Phospholipid Fatty Acyl Chain Asymmetry in the Membrane Bilayer of Isolated Skeletal Muscle Sarcoplasmic Reticulum†

Roger J. Bick,* W. Barry Van Winkle, Charlotte A. Tate, and Mark L. Entman
Section of Cardiovascular Sciences, Department of Medicine, Baylor College of Medicine, Houston, Texas 77030
J. Kent Blasie
Department of Chemistry, University of Pennsylvania, Philadelphia, Pennsylvania 19104
Leo G. Herbette‡
Departments of Radiology, Medicine, and Biochemistry, University of Connecticut Health Center, Farmington, Connecticut 06032

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ABSTRACT: We previously showed [Herbette, L. G., Blasie, J. K., DeFoor, P., Fleischer, S., Bick, R. J., Van Winkle, W. B., Tate, C. A., & Entman, M. L. (1984) *Arch. Biochem. Biophys. 234*, 235–242; Herbette, L. G., DeFoor, P., Fleischer, S., Pascolini, D., Scarpa, A., & Blasie, J. K. (1985) *Biochim. Biophys. Acta 817*, 103–122] that the phospholipid head-group distribution in the membrane bilayer of isolated sarcoplasmic reticulum is asymmetric. From these studies, both the total number of phospholipid head groups and the total lipid, as well as the head-group species for these lipids, were found to be different for each monolayer of the membrane bilayer. In this paper, we demonstrate for the first time that there is significant asymmetry in the distribution of unsaturated fatty acids between the two monolayers; i.e., the outer monolayer of the sarcoplasmic reticulum contained more unsaturated and polyunsaturated chains when compared to the inner monolayer. X-ray diffraction measurements demonstrated that the time-averaged fatty acyl chain extension for the outer monolayer was approximately 20% less than for the inner monolayer. This is consistent with the concept that the greater degree of unsaturation in the outer monolayer may provide for a decreased average fatty acyl chain extension for that layer. This architecture for the bilayer may be related to both the "resting" state mass distribution of the calcium pump protein within the membrane bilayer and possible "conformational" states of the calcium pump protein during calcium transport by the sarcoplasmic reticulum.

The sarcoplasmic reticulum membrane is responsible for the vectorial translocation of calcium from one side of the membrane bilayer to the other (Tada et al., 1978) supported by the hydrolysis of ATP (DeMeis & Vianna, 1979; Hasselbach, 1978; Tada et al., 1978; Yamamoto et al., 1979). This asymmetric functionality is brought about by the calcium pump ATPase which hydrolyzes ATP to form a phosphorylated intermediate, an important component of this calcium translocation reaction. The total electron density profile structure determined by X-ray diffraction and the neutron density profile structures of the various components of this membrane as determined by neutron diffraction are highly asymmetric. We have previously demonstrated the asymmetric nature of the phospholipid head-group distribution as well as the asymmetry in protein mass and total numbers of phospholipids between the outer and inner monolayers of the sarcoplasmic reticulum membrane bilayer (Herbette et al., 1984, 1985). Thus, it has been shown that the separate phospholipid and protein components of this membrane are organized so as to allow a distinct structural asymmetry for the membrane bilayer and at the same time preserve the total cross-sectional area of protein and lipid along the full extent of the membrane bilayer (Herbette et al., 1984, 1985). This structural asymmetry in the profile of the individual membrane components is consistent with several observations regarding the overall highly asymmetric nature of the sarcoplasmic reticulum (Campbell et al., 1980; Deamer & Baskin, 1969; DuPont et al., 1973; Hidalgo & Ikemoto, 1977; Ikemoto et al., 1968; Inesi & Asai, 1968; Opden Kamp, 1979, 1981; Saito et al., 1978; Tillack et al., 1974; Vale, 1980; Worthington & Liu, 1973).

Recent studies utilizing caged ATP and X-ray synchrotron radiation have allowed a determination of some structurally distinct reaction intermediates during the calcium transport cycle (Blasie et al., 1985). The membrane bilayer of the sarcoplasmic reticulum was shown to be highly asymmetric in the resting, phosphorylated, and transport states (Blasie et al., 1985). It appeared that reversible redistributions of the protein mass on the order of 5–10% of the total protein mass occurred along the profile axis during the calcium translocation reaction. In particular, significant redistributions of the calcium pump ATPase apparently occurred within the hydrocarbon core of the membrane bilayer during phosphorylation, indicating that the membrane bilayer of the sarcoplasmic reticulum must be a highly "flexible" component of its structure.

In this paper, we have demonstrated the asymmetry[1] of the phospholipid fatty acyl chains within the two monolayers of the membrane bilayer in skeletal muscle sarcoplasmic reticulum utilizing phospholipase digestion and 2,4,6-trinitrobenzenesulfonate (TNBS) labeling, comparing these results

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* Address correspondence to this author.
‡ Established Investigator of the American Heart Association.

[1] Asymmetry, as used in this paper, can refer either to the inequality in the amount of fatty acyl chains within the monolayers of the sarcoplasmic reticulum membrane bilayer or to the distribution of specific fatty acyl species between the monolayers. In this paper, specific reference will be made each time to the type of asymmetry being discussed.

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to X-ray diffraction measurements of the time-averaged fatty acyl chain extensions for each monolayer. The outer monolayer was found to contain less saturated and more unsaturated and polyunsaturated fatty acyl chains compared to the inner monolayer. Taken together, we have thus shown that the calcium pump ATPase, the phospholipid head groups, and the fatty acyl chains are all asymmetrically distributed in the sarcoplasmic reticulum bilayer, and a more complete structural picture of the sarcoplasmic reticulum membrane can now be obtained. The asymmetry in the amount of fatty acyl chains may be related to the mass distribution ("conformation") of the calcium pump ATPase before and during calcium translocation; this architecture may optimize calcium binding, uptake, and ATPase functions. The preferential high degree of unsaturation in the outer monolayer may be related to a requirement of differential "fluidity" within the membrane bilayer of the sarcoplasmic reticulum which may be related to optimal functioning of the calcium pump ATPase to transport calcium.

Materials and Methods

Sarcoplasmic Reticulum Membrane Preparation. Sarcoplasmic reticulum was isolated from rabbit white (fast) back and hind leg muscle and purified either by zonal density gradient centrifugation (Herbette et al., 1977) or by the method of Van Winkle et al. (1981). Sarcoplasmic reticulum vesicles were either used immediately or stored on ice for a maximum of 12 h. The membrane preparations used in this study correspond to light sarcoplasmic reticulum and will be referred to as sarcoplasmic reticulum in this report. Preparations were routinely examined by freeze–fracture electron microscopy and found to be greater than 98% right-side-out. Protein concentration was determined by the method of Lowry et al. (1951), using bovine serum albumin (BSA) as standard. Phospholipid phosphorus content was determined by the method of Bartlett (1959).

Phospholipase Experiments. Sarcoplasmic reticulum protein was incubated in a reaction mixture containing 1 mM *N*-[tris(hydroxymethyl)methyl]-2-aminoethanesulfonic acid (TES), pH 7.0, containing 100 mM KCl, 40 μM $CaCl_2$, 5 mg BSA, and 10 units of phospholipase A_2 (Sigma Chemical Co., St. Louis, MO: from bee venom or porcine pancreas). Sodium deoxycholate (0.1%) was added to some reactions so that both sides of the membrane were accessible to the phospholipase A_2. Reactions were carried out for up to 1 h and terminated by the addition of cold ethylene glycol bis(β-aminoethyl ether)-*N*,*N*,*N*′,*N*′-tetraacetic acid (EGTA) to a final concentration of 5 mM and immediate cooling of the samples in an ice bath. Aliquots of the A_2-treated preparations were examined by negative-stain electron microscopy, and the sarcoplasmic reticulum (SR) vesicles were found to remain intact within the time frame used. The samples were centrifuged for 30 min in a Beckman L3-40 ultracentrifuge at 50000*g*, and the supernatants were removed and retained. The resulting pellets were washed twice by resuspension in 1 mM TES, pH 7.0, and 100 mK KCl and centrifuged as above, and the supernatants from the washing steps were combined with the original supernatant. The pellets were resuspended in 1 mM TES, pH 7.0, and 100 mM KCl, and both the pellet and supernatant fractions were extracted twice with 4 volumes of chloroform/methanol (2:1) and once with 4 volumes of chloroform/methanol (1:2). The organic extracts, including the solid material at the interface, were filtered through glass wool, and the glass wool was washed with 1 volume of 2:1 chloroform/methanol. The organic extracts were taken to dryness under a stream of nitrogen and the residues dissolved in chloroform/methanol (2:1). Aliquots of the samples were then applied to silica gel type 60 thin-layer chromatography (TLC) plates and developed in chloroform/methanol/NH_4OH (60:35:8) in the first dimension and chloroform/methanol/acetone/acetic acid/water (6:2:8:2:1) in the second dimension if required for a better separation.

The developed plates were placed in a tank of iodine vapor and allowed to develop for 2 min to show all components of the samples. Ammonium molybdate, dissolved in sulfuric acid, was then sprayed on the plates to locate the phospholipids.

The plates showed that in the first few minutes of the reaction, there was a rapid increase in lysophosphatidylcholine (LPC) and lysophosphatidylethanolamine (LPE) and a corresponding decrease in phosphatidylcholine (PC) and phosphatidylethanolamine (PE). The formation of lysophosphatides then slowed, the rest of the PC and PE being hydrolyzed over the next hour (Figure 1). This rapid hydrolysis was taken to be due to the action of the phospholipase on the outer monolayer and the slower hydrolysis being due to the action of the phospholipase on the less accessible inner monolayer (see Results and Discussion). A further digestion reaction was initiated under the same conditions as above, and after the predetermined incubation time, the reaction was stopped with EGTA and the pellet(s) and supernatant(s) obtained as above. The supernatant was taken as containing the fatty acids originating from the outer monolayer of the membrane, being removed by the BSA, and the pellet being representative of the inner monolayer. The dried organic extracts were exposed to dry HCl gas for 5 min, then mixed with 1 mL of methanolic base, heated in a steam bath for 3 min, mixed with 1 mL of boron trifluoride, heated for a further 3 min, and extracted with petroleum ether. The petroleum ether was taken to dryness, and the residues were dissolved in carbon disulfide and run on a Hewlett Packard 5830A gas chromatograph at 285 °C, using a Silar column (Supelco, Bellefonte, PA). Detection was by flame ionization. These chromatographic traces were examined for retention times, and the *percent* of material in each peak was calculated (see Table I where the values are given as a percent of total obtained in the trace). All values were corrected for the solvent. To convert these percentages to moles of fatty acid per mole of protein, an internal standard (C21:0, heneicosanoic acid) was included in the assay procedure, and the peak areas could then be converted to micrograms of fatty acid and then to moles assuming the same recovery for native fatty acids as for the standard (Table II). A sodium deoxycholate treated preparation was run as control to show total, rapid hydrolysis of the phospholipids, when the membrane was made permeable to phospholipase A_2. These values were converted to moles, based on identification (known molecular weight) of the fatty acids obtained by gas chromatography. The data were then normalized to the amount of sarcoplasmic reticulum protein in order to express the results as moles of fatty acid per mole of calcium pump protein.

TNBS Labeling of SR Phospholipids. Sarcoplasmic reticulum protein was incubated at room temperature in the following medium and in the dark: 10 mM TES, pH 7.0, 100 mM KCl, and 1 mM TNBS (2,4,6-trinitrobenzenesulfonate; Sigma, St. Louis, MO). Phospholipids were separated and extracted as detailed under Phospholipase Experiments. PE–TNBS was visible as a bright yellow spot of high R_f distinct from the unreacted PE. These spots were analyzed for estimation of outer and inner membrane PE. The resulting values were also used to monitor the A_2 digestion of the SR membrane.

Plasmalogen Content. SR was exhaustively extracted in 2:1 chloroform/methanol, filtered through glass wool, followed with an aliquot of chloroform/methanol, and evaporated to dryness under a stream of nitrogen. Residues were reconstituted in chloroform/methanol and spotted on a 20 × 20 cm silica TLC plate(s). Separation was achieved with chloroform/methanol/water (65:25:4) in the first dimension: the plates were dried, placed in HCl vapor for 15 min, dried for a further 15 min, and run in the second dimension with chloroform/methanol/ammonium hydroxide/water (70:30:4:1) Visualization was with iodine vapor. Spots were marked and scraped into tubes, and phosphate (Bartlett, 1959) estimations were carried out to determine the content of plasmalogen in PC, PE, and phosphatidylserine (PS).

X-ray Diffraction Measurements and Model Refinement Analysis. Lamellar meridional X-ray diffraction from oriented membrane multilayers has previously provided the electron density profile structure for the isolated sarcoplasmic reticulum membrane derived to 10-Å resolution (Herbette et al., 1977). This continuous electron density profile structure was modeled by constructing step function equivalent profiles for the membrane bilayer and protein "knob" regions (Herbette et al., 1983, 1985; Pachence et al., 1979). A strip model containing six steps was needed to define the membrane bilayer region of the profile structure for the isolated sarcoplasmic reticulum membrane. The step function profile was Fourier transformed once, and the resulting calculated structure factor function was truncated to the same resolution as the experimentally derived profile structure. A second Fourier transformation was then obtained to provide the calculated profile structure, and a least-squares fit between the calculated and experimental continuous profile structures was obtained (Herbette et al., 1985). The fitting procedure was terminated when the least-squares deviation was less than 5%. This double Fourier transformation approach properly takes into account the resolution-limited artifacts inherent in the experimentally derived profile structures. From these step function models, only the time-averaged width (w in Table IV) of the hydrocarbon core region and the fatty acyl chain extensions (given as the ratio R_h in Table IV) for the inner and outer monolayers of the membrane bilayer were obtained. For width measurements, the total electron density profile structure is all that is required. For a determination of compositional asymmetry parameters, the total electron density profile structure must be separated into the profile structures of the separate components (protein, lipid, and water) in order to abstract information relevant to the number of lipid molecules residing in each monolayer of the sarcoplasmic reticulum membrane bilayer (Herbette et al., 1981, 1984, 1985). This determination of the separate contributions of the individual membrane components to the overall structure of the sarcoplasmic reticulum membrane was previously described in detail (Herbette et al., 1985) providing the R_m values in Table IV.

Results

The primary goal of this work has been to evaluate the asymmetry in the phospholipid bilayer of skeletal muscle sarcoplasmic reticulum fragments. The method utilized in these and previous experiments (Herbette et al., 1984) depended on analyses of phospholipids that are most susceptible to phospholipase A_2 hydrolysis and is based on the assumption that the phospholipids in the outer monolayer could be distinguished by this susceptibility. Because of the nature of the studies to be described, we reexamined our ability to analyze asymmetry by these methods utilizing independent means to validate our results. The results presented will demonstrate

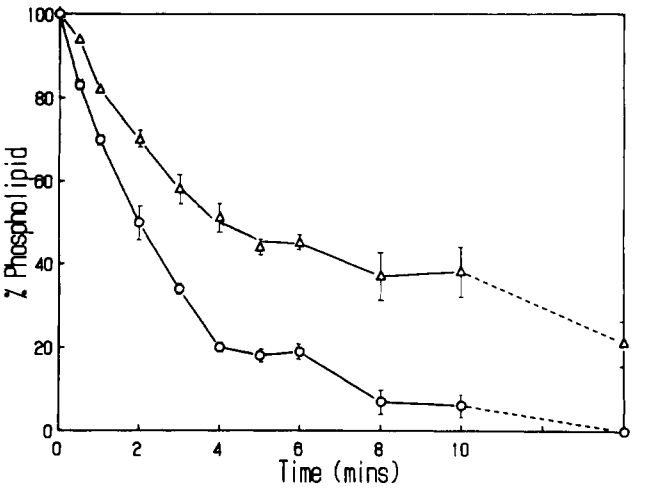

FIGURE 1: Plot of hydrolysis of the major SR membrane phospholipids by phospholipase A_2 action. Note that the break point is between 4 and 5 min. Reaction conditions as described under Materials and Methods. (○) PC; (△) PE.

Table I: Comparison of Phospholipid Values Obtained with TNBS and Phospholipase A_2 Experiments[a]

skeletal muscle	TNBS	A_2
total PC	64 ± 4	61 ± 2
total PE	17 ± 2.5	22 ± 3
total PS	12 ± 3	10 ± 2
inner PC	48.0	50.0
outer PC	52.0	50.0
inner PE	23.5	20.0
outer PE	76.5	80.0

[a] Values are percentage of total phospholipid.

that two independent biochemical methods agree with each other and with the accompanying X-ray diffraction data so that the method allows distinction between the inner and outer monolayer.

Phospholipase A_2 Digestion. A time course of phospholipase A_2 digestion is demonstrated in Figure 1. There is an initial rapid breakdown of the major phospholipids which appears to sharply reduce in velocity at 4 min. This was a consistent time point and suggested that the initial rapid phase represented hydrolysis of the outer bilayer. To further investigate the relationship between the "break point" in Figure 1 and outer bilayer hydrolysis, we took advantage of the highly reactive amine group of phosphatidylethanolamine. TNBS reacts specifically with the amine group of this phospholipid but is unable to penetrate through the phospholipid bilayer and therefore labels only outwardly orientated phosphatidylethanolamine. The labeled phosphatidylethanolamine is easily separated from unlabeled (see Materials and Methods) and allows an estimation of phosphatidylethanolamine in the inner and outer monolayer, as shown in Table I. In estimates using TNBS, labeled phosphatidylethanolamine as an index of outer monolayer phosphatidylethanolamine in the inner monolayer was estimated at 23.5% and the outer monolayer, 76.5%. Utilization of the phospholipase A_2 technique, examining the proportion of PE hydrolyzed at 4 min as an index of the outer monolayer, gave an estimate of 20% for the inner monolayer vs. 80% for the outer monolayer. Thus, these two independent methods gave the same estimates of phosphatidylethanolamine asymmetry.

Hydrolysis experiments identical with that seen in Figure 1 were performed on TNBS-labeled membranes and demonstrated that greater than 95% of the TNBS label was removed at the 4-min time period. Assuming that this represented a

Table II: Fatty Acyl Chains of Phospholipase-Treated Skeletal Muscle Sarcoplasmic Reticulum[a]

carbon no.	inner monolayer[b]	outer monolayer[b]
12:0	1.4 ± 0.3	1.1 ± 0.4
13:0	1.0 ± 0.2	0.6 ± 0.2
14:0	2.3 ± 0.2	1.6 ± 0.4
15:0	2.5 ± 0.2	1.6 ± 0.4
16:0	30.3 ± 2.6	21.8 ± 2.3
16:1	3.6 ± 0.0	2.5 ± 0.2
16:1 (trans)	0.1 ± 0.0	0.1 ± 0.0
18:0	6.8 ± 0.2	4.3 ± 0.1
18:1	22.5 ± 1.5	21.8 ± 2.3
20:0	0.1 ± 0.0	0.1 ± 0.0
18:2	14.3 ± 1.0	21.4 ± 1.8
20:1	0.1 ± 0.0	0.1 ± 0.0
21:0	0.1 ± 0.0	0.4 ± 0.1
18:3	0.2 ± 0.1	0.1 ± 0.0
22:0	0.4 ± 0.1	0.4 ± 0.0
22:1	1.0 ± 0.1	1.3 ± 0.1
20:4	8.8 ± 0.9	14.5 ± 0.9
23:0	0.1 ± 0.0	0.5 ± 0.1
24:0	0.7 ± 0.3	1.9 ± 0.1
24:1	2.4 ± 0.2	2.0 ± 0.4
22:4	0.6 ± 0.3	0.6 ± 0.1
22:6	0.8 ± 0.2	1.6 ± 0.1
saturated	45.7 ± 4.1	34.3 ± 4.1
unsaturated[c]	54.3 ± 4.3	65.7 ± 5.9
polyunsaturated[c]	(24.7 ± 2.5)	(38.2 ± 2.9)
saturated inside/outside	1:0.75	
unsaturated inside/outside	1:1.20	

[a] Data are the mean ± SE for five determinations. [b] Values indicated represent percentages of the total fatty acid recorded in the various chromatographic traces. [c] The percentages for unsaturated fatty acids include the polyunsaturated population of fatty acyl species.

Table III: Moles of Fatty Acyl Chain per Mole of Sarcoplasmic Reticulum Protein

carbon no.	inner monolayer	outer monolayer
12:0	1.08	0.72
13:0	0.72	0.38
14:0	1.64	0.96
15:0	1.82	1.02
16:0	21.92	14.20
16:1	2.64	1.68
16:1 (trans)	0.04	0.04
18:0	4.91	2.80
18:1	16.09	14.20
20:0	0.04	0.00
18:2	10.30	13.90
20:1	0.04	0.04
21:0	0.04	0.24
18:3	0.17	0.04
22:0	0.26	0.22
22:1	0.69	0.89
20:4	6.31	9.46
23:0	0.04	0.24
24:0	0.52	1.20
24:1	0.12	1.14
22:4	0.44	0.42
22:6	0.58	0.96
total[a]	70.41	64.75
saturated	32.99	21.98
unsaturated	37.42	42.77
polyunsaturated	17.80	24.78

[a] Total moles of fatty acid for the bilayer = 135; moles of polyphospholipid per mole of protein = 67.5.

reasonable estimate of hydrolysis of the outer monolayer, calculations similar to those previously done by ourselves and others were utilized to estimate phosphatidylcholine in the inner and outer monolayer in both labeled and unlabeled membranes. Utilizing the TNBS-labeled membranes, the estimation of the ratio of inner to out phosphatidylcholine was 48:52, whereas utilizing the unlabeled membranes and the 4-min break point, as in Figure 1, the estimate was 50:50.

Thus, the phosphatidylethanolamine asymmetry previously described by ourselves and others is independently confirmed by two methods with very close agreement. The use of TNBS demonstrates that the break point seen in Figure 1 is indeed related to hydrolysis of the major proportion of the external monolayer, thus allowing this method to be used for approximation of the ratios of external to internal monolayer. These results confirm the asymmetry of phospholipid head groups in bilayers previously reported by ourselves and others using multiple techniques (Fontaine et al., 1978; Herbette et al., 1984; Vale, 1980; Verkleij et al., 1973; Warren et al., 1974).

Asymmetry of Unsaturated Fatty Acyl Chains across the Lipid Bilayer. Estimates of the relative concentration of various fatty acyl groups in the inner and outer monolayer of sarcoplasmic reticulum membrane are represented in Tables II and III (Table II, relative proportion; Table III, molar concentration). The results indicate that the sarcoplasmic reticulum membrane bilayer contains 40% saturated and 60% unsaturated fatty acyl chains. In comparing the inner and outer monolayers, the inner monolayer contains a higher amount of C16:0 and significantly less C18:2 and C20:4. The outer monolayer contains approximately twice as many unsaturated chains as saturated chains while the inner monolayer contains approximately the same amount of saturated and unsaturated chains. The relative number of unsaturated chains for the inner monolayer compared to the outer monolayer is 1:1.2, whereas the corresponding ratio for saturated chains is 1:0.75. Polyunsaturated chains are also present to a greater extent in the outer monolayer, the ratio in this case for the inner to outer monolayer being 1:1.5. An increase in the degree of unsaturation for the outer monolayer is due almost entirely to increased concentrations of C18:2 and C20:4, both being present in an approximate ratio of 1.5 to 1 when compared to the inner monolayer.

In Table II, inequality in the amount of fatty acyl chain from the inner and outer monolayer of the membrane is shown. These results demonstrate that the molar ratio of fatty acyl chains in the inner compared to the outer monolayer is 1.08:1 ($p < 0.05$). This is consistent with our earlier calculations on the basis of phospholipid head-group distribution between monolayers of the sarcoplasmic reticulum membrane bilayer (Herbette et al., 1984, 1985). Overall, the molar ratio of unsaturated to saturated lipids in the membrane bilayer was 1.46:1. Since the phospholipid is composed of a head group and two fatty acyl chains, we can estimate from these results that there are 68 mol of phospholipid per mole of sarcoplasmic reticulum protein. This value is slightly lower than that from our earlier publications and may be accounted for by the fact that some phospholipid is undoubtedly lost during the extraction procedures. However, the samples all follow the same extraction procedures so that the losses are equivalent for all samples, and differences in the monolayers apparently are preserved since these results agree with our previous data which did not involve such extractions.

Plasmalogen Estimation. These experiments were undertaken to ensure that total phospholipid estimations were accurate with both total SR and phospholipase-treated phospholipids. PC was found to be 35% plasmalogen, PE 66%, and PS 15%. Totally diacyl and plasmalogenic phospholipids showed the recovery of lipids after phospholipase digestion to be 98 ± 5% (results not shown).

X-ray Diffraction Measurements. The step function equivalent profile for the membrane bilayer region of the

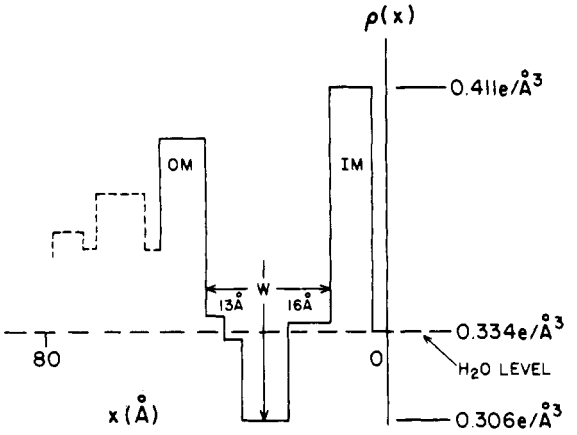

FIGURE 2: Step function equivalent profile for the sarcoplasmic reticulum membrane with the bilayer region given as a solid line; the dotted line step function region corresponds to the protein knob which is external to the membrane bilayer. w refers to the hydrocarbon core width; the downward vertical arrow indicates the center of the hydrocarbon core as previously determined by neutron diffraction and H_2O/D_2O exchange. The fatty acyl chain extension for each monolayer of the membrane bilayer is indicated and was taken directly from these step function equivalent profile structures. Step function equivalent profiles (not shown) were likewise obtained for reconstituted sarcoplasmic reticulum for lipid to protein ratios greater than 80 mol of lipid/mol of calcium pump ATPase and provided the values given in Table IV.

Table IV: Structure of the Sarcoplasmic Reticulum Membrane Bilayer[a]

	R_h[b]	w[c]	R_m[d]
isolated SR	0.8	29	1.17
reconstituted SR	1.4	38	1.12

[a] R_h and w parameters determined by X-ray diffraction methods as described in text; R_m parameters determined by neutron diffraction methods are taken from related studies (Herbette et al., 1981, 1984, 1985). [b] Ratio of the fatty acyl chain extensions for the outer and inner monolayers of the membrane bilayer. [c] Width of the hydrocarbon core in angstroms (Å). [d] Ratio of the number of lipid molecules in the inner compared to the outer monolayer.

isolated sarcoplasmic reticulum membrane is shown in Figure 2. The width of the hydrocarbon core, equal to 29 Å, was taken as the region between the phospholipid head groups labeled OM (for outer monolayer) and IM (for inner monolayer). The center of the hydrocarbon core was taken as the average position of the central trough of lowest electron density as obtained from the electron density profile structure. This position also corresponds to the average position of the central region of lowest neutron scattering density in the water profile structure as previously determined by neutron diffraction and H_2O/D_2O exchange (Blasie et al., 1985). On the basis of this reference point, the ratio (R_h) of the average fatty acyl chain extension for the outer vs. inner monolayer was equal to 0.8 (Table IV). Thus, the time-averaged extent of the outer monolayer fatty acyl chains is approximately 20% less than that for the inner monolayer.

The precise mass distribution of the calcium pump protein in the isolated sarcoplasmic reticulum dictates a precise structure for the membrane bilayer. When the structure of the membrane (i.e., the mass distribution of the calcium pump protein) is altered, the lipid bilayer structure should change accordingly. This is demonstrated by an analysis of the structure of the sarcoplasmic reticulum membrane reconstituted with lipids extracted from the isolated membrane. The step function equivalent profile for the membrane bilayer region of reconstituted sarcoplasmic reticulum (Herbette et al., 1981, 1983) was similarly obtained for comparison (not shown). Although this membrane system retains <80% unidirectionality of the calcium transport, the "conformation" (i.e., mass distribution within the membrane) of the calcium pump ATPase is significantly different from that in the native state (Herbette et al., 1983). The bilayer structure is also found to be different where the hydrocarbon core width is now equal to approximately 38 Å. The symmetry of the fatty acyl chain extension for the outer vs. inner monolayer is opposite to that for native sarcoplasmic reticulum with R_h equal to 1.4. Thus, the protein and lipid mass distributions for the sarcoplasmic reticulum membrane are strongly related.

Discussion

Relationship of Fatty Acyl Chain Composition to the Time-Averaged Structure for Isolated Sarcoplasmic Reticulum. The studies demonstrate for the first time that the outer monolayer of the sarcoplasmic reticulum membrane bilayer has a greater amount of unsaturated fatty acyl chains compared to the inner monolayer although such a possiblity has been previously suggested by spin probe studies (Coan, 1985). If the greater amount of unsaturation is accompanied by a greater degree of trans/gauche conformations for the fatty acyl chains in the outer monolayer, the time-averaged fatty acyl chain extension for the outer monolayer might be predicted to be less than that for the inner monolayer. The measured values for the chain extension for the outer and inner monolayers, as determined by X-ray diffraction measurements, support this view (Herbette et al., 1985). In addition, it has previously been shown that the average cross-sectional area for the calcium pump ATPase within the hydrocarbon core region is greater for the outer compared to the inner monolayer (Herbette et al., 1985), while more lipid is present in the inner monolayer. It is possible that the resting state conformation of the calcium pump ATPase may influence both the numbers of lipids and the distribution of different phospholipid species between both monolayers of the sarcoplasmic reticulum membrane bilayer, thus providing a stable membrane structure.

Possible Relationship of Structural Kinetics to Membrane Bilayer Architecture. This overall architecture for the sarcoplasmic reticulum as described above may also be related to phospholipid-dependent breakdown of the phosphorylated intermediate (EP) of the calcium pump ATPase as suggested by Warren and co-workers, who stated that "the lipid composition is probably an important structural feature for optimal function" (Warren et al., 1974). Meissner and Fleischer (1972) also found that there was a decrease in the amount of EP with removal of the phospholipids by phospholipase digestion.

It has been previously demonstrated by using synchrotron X radiation and caged ATP in sarcoplasmic reticulum membrane multilayers that different conformational states of the calcium pump ATPase apparently are formed during the calcium translocation process (Blasie et al., 1985). The conformation of the phosphorylated state of the calcium pump ATPase apparently differs from the resting state conformation such that there is a redistribution of protein mass from the outer phospholipid head-group region into the hydrocarbon core region of the membrane bilayer upon protein phosphorylation (Blasie et al., 1985). Such a rearrangement of protein mass may require a high degree of "fluidity" or "flexibility" in the membrane bilayer structure in order for the calcium transport cycle to function (Martonosi, 1964). The relatively high degree of unsaturation for the fatty acyl chain composition of the outer monolayer would meet such a requirement. In addition, since the changes in protein mass distribution upon

phosphorylation appear to be localized in specific regions of the membrane bilayer (Blasie et al., 1985), the differences in saturation/unsaturation for the two monolayers of the membrane bilayer may be a further requirement for proper functioning.

Phospholipid species asymmetry in sarcoplasmic reticulum (Coan, 1985; Herbette et al., 1984; Vale, 1980) and other membrane systems has previously been shown (Chiu & Babitch, 1977; Fontaine et al., 1978; Verkleij et al., 1973). Fatty acid asymmetry and fluidity differences between the monolayers of the bilayer of the sarcoplasmic reticulum are now demonstrated by two independent biochemical methods. Rothman and Lenard in their review (Rothman & Lenard, 1977) state that "for membranes where cholesterol is absent or present in small quantities, differences in membrane fluidity remain a real possibility" and "there is compelling evidence that biological membranes are vectorial structures; that is, their components are asymmetrically distributed between the two surfaces". This structural requirement may also apply to the sarcoplasmic reticulum membrane bilayer.

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Registry No. ATPase, 9000-83-3; calcium, 7440-70-2.

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