(1M)PE, dioleoylmonomethylphosphatidylethanolamine; DO(2M)PE, dioleoyldimethylphosphatidylethanolamine; Hepes, *N*-(2-hydroxyethyl)piperazine-*N*′-2-ethanesulfonic acid; Tris, tris(hydroxymethyl)aminomethane.

0006-2960/84/0423-0130$01.50/0

the idea that the increased coupling is associated with the polymorphic phase behavior of PE.

Materials and Methods

Synthetic and natural phospholipids were purchased from Avanti Polar-Lipids, Inc. (Birmingham, AL). Monogalactosyldiglyceride and digalactosyldiglyceride were obtained from Applied Science Laboratories, Inc. (State College, PA). Bio-Beads SM-2 were purchased from Bio-Rad Laboratories (Richmond, CA). A23187 was generously supplied by Dr. R. Hamill. All other reagents were obtained from Sigma.

Reconstitution of the Ca^{2+}-ATPase. Sarcoplasmic reticulum vesicles were prepared according to the method of MacLennan (1970). Partial purification of the Ca^{2+}-ATPase was performed by method II of Meissner et al. (1973). Ca^{2+}-ATPase was delipidated and depleted of proteolipids by solubilization in Triton X-100 and chromatography on a DE-52 column as described by Green (1975). The fractions containing Ca^{2+}-ATPase activity were combined and relipidated by addition of 0.5–1 mg of DOPC/mg of protein. Triton X-100 was removed by addition of 50 mg of Bio-Beads SM-2/mg of protein and incubation for 1 h at 0 °C. After separation of the Bio-Beads by filtration, the solution containing the purified protein was centrifuged at 150000*g* for 30 min. The pellet was resuspended in 0.3 M sucrose–1 mM Hepes (pH 7.4) at 10–15 mg/mL and stored at −70 °C.

Unless otherwise specified, reconstitution of the Ca^{2+}-ATPase was carried out as described previously (Knowles & Racker, 1975) with some minor modifications. In brief, the purified Ca^{2+}-ATPase was solubilized with 0.5–0.6 mg of sodium deoxycholate/mg of protein in 10% glycerol–10 mM Tris-HCl (pH 8.0)–0.5 M KCl. The insoluble material was removed by centrifugation at 150000*g* for 45 min. The soluble protein (1 mg) was mixed with 1–3 mg of lipids which were dissolved in potassium cholate at a weight ratio of 1/1 (lipid/detergent). The mixture was dialyzed against 0.4 M potassium phosphate, pH 7.4, for 17 h at 4 °C.

Ca^{2+} Uptake and ATP Hydrolysis. The transport assay contained 50 mM Tris-HCl (pH 7.5), 0.1 M KCl, 0.7 M sucrose, 10 mM $MgCl_2$, 0.1 mM $^{45}CaCl_2$ (20 μCi/μmol), 3 mM ATP, and 20 μg of protein/mL. The reaction was initiated at 20 °C by addition of reconstituted vesicles. The final volume of the assay was 250 μL. The transport was terminated by passing the reaction mixture through Dowex–Tris columns previously equilibrated with 0.25 M sucrose and 10 mg of bovine serum albumin. Vesicles loaded with $^{45}CaCl_2$ were eluted from the column with 0.25 M sucrose as described (Gasko et al., 1976). After addition of scintillation fluid, the radioactivity was counted in a Beckman scintillation counter.

ATP hydrolysis was measured in the same incubation mixture used for Ca^{2+} uptake in the presence of [γ-^{32}P]ATP (3 μCi/μmol) as described by Knowles & Racker (1975).

Freeze–Fracture Electron Microscopy. The freeze–fracture experiments were carried out with a Balzer 360 M freeze–etch device with a QSZ 101 quartz crystal thin-film monitor for Pt shadowing. Reconstituted vesicles were centrifuged at 150000*g* for 1 h at 2 °C and resuspended in a small volume of 0.4 M potassium phosphate, pH 7.4, in the presence or absence of 25% glycerol. Samples were incubated at various temperatures for 20 min in a water bath and then quickly frozen with liquid propane cooled to liquid nitrogen temperature. Samples were stored up to 3–4 h and then fractured at −105 °C. Pt/C shadowing was performed at 45 °C and C coating from above. The samples were observed with a Philips 300 electron microscope.

Lipid Analysis. Lipids were extracted from the dialyzed reconstituted vesicles with 25 volumes of chloroform–methanol (2:1) and quantitated by determination of inorganic phosphate according to Ames (1966). The lipids were separated by thin-layer chromatography with a solvent system of chloroform–methanol–acetic acid–water (65:43:1:3). The spots containing the phospholipids were scraped and eluted with chloroform–methanol (2:1) and quantitated by phosphate determination.

Protein Determination. Protein was determined by the method of Lowry et al. (1951) with bovine serum albumin as standard.

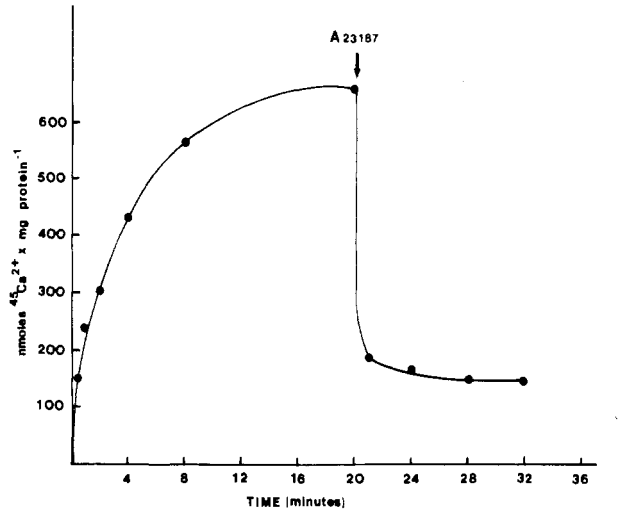

FIGURE 1: Reconstitution of Ca^{2+}-ATPase with a DOPE/DOPC molar ratio of 10. Reconstitution and assay was performed as described under Materials and Methods. The final lipid to protein weight ratio was 2/1. A23187 at a final concentration of 5 μg/mL was added to the incubation mixture at 20 min.

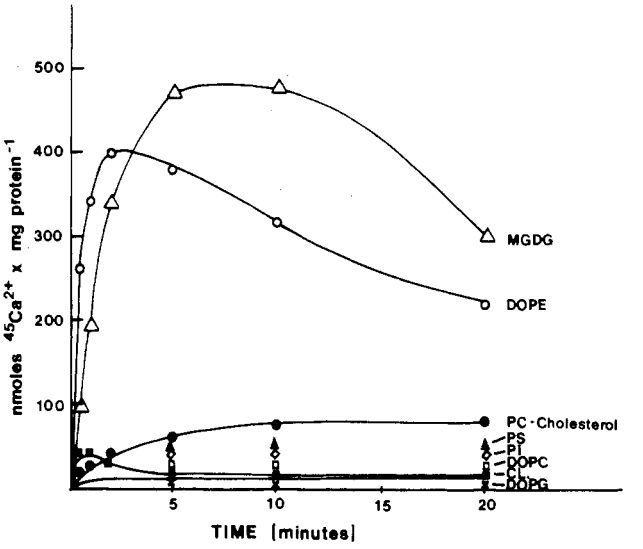

FIGURE 2: Reconstitution of Ca^{2+}-ATPase with several lipids. Experimental conditions were as described under Materials and Methods. The final lipid to protein weight ratio was 4.5.

Results

Ca^{2+}-ATPase reconstituted at a lipid to protein ratio of 2 (w/w) and at a molar ratio of DOPE/DOPC of about 10 catalyzed ATP-dependent Ca^{2+} uptake (Figure 1). Ca^{2+} accumulated inside the reconstituted vesicles was rapidly released upon addition of ionophore A23187.

In order to determine the lipid requirement for ATP-dependent Ca^{2+} uptake, the Ca^{2+}-ATPase depleted of proteo-

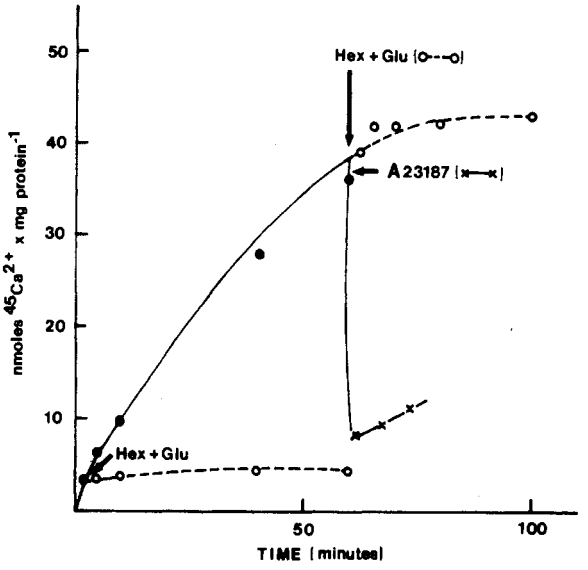

FIGURE 3: Leakiness of Ca^{2+}-ATPase reconstituted with DOPC. Vesicles were loaded with $^{45}Ca^{2+}$ for 60 min at 20 °C, and then 20 mM glucose and 50 units of hexokinase or 5 μg/mL A23187 were added to the incubation mixture. The lipid to protein weight ratio was 3.5.

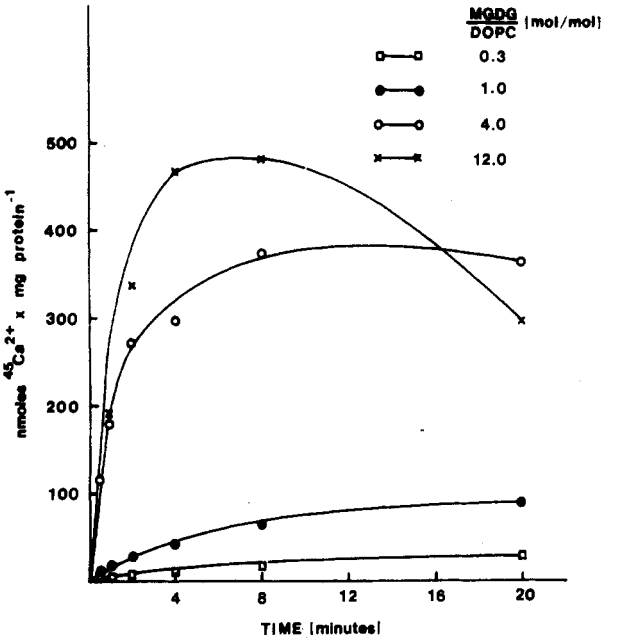

FIGURE 4: Ca^{2+} uptake of Ca^{2+}-ATPase reconstituted with mixtures of MGDG/DOPC at several molar ratios. The final lipid to protein ratio was 3.5.

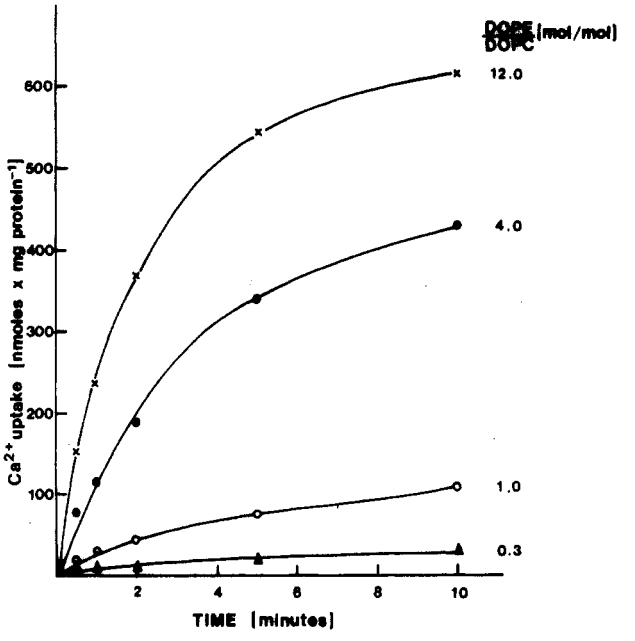

FIGURE 5: Ca^{2+} uptake of Ca^{2+}-ATPase reconstituted with mixtures of DOPE/DOPC at several molar ratios. The final lipid to protein ratio was 3.3.

lipids was reconstituted with several synthetic and natural lipids. Figure 2 demonstrates that Ca^{2+}-ATPase reconstituted with DOPE or MGDG catalyzed an initial high rate of ATP-dependent Ca^{2+} uptake. Similar rates of Ca^{2+} uptake were obtained when the Ca^{2+}-ATPase was reconstituted with mixtures of DOPE/MGDG at several molar ratios (data not shown). On the other hand, Ca^{2+}-ATPase reconstituted with lipids such as DOPC, DOPG, PI, PS, DOPC cholesterol, or CL catalyzed Ca^{2+}-dependent ATP hydrolysis but low rates of ATP-dependent Ca^{2+} uptake (Figure 2). The low rates of Ca^{2+} uptake in reconstituted vesicles made with the above lipids may have been due to the formation of leaky vesicles that are unable to accumulate Ca^{2+}. In order to test the degree of leakiness, reconstituted vesicles with DOPC were allowed to slowly accumulate $^{45}Ca^{2+}$ over a period of 60 min in the presence of ATP. Ca^{2+} uptake was then stopped by removing ATP from the incubation mixture upon addition of hexokinase and glucose. As shown in Figure 3 after a short lag period the rate of Ca^{2+} uptake stopped, and there was no significant $^{45}Ca^{2+}$ efflux. Addition of hexokinase and glucose to the incubation mixture prior to the initiation of the transport assay blocked the ATP-dependent Ca^{2+} uptake (Figure 3). However, addition of A23187 induced a rapid release of Ca^{2+}. This experiment suggests that the slow rate of Ca^{2+} transport in vesicles reconstituted with DOPC is not due to leakage.

Properties of the Ca^{2+}-ATPase Reconstituted Vesicles. It was shown (Cullis et al., 1982; Shipley et al., 1973) that DOPE and MGDG tend to adopt nonbilayer structures. This may explain why reconstituted vesicles made with these lipids which gave high initial rates of Ca^{2+} uptake released accumulated Ca^{2+} under the experimental conditions shown in Figure 2. On the other hand, DOPC, DOPG, PS, and PI tend to form stable lipid bilayer structures (Israelachvili et al., 1980; Luzzati & Tardieu, 1974). We have therefore analyzed vesicles reconstituted at various molar ratios of DOPE/DOPC and MGDG/DOPC at different lipid to protein ratios and at different temperatures with respect to both Ca^{2+} accumulation and Ca^{2+} release. Ca^{2+}-transport assays in reconstituted vesicles made with several molar ratios of MGDG/DOPC have indicated that inclusion of DOPC reduces the rate and extent of Ca^{2+} uptake as well as the release of $^{45}Ca^{2+}$ accumulated into the vesicles (Figure 4). A ratio of MGDG/DOPC of about 4 was optimal for obtaining stable vesicles with a high rate of Ca^{2+} uptake. Under similar conditions vesicles reconstituted with DOPE and DOPC at several molar ratios demonstrated negligible release of $^{45}Ca^{2+}$ (Figure 5). Longer incubation times did not result in significant Ca^{2+} release (data not shown). Furthermore, it is shown that the rate and extent of Ca^{2+} uptake decrease as the DOPE/DOPC ratios are reduced. Determinations of the coupling of Ca^{2+} uptake to ATP hydrolysis (Ca^{2+}/ATP) as a function of DOPE/DOPC and MGDG/DOPC ratios are shown in Figure 6. Coupling ratios up to 1–1.2 were seen at DOPE/DOPC or MGDG/DOPC molar ratios of 12 (Figure 6).

Since coupling of the Ca^{2+}-ATPase appears to depend on the presence of lipids capable of adopting hexagonal II

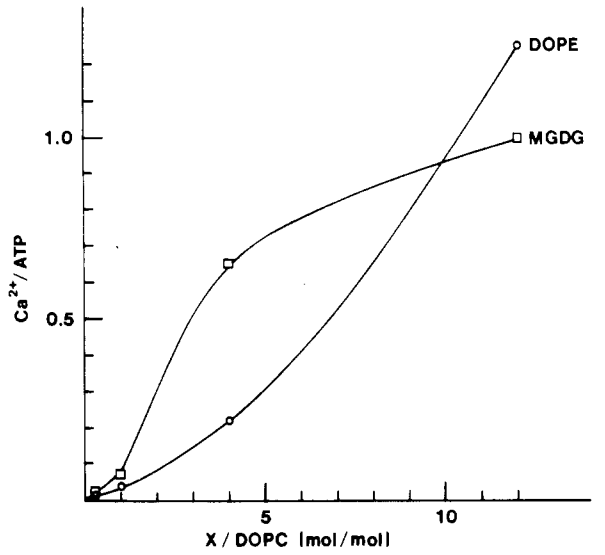

FIGURE 6: Coupling ratios (Ca^{2+}/ATP) of the Ca^{2+}-ATPase reconstituted into lipid vesicles made with various ratios of DOPE/DOPC or MGDG/DOPC. X represents either DOPE or MGDG. The lipid to protein ratio was 3.3. The Ca^{2+}/ATP ratios were calculated by measuring the initial rates of Ca^{2+} uptake and ATP hydrolysis.

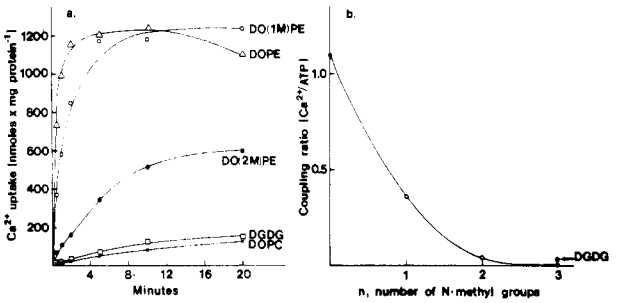

FIGURE 7: (a) Influence of the degree of N-methylation of DOPE and DGDG on the ATP-dependent Ca^{2+} uptake. Ca^{2+}-ATPase was reconstituted with the specified lipids at a lipid to protein ratio of 3.5. The incubation temperature was 30 °C. (b) Dependence of the coupling ratio on the degree of N-methylation of DOPE and DGDG. Initial rates of Ca^{2+} uptake and ATP hydrolysis were employed for the calculation of the coupling ratios. Conditions were the same as described in part a.

structures (e.g., MGDG and DOPE) which in turn are determined by the geometrical features of the lipid molecules (Israelachvili et al., 1980; Wieslander et al., 1978), we have performed reconstitution experiments with dioleoylphospholipids of increasing degree of N-methylation from DOPE to DOPC in order to analyze the effect of size of the polar head on the coupling of the Ca^{2+}-ATPase. Figure 7 shows that Ca^{2+}-ATPase reconsituted with dioleoylphospholipids of increasing degrees of N-methylation (DOPE to DOPC) revealed a progressive reduction in the ATP-dependent Ca^{2+} uptake as well as the coupling ratio. Of particular interest is that DGDG, which has a larger polar head than MGDG, was not very efficient in yielding reconstituted vesicles with high rates of Ca^{2+} uptake or coupling ratios (Figure 7).

Experiments on the ATP-dependent Ca^{2+} uptake as a function of temperature have revealed that release of $^{45}Ca^{2+}$ is enhanced as the incubation temperature is raised. Reconstituted vesicles made with mixtures of DOPE/DOPC at molar ratios of 12 showed negligible Ca^{2+} release at 10 and 25 °C. When the temperature was raised to 37 °C, the initial rate of Ca^{2+} uptake was enhanced, but the rate of $^{45}Ca^{2+}$ release was greatly increased (Figure 8a). In vesicles reconstituted with mixtures of MGDG/DOPC at molar ratios of 12, the release of $^{45}Ca^{2+}$ was evident even at 20 °C and became very rapid at 37 °C (Figure 8b). Freeze–fracture electron microscopy of reconstituted vesicles made with mixtures of DOPE/DOPC at a molar ratio of 12 and incubated at 20 °C for 20 min revealed vesicular structures (Figure 9a); however, incubation of the same preparation at 37 °C for 20 min induced fusion of vesicles (Figure 9b,c). Formation of hexagonal II structures was only observed in functional vesicles reconstituted in the presence of 5 mM Tris, pH 7.4, and 0.25 M sucrose instead of 0.4 M potassium phosphate, pH 7.4 (Figure 9d).

Experiments on the reconstitution of the Ca^{2+}-ATPase at different lipid to protein ratios have shown that the release of $^{45}Ca^{2+}$ increased when the lipid to protein ratio was raised from 2.7 to 4.6 (Figure 10) in the reconstituted vesicles. This finding suggests that the Ca^{2+}-ATPase stabilizes the vesicular structure. The Ca^{2+}/ATP coupling ratio decreased from 1.2 to 0.9 when the lipid to protein ratio was raised from 2.7 to 4.6. The coupling ratios remained almost constant for about 4 min when little Ca^{2+} release was observed. When Ca^{2+} release was pronounced, the ratio already dropped after 1 to 2 min.

Discussion

The lipid requirement for coupling the Ca^{2+}-ATPase has been previously analyzed in sarcoplasmic reticulum as well as in Ca^{2+}-ATPase reconstituted into phospholipid vesicles. ATP hydrolysis catalyzed by the Ca^{2+}-ATPase does not appear to require specific lipids provided that the lipid is above the gel to liquid-crystal phase transition temperature (Knowles et al., 1976; Moore et al., 1981; Hesketh et al., 1976) and forms a bilayer which is neither too thick nor too thin (Caffrey & Feigenson, 1981). However, the ATP-dependent Ca^{2+} uptake seems to be sensitive to the lipid composition of the membrane. Of particular interest is the role of PE. Knowles et al. (1975) demonstrated that reconstitution of the Ca^{2+}-ATPase with acetyl-PE abolished both ATP hydrolysis and Ca^{2+} uptake which was restored by addition of suitable amounts of stearoylamine and oleoylamine. Furthermore, Knowles & Racker (1975) have shown that the coupling ratio (Ca^{2+}/ATP) of the reconstituted Ca^{2+}-ATPase depends upon the ratio of PE/PC in the lipid mixture. Recently, Hidalgo et al. (1982) have shown that blockage of the amino group of PE with fluorescamine in sarcoplasmic reticulum vesicles resulted in low coupling of the Ca^{2+}-ATPase. These findings showed that the amino group of PE was essential for coupling the Ca^{2+}-ATPase of sarcoplasmic reticulum.

In this work we show that Ca^{2+}-ATPase reconstituted with MGDG as well as DOPE catalyzes ATP-driven Ca^{2+} uptake. Ca^{2+}-ATPase reconstituted with lipid mixtures at several ratios of DOPE/DOPC or MGDG/DOPC have revealed that the ATP-driven Ca^{2+} uptake and coupling ratios were progressively enhanced when the molar ratios of DOPE/DOPC or MGDG/DOPC in the reconstituted vesicles were increased (Figures 4–6). Other lipids, including DOPC, DOPG, PI, PS, CL, or DOPC and cholesterol mixtures are not very effective (Figure 2).

It appears that the quantitative requirement for PE can be diminished by increasing the degree of unsaturation of PE. For example, Knowles & Racker (1975) found that the highest Ca^{2+} uptake mediated by reconstituted vesicles was obtained when the molar ratio of soybean PE/PC was about 4. In sarcoplasmic reticulum PE accounts for 20–30% of the total lipid, but PE is highly unsaturated. The transition temperature of bilayer to hexagonal II structures of PE from sarcoplasmic reticulum is about −10 °C in contrast to the transition tem-

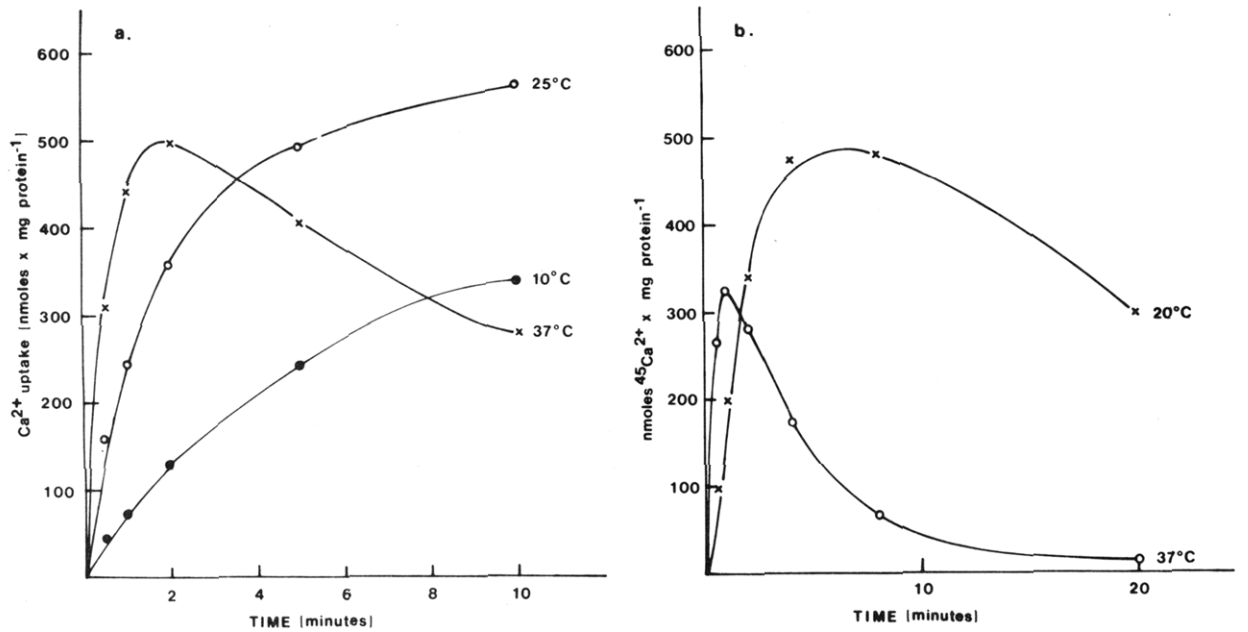

FIGURE 8: (a) Effect of temperature on the leakage of the $^{45}Ca^{2+}$ accumulated by reconstituted vesicles made with mixtures of DOPE/DOPC at a molar ratio of about 12. The lipid to protein ratio was 3.4. (b) Effect of temperature on the leakage of the $^{45}Ca^{2+}$ accumulated by reconstituted vesicles made with mixtures of MGDG/DOPC at a molar ratio of about 12. The lipid to protein ratio was 3.3.

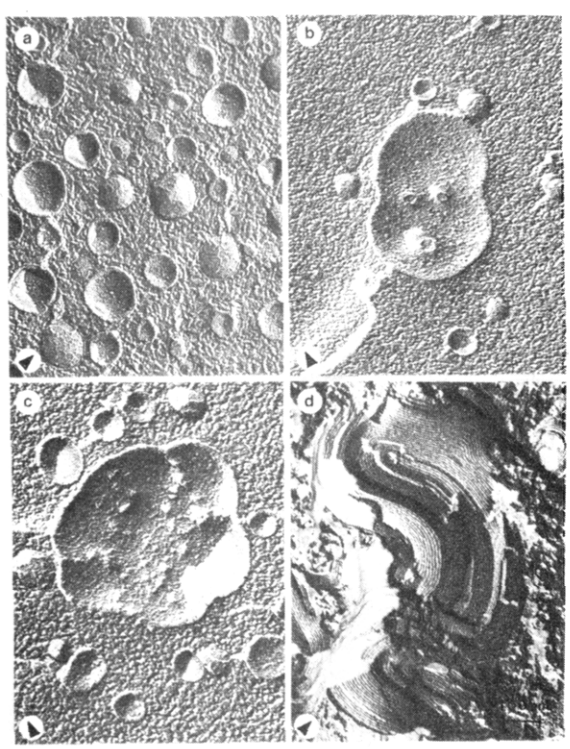

FIGURE 9: (a) Freeze–fracture electron microscopy of Ca^{2+}-ATPase reconstituted vesicles made with mixtures of DOPE/DOPC at a molar ratio of about 12. The lipid to protein ratio was 3.4. Freezing of the sample was carried out in the presence of 25% glycerol and after incubation of the vesicles at 20 °C for 20 min. (b, c) Freeze–fracture electron microscopy of Ca^{2+}-ATPase reconstituted vesicles prepared as described in Figure 8a, except that the vesicles were incubated at 37 °C for 20 min. (d) Reconstituted vesicles prepared as described in Figure 8b, except that the dialysis buffer was 5 mM Tris, pH 7.4, and 0.25 M sucrose instead of 0.4 M potassium phosphate, pH 7.4.

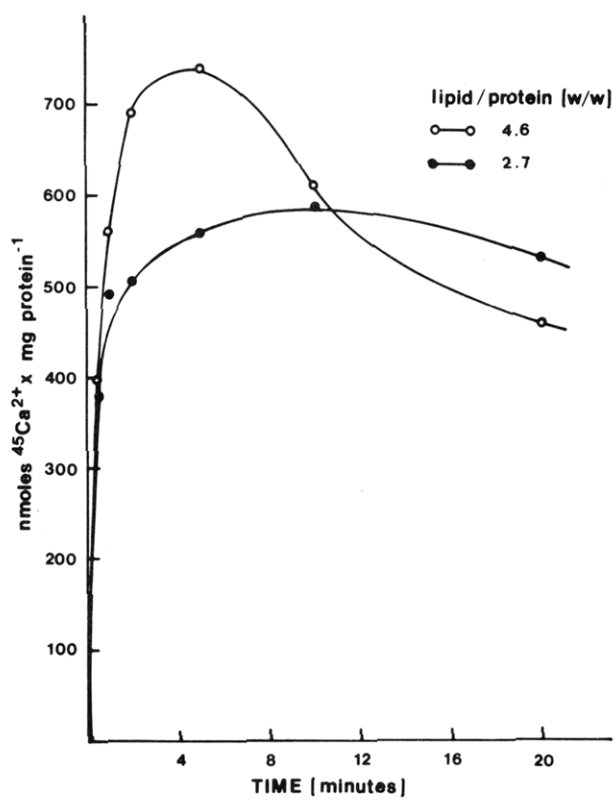

FIGURE 10: Ca^{2+} uptake of the Ca^{2+}-ATPase reconstituted vesicles prepared at different lipid to protein ratios. The major phospholipid was DOPE. Ca^{2+} uptake was carried out at 20 °C as described under Materials and Methods.

perature of DOPE which is about 10 °C (Cullis et al., 1982).

DOPE and MGDG are lipids that tend to destabilize the lipid bilayer by inducing the formation of nonbilayer structures (Cullis et al., 1982). This property is directly related to the polymorphic phase behavior exhibited by DOPE and MGDG [transition of bilayer to hexagonal II structures (H II)] (Cullis et al., 1982; Wieslander et al., 1978; Shipley et al., 1973). This polymorphic phase behavior is determined at least in part by the geometrical features of PE and MGDG (cone-shaped molecules) which in turn is dependent on the size of the polar head, degree of unsaturation of the hydrocarbon chain, presence of cholesterol, and temperature (Israelachvili et al., 1980; Tilcock et al., 1982).

In this work the Ca^{2+}-ATPase reconstituted with lipids of increasing degrees of methylation from DOPE to DOPC or

increasing degrees of glycosylation from MGDG to DGDG revealed a progressive decrease in both ATP-dependent Ca^{2+} uptake and coupling ratios (Figure 7). This increase in size of the polar head reduces the cone shape of MGDG and DOPE, and instead the lipids tend to adopt cylindrical shapes which in turn increases the hexagonal II temperature transitions (Seddon et al., 1983). In conclusion, our findings support the idea that good coupling of the Ca^{2+}-ATPase requires the presence of cone-shaped lipids (e.g., DOPE and MGDG) rather than lipids with cylindrical shapes like DOPC, DOPG, DGDG, etc. It is possible that the coupling of the Ca^{2+}-ATPase is controlled by a specific arrangement of cone-shaped lipids at the lipid to protein interface. Furthermore, our findings also support the idea that the amino group of PE is not essential for coupling the Ca^{2+}-ATPase since MGDG is able to substitute for DOPE in coupling the Ca^{2+}-ATPase under the experimental conditions used. Warren et al. (1974), Zimniak & Racker (1978), and Anderson et al. (1983) have shown that significant rates of Ca^{2+} uptake can be observed when the Ca^{2+}-ATPase is reconstituted into phosphatidylcholine vesicles by using other methods.

^{31}P NMR and ^{1}H NMR experiments with sarcoplasmic reticulum vesicles have strongly suggested the presence of nonbilayer structures (Davis & Inesi, 1971; Cullis & de Kruijff, 1979). It is possible that modification of PE with fluorescamine in sarcoplasmic reticulum or reconstitution of Ca^{2+}-ATPase with acetyl-PE has modified the specific arrangement adopted by PE in the vicinity of the Ca^{2+}-ATPase. The ability of stearoylamine and oleoylamine to restore ATP hydrolysis and Ca^{2+} transport of the Ca^{2+}-ATPase reconstituted with acetyl-PE may be due to the fact that these amines induce nonbilayer structures (Hope & Cullis, 1981).

We have also shown that vesicles reconstituted with high molar ratios of DOPE/DOPC or MGDG/DOPC and at a high lipid to protein ratio became leaky and released the Ca^{2+} accumulated inside the vesicle when the temperature of the incubation mixture was raised to 37 °C. Under similar conditions, freeze–fracture electron microscopy of reconstituted vesicles made with mixtures at high DOPE/DOPC ratios and incubation at 37 °C have revealed fusion of vesicles as well as hexagonal II structures (Figure 9).

Vesicles reconstituted with mixtures of MGDG/DOPC at high molar ratios exhibited greater $^{45}Ca^{2+}$ leakage (Figures 4 and 8b) than vesicles made with equivalent molar ratios of DOPE/DOPC (Figures 5 and 8a). This may be due to the fact that MGDG has a lower transition temperature (bilayer to H II structures) than DOPE (Shipley et al., 1973; Tilcock et al., 1982). These findings suggest that the $^{45}Ca^{2+}$ release is due to fusion of reconstituted vesicles and their further transformation to hexagonal II structures that takes place only after the temperature is raised to 37 °C. Of particular interest is that the Ca^{2+} release was prevented when reconstituted vesicles were made at low lipid to protein ratios (Figure 10) which suggests that Ca^{2+}-ATPase stabilizes the bilayer structure of the reconstituted vesicles.

Registry No. ATPase, 9000-83-3; DOPE, 2462-63-7; DOPC, 10015-85-7; DO(1M)PE, 87803-74-5; DO(2M)PE, 87803-75-6; DOPG, 62700-69-0; cholesterol, 57-88-5; ATP, 56-65-5; Ca, 7440-70-2.

References

Ames, B. N. (1966) *Methods Enzymol. 8*, 115–117.

Andersen, J. P., Skriver, E., Mohrous, T. S., Moller, J. V. (1983) *Biochim. Biophys. Acta 728*, 1–10.

Caffrey, M., & Feigenson, G. W. (1981) *Biochemistry 20*, 1949–1961.

Cullis, P. R., & de Kruijff, B. (1979) *Biochim. Biophys. Acta 559*, 399–420.

Cullis, P. R., de Kruijff, B., Hope, M. J., Verkleij, A. J., Nayar, R., Forsen, S. B., Tilcock, C., Madden, T. D., & Bolly, M. B. (1982) *Membrane Fluidity in Biology*, Vol. 1, pp 39–81, Academic Press, New York.

Davis, D. G., & Inesi, G. (1971) *Biochim. Biopys. Acta 241*, 1–8.

Gasko, O. D., Knowles, A. F., Shertzer, H. G., Suolinna, E.-M., & Racker, E. (1976) *Anal. Biochem. 72*, 57–65.

Green, N. M. (1975) in *Calcium Transport and Contraction and Secretion* (Carafoli, E., et al., Eds.) pp 339–348, North-Holland, Amsterdam.

Hesketh, T. R., Smith, G. A., Houslay, M. D., McGill, K. A., Birdsall, N. J. M., Metcalfe, J. C., & Warren, G. B. (1976) *Biochemistry 15*, 4145–4151.

Hidalgo, C., Petrucci, D. A., & Vergara, C. (1982) *J. Biol. Chem. 257*, 208–216.

Hope, M. J., & Cullis, P. R. (1981) *Biochim. Biophys. Acta 640*, 82–90.

Israelachvili, J. N., Marcelja, S., & Horn, R. G. (1980) *Q. Rev. Biophys. 13*, 121–200.

Knowles, A. F., & Racker, E. (1975) *J. Biol. Chem. 250*, 3538–3544.

Knowles, A. F., Kandrach, A., Racker, E., & Khorana, H. G. (1975) *J. Biol. Chem. 250*, 1809–1813.

Knowles, A. F., Eytan, E., & Racker, E. (1976) *J. Biol. Chem. 251*, 5161–5165.

Lowry, O. H., Rosenbrough, N. J., Farr, A. L., & Randall, R. J. (1951) *J. Biol. Chem. 193*, 265–275.

Luzzati, V., & Tardieu, A. (1974) *Annu. Rev. Phys. Chem. 25*, 79–94.

MacLennan, D. H. (1970) *J. Biol. Chem. 245*, 4508–4518.

Meissner, G., Conner, G. E., & Fleischer, S. (1973) *Biochim. Biophys. Acta 298*, 246–269.

Moore, B. M., Lentz, B. R., Hoechli, M., & Meissner, G. (1981) *Biochemistry 20*, 6810–6817.

Racker, E. (1979) *Methods Enzymol. 55*, 699–711.

Racker, E., & Eytan, E. (1975) *J. Biol. Chem. 250*, 7533–7534.

Seddon, J. M., Cevc, G., & Marsh, D. (1983) *Biochemistry 22*, 1280–1289.

Shipley, G. G., Green, J. P., & Nichols, B. W. (1973) *Biochim. Biophys. Acta 311*, 531–544.

Tilcock, C. P. S., Bolly, M. B., Forren, S. B., & Cullis, P. R. (1982) *Biochemistry 21*, 4596–4601.

Warren, G. D., Toon, P. A., Birdsall, N. J. M., Lee, A. G., & Metcalfe, J. C. (1974) *Proc. Natl. Acad. Sci. U.S.A. 71*, 622–626.

Wieslander, A., Ulmius, J., Lindblom, G., & Fontell, K. (1978) *Biochim. Biophys. Acta 512*, 241–253.

Zimniak, P., & Racker, E. (1978) *J. Biol. Chem. 253*, 4631–4637.