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Fourier-Transform Infrared Studies of CaATPase/Phospholipid Interaction: Survey of Lipid Classes†

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ABSTRACT: CaATPase from rabbit skeletal muscle has been isolated, purified, delipidated, and reconstituted with retention of ATPase activity into lipid vesicles consisting respectively of 1,2-dipalmitoylphosphatidylethanolamine, 1-palmitoyl-2-oleoylphosphatidylethanolamine (POPE), 1-stearoyl-2-oleoylphosphatidylcholine (SOPC), and egg sphingomyelin. The effect of the enzyme on phospholipid order and melting characteristics were determined with Fourier-transform infrared spectroscopy. Taken together with prior data from this laboratory for 1,2-dipalmitoylphosphatidylcholine and 1,2-dioleoylphosphatidylcholine (DOPC), as well as for native sarcoplasmic reticulum (SR), three types of lipid response to protein incorporation have been observed: (1) Phospholipids with high levels of acyl chain unsaturation (DOPC or native SR) have their lipid acyl chains slightly ordered by CaATPase incorporation. The effect of protein on the gel–liquid crystal phase transition cannot be easily determined, since the cooperative melting even in these systems occurs at temperatures well below 0 °C. (2) Phospholipids with saturated acyl chains show slightly lowered melting temperatures and reduced cooperativity of melting upon CaATPase insertion. In addition, protein induces (at most) slight disorder into the acyl chains at temperatures removed from the lipid melting point. (3) The strongest response is observed for phospholipids containing one saturated and one unsaturated chain (POPE or SOPC) or heterogeneous systems with low levels of unsaturation (egg sphingomyelin). In these cases, relatively low protein levels diminish the magnitude of or completely abolish the phospholipid phase transition. In addition, substantial disorder is introduced into the acyl chain compared with the pure lipid both above and below its transition temperature. The current data suggest that specific models for lipid/protein interaction based on a single system will not be easily generalized. The interaction between lipid and protein appears to be a strong function of acyl chain unsaturation levels, so that the relative magnitudes of lipid/lipid and lipid/protein interactions, which determine lipid response to protein insertion, become strongly dependent upon both the particular lipids and proteins used.

The well-documented dependence of membrane-bound enzyme activity on the chemical structure or physical state of the phospholipid environment has resulted in many physical studies aimed at elucidation of the molecular basis of lipid/protein interaction [for reviews, see Gennis & Jones (1977) and Parsegian (1982)]. Spectroscopic investigations are generally directed toward determination of changes in the conformation or dynamics of either the lipid or protein component upon their association in reconstituted systems.

To date, the majority of studies involving reconstituted complexes have utilized a small fraction of the available lipid classes, frequently phosphatidylcholines with two saturated acyl chains. Yet this acyl chain distribution is not representative of native membrane environments. In particular, naturally occurring lipids often contain a saturated acyl chain at the *sn*-1 carbon and an unsaturated moiety at the *sn*-2. In addition, native membranes are heterogeneous with regard to lipid head group composition. The purpose of this study is to determine the effect of varying lipid composition (both head group and acyl chain) on protein-induced perturbation of lipid order and thermotropic properties.

The protein chosen for this investigation is CaATPase (ATP phosphohydrolase, EC 3.6.1.3) isolated from rabbit sarcoplasmic reticulum (SR).[1] This protein was selected for two reasons. First, techniques have been developed in several laboratories (Warren et al., 1974a,b; Hidalgo et al., 1976) for replacement of the native SR lipids with a variety of exogenous

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[1] Abbreviations: SR, sarcoplasmic reticulum; DPPE, 1,2-dipalmitoylphosphatidylethanolamine; POPE, 1-palmitoyl-2-oleoylphosphatidylethanolamine; SOPC, 1-stearoyl-2-oleoylphosphatidylcholine; ESPH, egg sphingomyelin; DPPC, 1,2-dipalmitoylphosphatidylcholine; DOPC, 1,2-dioleoylphosphatidylcholine; FT-IR, Fourier-transform infrared; ESR, electron spin resonance; NMR, nuclear magnetic resonance; Tris, tris(hydroxymethyl)aminomethane.

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lipids, with retention of enzymatic activity. Furthermore, substantial controversy as to the effect of CaATPase on lipid order/dynamics is apparent from the literature. ^{31}P NMR and ESR experiments (Selinsky & Yeagle, 1984; Thomas et al., 1982; Fernandez et al., 1980) of lipid properties in reconstituted systems were interpreted in terms of two distinct lipid populations—one with motional characteristics only slightly altered from pure lipid and the other with properties of an immobilized component. In contrast, other studies of native SR and reconstituted CaATPase using both ^{31}P and ^{2}H NMR (McLaughlin et al., 1981; Oldfield et al., 1978; Rice et al., 1979) have concluded that a single homogeneous lipid environment exists, at least on the time scale of the NMR experiments.

This study uses an independent method (FT-IR spectroscopy) to monitor changes in lipid configuration in systems where CaATPase has been reconstituted into a variety of phospholipid environments. The experimental technique effects several advantages. First, probe molecules, which might possibly perturb lipid order, are not required. As the IR spectrum of phospholipids is a sensitive function of the physical state and environment of the molecule, the technique is useful for probing subtle changes in lipid order and packing (Cameron et al., 1979; Mantsch et al., 1982a,b). Second, both lipid configuration and protein secondary structure are monitored in the experiment. Finally, small amounts of material ($\sim$1 mg of complex) suffice for data collection. A previous report from this laboratory (Mendelsohn et al., 1984) has shown that the physical state of the acyl chains within a given lipid class (PC's) markedly affects the response of lipid to CaATPase incorporation. 1,2-Dioleoylphosphatidylcholine (DOPC) is slightly ordered by protein, while 1,2-dipalmitoylphosphatidylcholine (DPPC) has its gel–liquid crystal phase transition rendered less cooperative and shifted to lower temperature as the level of protein incorporation is increased.

In this work, further studies delineating the effect of lipid head group and level of acyl chain unsaturation on the response of the lipid acyl chains to incorporation of CaATPase are reported, through experiments where the protein is reconstituted with 1-palmitoyl-2-oleoylphosphatidylethanolamine (POPE), 1,2-dipalmitoylphosphatidylethanolamine (DPPE), 1-stearoyl-2-oleoylphosphatidylcholine (SOPC), and egg sphingomyelin (ESPH). This series of molecules encompasses the main classes that occur in native SR (Marai & Kuksis, 1973; Hidalgo et al., 1976).

Materials and Methods

Lipids were purchased from Avanti Polar Lipids (Birmingham, AL) and evaluated for purity by thin-layer chromatography. Solvents were of the highest quality commercially available and were used without further purification. Water was doubly distilled.

Sample Preparation. Purified sarcoplasmic reticulum was obtained as described in a previous study (Mendelsohn et al., 1984). The endogenous lipids were exchanged by the deoxycholate-mediated lipid exchange protocol of Warren et al. (1974a) as modified by Hidalgo et al. (1976). The following weight ratios (protein/deoxycholate/lipid) were used for the samples indicated: SOPC, 3.5:1:1; POPE (lipid/protein mole ratio = 25:1), 3:1:1; POPE (lipid/protein mole ratio = 50:1), 3:1.1:1; DPPE, 2:1:1; ESPH, 3:1.2:1. Samples were incubated at 37 °C for 30 min except for POPE/CaATPase (lipid/protein mole ratio = 50:1), which was incubated at 0 °C for 2 h, and ESPH, which was incubated at 41 °C for 30 min and then at 0 °C for 1.5 h. Studies with radioactive deoxycholate showed the following molar ratios of detergent/protein remaining in the final complexes: POPE, 3.8:1; ESPH, 3.4:1.

Vesicles were assayed for lipid acyl chain length by gas chromatography of their methylated acyl chains. SOPC- and DPPE-containing complexes were transesterified as previously described (Mendelsohn et al., 1984). POPE-containing complexes were transesterified according to the method of Metcalfe and Schmitz (1961) with Supelco, Inc., BF_3 reagent (1 mL of reagent/mg of lipid). ESPH systems were transesterified according to Garver and Sweeley (1965). The lipids were extracted from the protein complex with 25:65 v/v MeOH/$CHCl_3$ followed by three extractions with diethyl ether. The extracts were combined and refluxed for 18 h in the presence of 5 mL of aqueous MeOH–HCl reagent (10:1:1 v/v/v MeOH/HCl/H_2O) at 80 °C. All methyl esters were extracted with diethyl ether and analyzed on a Perkin-Elmer isothermal chromatograph equipped with a column of 15% diethyleneglycol succinate on 80-mesh Carbosorb at a column temperature of 260 °C.

FT-IR. FT-IR spectra were obtained with a Mattson Instruments Sirius 100 FT-IR spectrophotometer interfaced with a Starlab supermicro computer. Samples were placed in a 25-μm Harrick cell equipped with CaF_2 windows mounted in a thermal jacket. Cell temperature was monitored with a digital thermometer from Bailey Instruments. Normally, 100 interferograms were coadded, apodized with a triangular function, and fast Fourier transformed to yield a spectrum with 4-cm^{-1} resolution. One level of zero filling was carried out. The resulting spectra were solvent subtracted in the C–H stretching region to remove the intense but nearly linear water absorption that underlies the 2800–3000-cm^{-1} region. Baseline leveling was then performed as necessary. Peak positions were determined with a center of gravity algorithm (Cameron et al., 1982) or a three-point parabolic fitting routine supplied with the FT-IR software. Multilamellar lipid vesicles were suspended in a 20 mM Tris–maleate buffer (pH 7.2) and lipid/protein complexes were suspended in a D_2O–Tris maleate buffer (pH 7.2).

CaATPase Activity. ATPase activities were measured with the coupled enzyme assay of Warren et al. (1974b). The activities varied somewhat from preparation to preparation. Typical values for the indicated preparations at 37 °C were as follows: SOPC/CaATPase (lipid/protein mole ratio = 100:1), 7.0 IU; POPE/CaATPase (lipid/protein mole ratio = 50:1, 4.1 IU; DPPC/CaATPase (lipid/protein mole ratio = 30:1), 2.8 IU. Activities for CaATPase in native SR range from 4 to 15 IU.

Results

Biochemical Characterization of Complexes. Gel electrophoresis patterns [not shown, but see Mendelsohn et al. (1984)] from purified SR demonstrate CaATPase to be the main protein present. In reconstituted vesicles, CaATPase constitutes 90–95% of the total protein. The ATPase activity levels of protein at 37 °C ranged from 4 to 15 IU.

The fatty acid compositions of the lipid acyl chains for several preparations used in the current work as determined by gas chromatography are given in Table I. Included for comparison are data for the DPPC/CaATPase complex used in an earlier FT-IR investigation (Mendelsohn et al., 1984). As shown in Table I, complexes prepared via deoxycholate-mediated lipid exchange followed by sucrose density gradient purification demonstrate that high levels of incorporation of the desired lipid species have been achieved. Since egg sphingomyelin has not, to our knowledge, previously been reconstituted with CaATPase, further demonstration of the success of the reconstitution protocols was deemed necessary.

Table I: Fatty Acid Compositions for Some Preparations Used in This Work

		lipid/protein mole ratio for							
	purified	POPE		egg sphingomyelin		DPPE		DPPC	
fatty acid[a]	SR, 30:1	lipid (pure)	complex, 50:1	lipid (pure)	complex, 30:1	lipid (pure)	complex, 70:1	lipid (pure)	complex, 30:1
12:0	0.9								1.3
14:0	0.9	2.8	2.2	14.4	20.0	10.6	19.3		0.5
16:0	30.4	51.2	41.9	44.9	63.0	79.3	77.9	>99	95.8
18:0	8.2		9.4	9.6	5.9	2.1			0.9
18:1	22.6	46.0	44.6	9.3		8.0	0.7		0.8
18:2	34.7		1.3	14.6	2.7				0.7
18:3	2.4								
24:0 + 24:1				7.2	3.7				
unknown			0.6		4.7		2.1		

[a] Number of carbon atoms:number of C=C bonds.

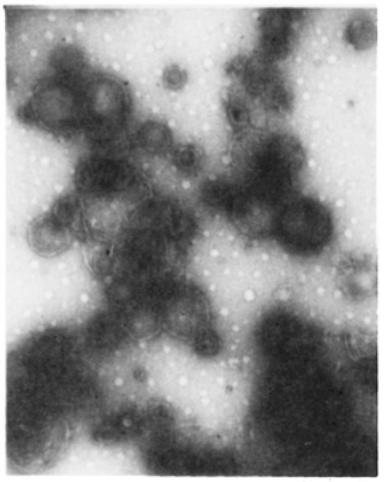

FIGURE 1: Electron micrograph of POPE/CaATPase vesicles (lipid/protein molar ratio = 50:1). The magnification was 25500×, and the size distribution and vesicle shape was typical of the sample. Slotted EM grids were polyvinyl-coated (polyvinyl formal/0.25% ethylene dichloride), carbon stabilized at 10^{-6} Torr with 500 Å of carbon. The samples were developed on the prepared grid for 90 s. Wet grids were treated with uranyl acetate (2%) and dried thoroughly in a desiccator. Samples were viewed in a Phillip EM 200 TEM electron microscope at 80–100 kV at a resolution of 4–5 Å.

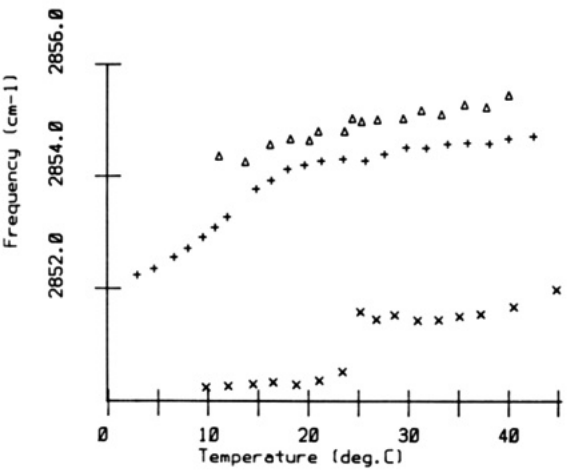

FIGURE 2: Plots of the temperature dependence of the lipid CH_2 symmetric stretching frequency for POPE/CaATPase complexes and the POPE multibilayers (×). The lipid/protein ratios represented in the complexes are 25:1 (△) and 50:1 (+).

Thin-layer chromatograms (solvent system $CHCl_3$–MeOH–NH_4OH, 65:25:5 v:v:v) of the complexes followed by lipid phosphorus determination of the various fractions were therefore carried out for this complex. The proportion of sphingomyelin in the lipid fraction was about 80%. This value, when compared with the sphingomyelin content of native SR [about 3–5% as per Marai & Kuksis (1973)] and taken in conjuction with the data in Table I, confirms the formation of a sphingomyelin/CaATPase complex.

Electron micrographs show the lipid/protein complexes to exist primarily in the form of unilamellar vesicles, with an average diameter of about 1.2 μm. Typical micrographs (negative staining) for the POPE/sphingomyelin complexes are shown in Figure 1. The vesicular nature of the complex is evident. No signs of multilamellar vesicle formation are observed. In other systems, similar results were achieved. However, for the SOPC complex considerable fragmentation was noted.

Fourier-Transform Infrared Spectroscopy. (A) POPE/CaATPase Complexes. FT-IR spectra of the C–H stretching region for pure POPE and for a 50:1 POPE/CaATPase complex were obtained for various temperatures. The origin of the main spectral features arising from the lipid acyl chains is well-known (Cameron et al., 1980; Snyder et al., 1982). The asymmetric and symmetric methyl C–H stretching vibrations appear near 2956 and 2872 cm^{-1}, while the symmetric and antisymmetric methylene CH_2 stretching vibrations are observed near 2920 and 2850 cm^{-1}, respectively. Also observed are carbon–hydrogen C–H stretching modes from –CH=CH– moieties of unsaturated acyl chains at 3020 cm^{-1} and a broad Fermi resonance band appearing as a shoulder at 2900 cm^{-1} (Snyder et al., 1978). The feature appears most clearly in the Raman spectra of phospholipids (at 2880 cm^{-1}), where it is the most intense band in the gel-phase spectra of phosphatidylcholines. Although the protein component in the lipid/protein complexes contributes no distinct peaks to the observed C–H stretching frequencies, part of the band intensity underlying the 2920-cm^{-1} feature is derived from CaATPase.

As the temperature of either the pure lipid or the complex is raised, small alterations in the position or width of the lipid C–H stretching vibrations are evident in the spectra. These have been used to follow alterations in lipid order and melting (Cameron et al., 1981; Mantsch et al., 1981). Although the changes in frequency are much less than the resolution utilized (4 cm^{-1}) in the current experiments, they may, using protocols developed by Cameron et al. (1982), be measured with a precision better than 0.1 cm^{-1}. The vibration most suitable for following lipid structural changes is the CH_2 symmetric stretching mode near 2850 cm^{-1}. This vibration has little or no underlying contribution from protein and so is useful for studies of the lipid component. Temperature-induced variations in the frequency of this band in pure POPE and in two POPE/CaATPase complexes are shown in Figure 2.

The sharp discontinuity in the plot of frequency vs. temperature for POPE at 25 °C is evidence for the occurrence of the gel–liquid crystal phase transition for this molecule. Differential scanning calorimetry experiments (not shown)

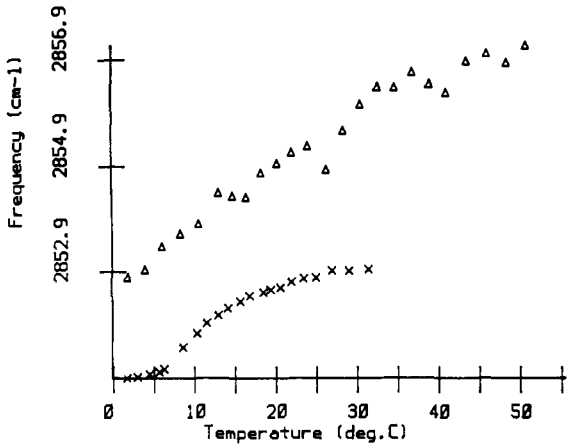

FIGURE 3: Plots of the temperature dependence of the lipid CH_2 symmetric stretching frequency for the SOPC/CaATPase complex (△) and for SOPC multibilayers (×). The lipid/protein molar ratio in the complex is 100:1.

reveal an endothermic transition at 25 °C, consistent with the interpretation of the FT-IR results. It is noted that an earlier fluorescence study (Eibl & Wooley, 1979) showed a somewhat lower value for T_m. The origin of the increase in the symmetric stretching frequency upon the introduction of gauche rotamers into the hydrocarbon chains has been addressed by Snyder et al. (1982) and can be traced to alterations in the interaction constants between C–H stretching coordinates on adjacent methylene groups when the lipid physical state is altered.

FT-IR melting curves for the complexes (Figure 2) reveal that incorporation of CaATPase into POPE vesicles results in the complete abolition of the phase transition at at lipid/protein ratio of 25:1. In addition, the measured frequency at 2850 cm^{-1} is increased substantially both above and below T_m compared with pure POPE. At a lipid/protein ratio of 50:1, there is evidence for a melting process below 10 °C and a weaker indication for a residual transition at about 28 °C. The latter is close to the experimentally observable limit. The data thus indicate the presence of substantial protein-induced disorder in both the gel and liquid crystalline phases of the lipid. As the level of protein is increased from 2 to 4%, an increase in the frequency of the 2850-cm^{-1} band is noted at all temperatures.

(B) SOPC/CaATPase Complexes. DSC studies of SOPC (not shown) reveal a fairly broad endotherm centered at temperatures from 7 to 12 °C accompanied by a run-to-run variation in transition width dependent on sample thermal history. The width of the transition has been related by previous workers (Phillips et al., 1972; Davis et al., 1980) to the presence of sample-to-sample variation in the amount of OSPC present in the initial lipid.

Prolonged equilibration of the gel phase results in a melting profile centered at 12 °C (Figure 3) as determined by FT-IR. Addition of CaATPase produces drastic changes in the thermal response of the system. As shown in Figure 3, the transition of the SOPC is totally abolished at lipid/protein ratios of 100:1. In addition, the introduction of substantial disorder into the hydrocarbon chains at temperatures corresponding to both the gel and liquid crystalline state of the pure lipid is evident from the increased CH_2 frequency at all temperatures.

(C) Egg Sphingomyelin/CaATPase Complexes. The FT-IR spectra of egg sphingomyelin in the C–H stretching region reveal the same general features as the phosphatidylcholines. The CH_2 symmetric stretching mode is again useful as a probe of structural alterations. As shown in Figure 4, the sphingomyelin exhibits a response to the addition of protein similar to that seen for POPE and SOPC. The pure lipid exhibited a resonably sharp discontinuity at a temperature of 37 °C, consistent with the calorimetrically determined transition temperature (G. Anderle and R. Mendelsohn, unpublished results). Addition of protein (lipid/protein ratio = 30:1) substantially reduces the cooperativity of the transition and introduces substantial disorder into the acyl chains at all temperatures as seen from the FT-IR data in Figure 4.

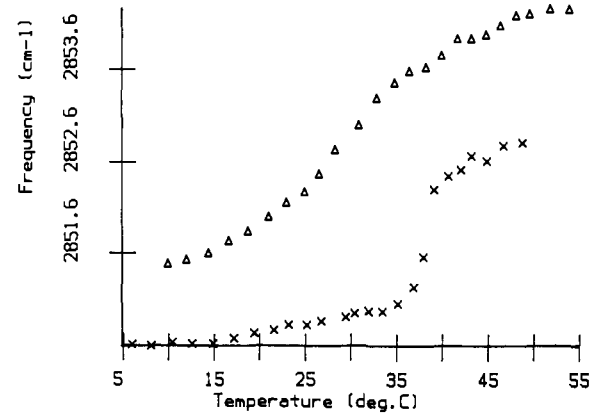

FIGURE 4: Plots of the temperature dependence of the lipid CH_2 symmetric stretching frequency for an egg sphingomyelin/CaATPase complex at a lipid/protein mole ratio of 30:1 (×) and for egg sphingomyelin vesicles (△).

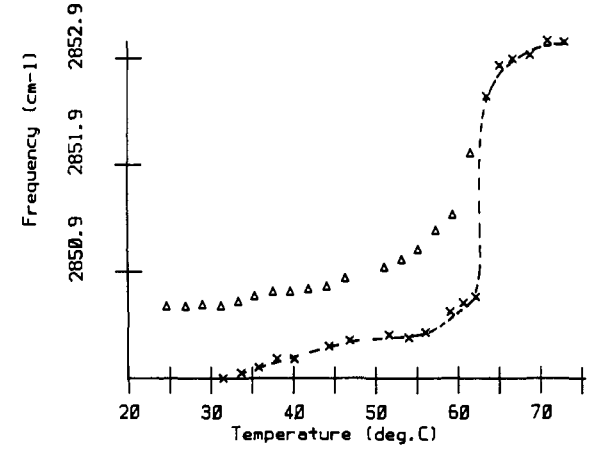

FIGURE 5: Plots of the temperature dependence of the lipid CH_2 symmetric stretching frequency for a DPPE/CaATPase complex at a lipid/protein mole ratio of 70:1 (△) and for DPPE multibilayers (×).

(D) DPPE/CaATPase Complexes. The response of DPPE (Figure 5) to CaATPase incorporation differs from the three lipids studied above and resembles previous investigations with DPPC (Mendelsohn et al., 1984). Pure DPPE melts sharply at 63 °C, in good accord with prior calorimetric (Wilkinson & Nagle, 1981) and other (Shimshick & McConnell, 1973; Mantsch et al., 1983) physical investigations. As CaATPase is known to undergo thermal denaturation at about 50 °C, FT-IR samples of DPPE/CaATPase were not heated above this level, so that the effect of protein was explored only on the gel phase of DPPE. Some disordering of the lipid gel phase is noted (Figure 5), and the beginning of a lipid melting process is also evident from the data. It appears that a slight reduction occurs in T_m, along with the aforementioned disordering.

Discussion

The current results, taken in conjunction with prior IR studies of CaATPase/lipid complexes, both from our laboratory (Mendelsohn et al., 1984) and others (Chapman et al.,

1980), point to the occurrence of various types of protein-induced perturbations on lipid order and melting characteristics. Three types of phospholipid response have been observed as follows:

(i) For phospholipid environments with high levels of unsaturation (DOPC or native SR), the lipid acyl chains are slightly ordered by protein incorporation. The effect on T_m cannot be easily studied, since the melting of these highly unsaturated systems occurs at temperatures well below 0 °C.

(ii) For phospholipids with no unsaturation (DPPC or DPPE), the effect of protein is to lower T_m slightly and to induce, at most, slight disorder into the acyl chains at temperatures removed from the lipid melting.

(iii) The strongest response is observed for phospholipids containing one saturated and one unsaturated chain (e.g., POPE or SOPC). In these cases, relatively low levels of protein diminish the magnitude of or completely abolish the phase transition. In addition, substantial disorder is introduced into the acyl chains compared with pure lipid both above and below T_m.

To correlate the current observations with prior work required some discussion of the contradictions and uncertainties in the literature concerning lipid/protein interaction in general and CaATPase/lipid interactions in particular. For example, the ^{31}P NMR studies of Selinsky and Yeagle (1984) were interpreted in terms of two distinct lipid populations, one with characteristics only slightly perturbed from pure lipid and the other with characteristics of an immobilized component. In contrast, Oldfield and coworkers (Oldfield et al., 1978) and McLaughlin et al. (1981), using ^2H NMR spectroscopic studies of CaATPase/lipid complexes, concluded that a single homogeneous lipid environment exists, at least on the time scale of the NMR experiments. If both these experimental results are correct, their reconciliation requires that the motional characteristics of the phosphorus in the head group be different from those of deuterium atoms incorporated into the acyl chains. Specific head group/protein interactions may slow the phosphorus motions while leaving the chains relatively unhindered. However, this type of explanation cannot reconcile differences on similar samples as monitored by a single technique. For example, in contrast to the work of Selinsky and Yeagle (1984), McLaughlin et al. (1982) have examined oriented multilayers from isolated and reconstituted SR using ^{31}P NMR techniques and observed that 100 ± 7% of the phospholipid molecules in the membrane can be accounted for in the spectra. More recently, Pates et al. (1985), in accord with McLaughlin et al. (1982), observed no broad spectral component attributable to strong lipid/protein interactions in either native SR membranes or rod outer segments.

Other techniques such as fluorescence spectroscopy measure local protein-induced perturbations on lipid bilayers on a time scale of 10^{-8} s, so that motions faster than those sensed by NMR are monitored. Hudson and Wolber (1982) have suggested, on the basis of measurements of the interaction between fluorescent lipid chain analogues and M13 bacteriophage coat protein, that the NMR and fluorescence results are compatible if the protein increases the angular heterogeneity of some environments, increases the order of some environments, and does not appreciably slow translational diffusion of fluid lipid.

What additional information is available from the current FT-IR experiments that may help to reconcile the above contradictions? First, the observation that different lipid types show qualitatively different behavior depending upon the degree of acyl chain unsaturation indicates that specific models for lipid/protein interaction derived from a single experimental system are unlikely to be successfully generalized. For the most part, the FT-IR experiments for phospholipids containing moderate or low levels of unsaturation reveal protein-induced disordering of the lipid acyl chains. The experimental data for POPE (Figure 2) are particularly important in this regard. At a lipid/protein mole ratio of 25:1 (where most of the lipid is presumably in contact with the protein surface), the lipid melting event is totally abolished. At a lipid/protein mole ratio of 50:1 (where there are presumably 20–30 molecules of lipid not in direct contact with protein), a residual melting event appears at about 10 °C (15 °C below the melting of pure lipid). This must arise from the residual (bulk) POPE that is disordered by the remainder of the system (i.e., by the first 20–30 molecules of lipid plus protein), as judged by its CH_2 frequency at all temperatures compared with pure POPE. A useful model appears to be one of progressive disordering as one passes from the bulk lipids to lipid in the vicinity of protein (Lentz et al., 1985). The dynamic behavior of the system (rate of interconversion of gauche rotamers or rate of acyl chain libration) cannot be deduced easily from FT-IR frequency data. Half-widths might be appropriate here (Casal et al., 1980), but the fact that the observed bands are superpositions over all chain positions precludes the possibility of quantitative interpretation. Experiments with lipids specifically deuterated at particular chain positions, although technically complicated by reduced signal levels from the CD_2 groups, may be appropriate to address the question of phospholipid motion via FT-IR spectroscopy. Experiments along these lines will at least help to determine the relative strengths of lipid/protein interaction at various depths in the lipid bilayer.

The observed protein-induced disordering effects are even more pronounced for the SOPC/CaATPase complexes. The ability of protein to abolish the lipid cooperative melting in this instance clearly extends over several layers of surrounding lipid. As judged by the thermotropic behavior of the 100:1 complex (Figure 3), the protein completely abolishes the melting of 100 lipid molecules. In contrast to POPE and SOPC, the data for both DPPC (Mendelsohn et al., 1984) and DPPE (Figure 5) indicate a reduced range of protein-induced preturbations. At a mole ratio of 30:1, DPPC still shows a substantial melting event, albeit at temperatures reduced from the pure lipid. The molecular origin of the dependence of the effects observed on the particular lipid class is not clear, although some speculations may be useful. The determining factors are presumably the nature of the surface presented to a lipid molecule by the hydrophobic regions of membrane proteins and the ability of lipid acyl chains to bend to accommodate themselves to the surface. The observed slight protein-induced increase in order in native SR and in highly unsaturated lipid chains may be due to restricted formation of certain gauche rotamers in the presence of protein. The observed decrease in the midpoint temperature and broadening of the melting of saturated lipids is reasonably explained in terms of protein disruption of lipid packing. Such disruption would tend to reduce the cooperativity of the lipid melting as well as to lower T_m, depending on the relative magnitudes of lipid/lipid and lipid/protein interactions in the two lipid phases.

The result most difficult to understand is the strong disordering effect in lipid systems with one unsaturated and one saturated chain. This case appears to be physiologically the most relevant, as the native lipids contain high proportions of saturated *sn*-1 chain and unsaturated *sn*-2 chain. It is possible that the single unsaturated chain facilitates the initial interaction of protein with lipid, because the lipid gel phase is not

as well packed as in saturated systems. Subsequently, the introduction of disorder into the saturated chain is made easier because of the relatively poorer acyl chain packing in the vicinity of protein.

Registry No. DPPE, 3026-45-7; POPE, 26662-94-2; SOPC, 6753-56-6; ATPase, 9000-83-3.

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