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THE DETERMINATION OF THE SEPARATE Ca^{2+} PUMP PROTEIN AND PHOSPHOLIPID PROFILE STRUCTURES WITHIN RECONSTITUTED SARCOPLASMIC RETICULUM MEMBRANES VIA X-RAY AND NEUTRON DIFFRACTION

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We have previously compared the electron density profiles for several highly-functional reconstituted sarcoplasmic reticulum membranes with that for the isolated sarcoplasmic reticulum membrane (Herbette, L., Scarpa, A., Blasie, J.K., Wang, C.T., Saito, A. and Fleischer, S. (1981) Biophys. J. 36, 47–72). In this paper, we compare the separate calcium pump protein profile within these reconstituted sarcoplasmic reticulum membranes, as derived by X-ray and neutron diffraction methods, with that within isolated sarcoplasmic reticulum membranes. In addition, the time-average perturbation of the lipid bilayer by the incorporated calcium pump protein within these reconstituted sarcoplasmic reticulum membranes has been determined in some detail.

Introduction

An analysis of the lamellar X-ray or neutron diffraction from hydrated oriented membrane multilayers can provide the respective total scattering profile of the membrane. The total scattering profile is the sum of the scattering profiles for the various constituents comprising the membrane i.e., water, lipid and protein [1]. It is possible to determine the separate scattering profiles for the individual membrane constituents utilizing a model refinement analysis of the X-ray diffraction data where the ratios of the membrane components are varied [2–4] or alternatively by a direct analysis of neutron diffraction data where selected membrane components are deuterated and isomorphously exchanged for the protonated components [5].

Reconstitution of the native membrane affords advantages for both the X-ray and neutron diffraction approaches: (a) reconstitution allows a simplification of the structural analysis by reducing the number of membrane components to those essential for preserving the functional capabilities of the membrane (i.e. a single essential protein with a single species of lipid [2,4,6]), (b) the ratio of the lipid to protein in the reconstituted membrane can be manipulated [2,4,6,7] and (c) reconstitution allows the tailor-making of membranes with deuterium labelling in either the protein and/or the lipid at various positions within the protein or lipid molecules [3,5].

Lamellar X-ray and neutron diffraction were obtained from hydrated oriented multilayers formed from lipid vesicles composed of purified sarcoplasmic reticulum lipid and dioleylphosphatidylcholine (DOPC) mixtures and from unilamel-

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Abbreviation: DOPC, dioleylphosphatidylcholine.

lar membraneous vesicles composed of the purified sarcoplasmic reticulum calcium pump protein (> 95% pure Ca^{2+}- and Mg^{2+}-sensitive ATPase [7]) reconstituted with purified sarcoplasmic reticulum lipid/DOPC mixtures. The separate water, lipid and protein contributions to the reconstituted membrane profile were obtained directly by neutron diffraction at lower resolution (approx. 28 Å). These neutron diffraction studies revealed an asymmetry in the water, lipid and protein profile structures within the reconstituted sarcoplasmic reticulum membrane. Model refinement of the corresponding electron density profiles for these reconstituted sarcoplasmic reticulum membranes provided separate water, lipid and protein profiles at relatively higher resolution (14 Å) which were consistent with those obtained directly by neutron diffraction. The asymmetry in the profile structure of the reconstituted sarcoplasmic reticulum membranes was the result of a localization of a greater portion of the calcium pump protein (and correspondingly less lipid) in the extravesicular half of the reconstituted sarcoplasmic reticulum membrane profile than in the intravesicular half. This asymmetry in the calcium pump protein profile must necessarily arise from an asymmetry in the vectorial distribution of calcium pump protein molecules in the membrane profile which thereby results in the preservation of up to 80% of the ATP-induced Ca^{2+} transport properties of these particular reconstituted sarcoplasmic reticulum membranes (see Ref. 6) as compared to isolated sarcoplasmic reticulum membranes [8].

Methods

Unilamellar reconstituted sarcoplasmic reticulum vesicular dispersions were prepared as previously described [6,7] except that 60 : 40 mixtures of sarcoplasmic reticulum lipid and DOPC (protonated or deuterated) were used in place of the sarcoplasmic reticulum lipid dispersion normally used in reconstitution. In control experiments, sonicated dispersions of 60 : 40 sarcoplasmic reticulum lipid/DOPC (protonated or deuterated) mixtures were prepared [7]. Hydrated oriented multilayers were formed from these reconstituted sarcoplasmic reticulum and lipid dispersions as described in detail [4,8]. All multilayer samples were equilibrated at 84–92% relative humidity under optimal conditions for preserving calcium transport and ATPase activity as measured in redispersions of membrane multilayers [3,8] and directly in sarcoplasmic reticulum membrane multilayers [8,23].

Lamellar X-ray diffraction data from these oriented multilayers were collected on film and were corrected for background scattering as previously described [8]. The lamellar X-ray diffraction $I(s)$ was additionally corrected by s^2 where $s = 2 \sin \theta/\lambda$; one factor of s arises from the intersection of the reciprocal lattice for the cylindrically curved multilayer specimen with the Ewald sphere and the other is due to the arcing of the reflections on the surface of the Ewald sphere due to the mosaic spread of the multilayer [9] which was typically found to be ± 10–$15°$ depending on the water content of the specimen [3]. Lamellar neutron diffraction data from identically prepared oriented multilayers were collected on the low angle diffractometer at the High Flux Beam Reactor at Brookhaven National Laboratory operating at 2.36 Å [10]. A two-dimensional position sensitive counter [11] was used to collect the data and the experimental geometry was identical to that previously described [4]. Lamellar neutron diffraction data was collected via ω-scans and corrected for background scattering via a cubic spline fit algorithm [12] with appropriate modifications as previously described [5]. The lamellar neutron diffraction $I(s)$ was additonally corrected by s^1 only since the latter correction described above for the lamellar X-ray diffraction was removed because the lamellar neutron reflections were integrated over the full extent of the reflection arc as collected on the face of the two-dimensional counter. This data reduction provided the X-ray and neutron lamellar structure factor moduli $|F(s)|$ arising from their respective multilayer unit cell scattering profiles.

The lower-angle lamellar X-ray structure factor moduli for the reconstituted sarcoplasmic reticulum membrane multilayers were phased by the swelling method [13] while the higher-angle moduli were phased by the GFSDM procedure [14] which takes into account the lattice disorder present in these reconstituted sarcoplasmic reticulum membrane multilayers as described previously [4]. The

lamellar neutron structure factor moduli for the reconstituted sarcoplasmic reticulum membrane multilayers and the lipid multilayers were phased by the swelling method as were the X-ray moduli for the lipid multilayers. For the reconstituted sarcoplasmic reticulum and lipid multilayers, a minimum of two multilayer periodicities and two $H_2O/^2H_2O$ ratios were used to phase the neutron moduli while a minimum of three multilayer periodicities were used to phase the X-ray moduli for the lipid multilayers. These phased structure factor moduli were then used to provide the various unit cell X-ray and neutron scattering profiles for the reconstituted sarcoplasmic reticulum and lipid multilayers via standard Fourier procedures [9].

The unit cell water profiles were provided directly via the difference profiles between the scaled unit cell neutron scattering profiles from multilayers hydrated with different $H_2O/^2H_2O$ ratios at the same periodicity as previously discribed [15]. The distribution of deutrium atoms of the labelled DOPC molecules within the multilayer unit cell profile was obtained via either (a) the difference profiles between the scaled unit cell neutron scattering profiles from multilayers containing specifically deuterated versus protonated DOPC where the scaling was achieved via the water profiles for the two multilayers [16] or (b) the model refinement analysis of the difference in the phased structure factor moduli for the specifically deuterated versus the protonated multilayers [17–19]. This model refinement analysis assumed Gaussian distributions of deuterium label in the unit cell difference profile of (a) above where the number of independent label positions in the membrane profile was assumed to be two (one per lipid monolayer); the positions, widths and amplitudes of the Gaussian maxima were otherwise treated as variable parameters and the scaling of the two sets of structure factors was treated as a variable parameter restricted to lie reasonably close to that determined via the water profiles in (a) above.

The separate X-ray and neutron scattering profiles for the lipid within the reconstituted sarcoplasmic reticulum membrane profiles were calculated at approx. 28 Å and 14 Å resolution via an appropriate perturbation of the respective symmetric sarcoplasmic reticulum lipid/DOPC bilayer profiles to accurately reflect the experimentally determined asymmetry in the density and positions of the various deuterium labels of the DOPC molecules within these reconstituted sarcoplasmic reticulum membrane profiles. It was assumed that the distributions of DOPC and sarcoplasmic reticulum lipid in these reconstituted sarcoplasmic reticulum membrane profiles were similar and that the phospholipid polar headgroup separation across the reconstituted sarcoplasmic reticulum membrane profile and the sarcoplasmic reticulum lipid/DOPC bilayer profile were identical (as is readily apparent from an inspection of the reconstituted sarcoplasmic reticulum and sarcoplasmic reticulum lipid/DOPC bilayer X-ray scattering profiles, see Results).

The low-resolution (approx. 28 Å) neutron scattering profile for the calcium pump protein within the reconstituted sarcoplasmic reticulum membrane profile was calculated directly via subtraction of the separate water and calculated lipid profiles determined for these membranes from the total neutron scattering profile determined for these reconstituted sarcoplasmic reticulum membranes. All profiles utilized in this subtraction procedure were at comparable resolution (approx. 28 Å) and were scaled together so as to accurately reflect the composition of these reconstituted sarcoplasmic reticulum membranes.

The lower-resolution (approx. 28 Å) and higher-resolution (approx. 14 Å) separate X-ray scattering profiles for the water, lipid and calcium pump protein components of these reconstituted sarcoplasmic reticulum membranes were also determined via a model refinement analysis of the derived total X-ray scattering profiles for these reconstituted sarcoplasmic reticulum membranes at four different lipid/protein ratios. The refinement analysis incorporated only the compositional asymmetry for the lipid bilayer within these reconstituted sarcoplasmic reticulum membranes as indicated by the neutron diffraction analysis (e.g., the R_m values from Table I). These reconstituted sarcoplasmic reticulum membrane profiles were derived previously [4] and the model refinement analysis utilized has also been previously described in detail [2].

Results

(A) Lipid bilayer profiles and deuterium label distributions

Structural studies were performed on the sarcoplasmic reticulum lipid/DOPC bilayers (lamellar diffraction data not shown) in order that a comparison could be made between the profile structure of the lipid bilayer itself (in the absence of protein) and the profile structure of the sarcoplasmic reticulum lipid/DOPC bilayer within the reconstituted sarcoplasmic reticulum membranes (in the presence of calcium pump protein). The various structural features of the sarcoplasmic reticulum lipid/DOPC lipid bilayer itself are illustrated in Fig. 1. The electron density (or X-ray scattering) profiles for the sarcoplasmic reticulum lipid bilayer (dotted line) and the sarcoplasmic reticulum lipid/DOPC (protonated or specifically deuterated) bilayer (solid line) are shown in Fig. 1a at approx. 13 Å resolution. The polar headgroup separation is approx. 40 Å across the bilayer in both cases while the density of the hydrocarbon core of the DOPC-containing bilayer is significantly greater than that of the sarcoplasmic reticulum lipid bilayer.

The neutron scattering profiles for the sarcoplasmic reticulum lipid/DOPC (protonated) bilayer at two $H_2O/^2H_2O$ ratios are shown in Fig. 1b at approx. 13 Å resolution. The direct difference between these two scaled profiles provides the unit cell water profile for this lipid bilayer shown in Fig. 1c. Water is excluded from the hydrocarbon core of these lipid bilayers as expected and is localized in the polar headgroup layers and between the lipid bilayers in the unit cell profile.

The difference profile between the neutron scattering profiles for the sarcoplasmic reticulum lipid/DOPC (deuterated at the C-2 position in the fatty-acid chains) bilayer and the sarcoplasmic reticulum lipid/DOPC (protonated) bilayer is shown at approx. 13 Å resolution in Fig. 1d. The positive peak positions at ± 18 Å clearly indicate the position of the label at C-2 in the bilayer profile at this resolution. A similar difference profile is shown in Fig. 1e where the DOPC was specifically deuterated in the C-9 and C-10 positions of the fatty-acid chains. The positive peak positions at ± 8 Å clearly indicate the positions of the label at C-9 and C-10 in the bilayer profile at this resolution. The second difference profile between Figs. 1e and 1d (i.e. 1e minus 1d) shown in Fig. 1f clearly indicates the label at C-2 positions as positive peaks (at ± 8 Å) and the label at C-9 and C-10 positions as negative troughs (at ± 18 Å).

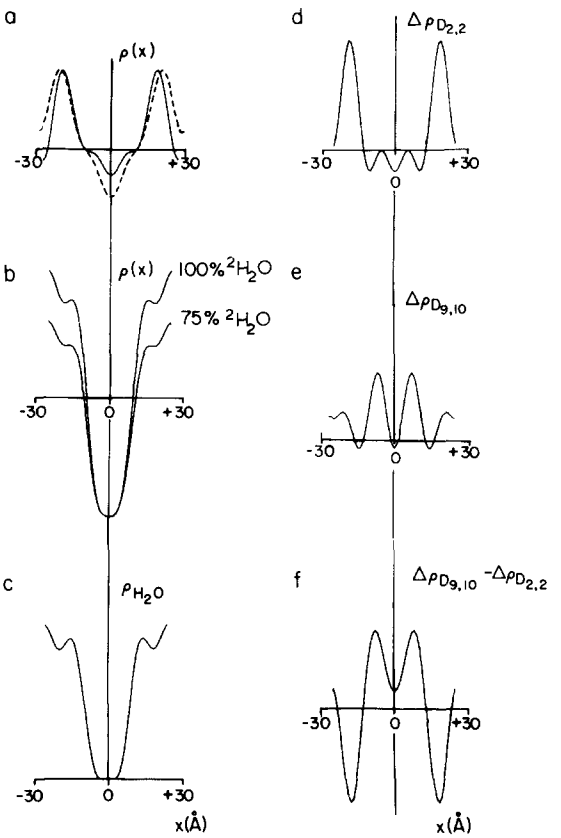

Fig. 1. Summary of the profile structure of the sarcoplasmic reticulum lipid/DOPC bilayer. In (a), the higher resolution (~ 13 Å) electron density profiles for the sarcoplasmic reticulum lipid bilayer (dotted line) and the sarcoplasmic reticulum lipid/DOPC bilayer (solid line) are shown superimposed. In (b), the neutron scattering profiles for the sarcoplasmic reticulum lipid/DOPC bilayer hydrated with two different $H_2O/^2H_2O$ ratios are shown at ~ 13 Å resolution. Their direct difference provides the unit cell water profile shown in (c). The direct difference profile between the neutron scattering profiles for the sarcoplasmic reticulum lipid/DOPC bilayer containing specifically deuterated versus protonated DOPC are shown in (d) where the deuteration occurred at the C-2 position in the fatty-acid chains and similarly in (e) for the C-9 and C-10 positions. The second direct difference profile (profiles (e) minus (d)) is shown in (f).

TABLE I

LABEL DISTRIBUTIONS IN THE MEMBRANE PROFILE

Label	Position (Å) [a]	Center of lipid hydrocarbon core (Å) [b]	R_h [c]	R_m [d]
Sarcoplasmic reticulum lipid: DOPC bilayer				
$D_{9,10}$	8 ± 1			1
		0	1	
$D_{2,2}$	18 ± 1			1
Reconstituted sarcoplasmic reticulum				
$D_{2,2}$	11.49 ± 1			1.12
		27	1.4	
$D_{9,10}$	17.39 ± 1			1.12

[a] Label positions within the single membrane profiles as measured from $x = 0$ of the unit cell profiles.

[b] As estimated from (a) the unit cell water profiles and (b) the electron density minimum of the single membrane electron density profiles.

[c] Ratio of fatty acyl chain extensions C_2–$C_{9,10}$ for the outer (facing $|x| = D/2$ for reconstituted sarcoplasmic reticulum) vs. inner (facing $|x| = 0$ Å for reconstituted sarcoplasmic reticulum) monolayer.

[d] Ratio of number of lipid molecules for the inner vs. outer monolayer.

Since the sarcoplasmic reticulum lipid/DOPC lipid bilayer profile is symmetric, the distances between the C-2 and the C-9 and C-10 positions in each monolayer of the bilayer are necessarily equal. These data are summarized in Table I; a model refinement analysis of these data (see Methods) was not necessary because of the higher resolution (approx. 13 Å) of these data as compared with the relative positions of these labels in the bilayer profile.

(B) Reconstituted sarcplasmic reticulum membrane profiles and deuterium label distributions

The lamellar neutron diffraction data from oriented reconstituted sarcoplasmic reticulum membrane multilayers (lipid/protein mole ratio = 118) are shown in Fig. 2 where the DOPC used in the reconstitution was either protonated or specifically deuterated at either the C-2 or the C-9 and C-10 positions in the fatty-acid chains. Major differences in the amplitudes of the various lamellar reflections (orders 1–4) shown here clearly occur depending on the specific sites of DOPC deuteration.

Our earlier structural studies on reconstituted sarcoplasmic reticulum multilayers at these higher lipid/protein ratios [4] indicated that the multilayer unit cell profile contained the two apposed single-membrane profiles of a flattened unilamellar membrane vesicle thereby causing the unit cell profile projection to be centrosymmetric. These conclusions were based on the nature of the lamellar X-ray and neutron diffraction data and electron microscopy of the fixed reconstituted sarcoplasmic reticulum membrane multilayers. The especially strong odd order lamellar neutron reflections shown in Fig. 2 add strong support to our earlier conclusions.

The various structural features of the reconstituted sarcoplasmic reticulum membrane profile (lipid/protein ratio = 118) are illustrated in Fig. 3. The electron density (or X-ray scattering) profiles for these reconstituted sarcoplasmic reticulum membranes reconstituted with either sarcoplasmic reticulum lipids (dotted line) or the sarcoplasmic reticulum lipid/DOPC (deuterated or protonated) mixture (solid line) are shown in Fig. 3a at approx. 14 Å resolution. The reconstituted sarcoplasmic reticulum single-membrane profile is contained within 0 Å $\leqslant |x| \leqslant$ 60 Å and the electron density of the hydrocarbon core region of the membrane profile is again seen to be somewhat more dense for the DOPC-containing membranes as was the case for the lipid bilayers in the absence of protein. The phospholipid headgroups which should dominate the positive features of these reconstituted sarcoplasmic reticulum membrane profiles (at these higher lipid/protein ratios, see Ref. 4) are seen to be separated by approx. 40 Å across the single reconstituted sarcoplasmic reticulum membrane profile essentially identical to that of the protein-free lipid bilayers.

The unit cell neutron scattering profiles at two different $H_2O/^2H_2O$ ratios for these reconstituted sarcoplasmic reticulum multilayers are shown in Fig. 3b at approx. 28 Å resolution. The resulting unit cell water profile is shown in Fig. 3c. A greater amount of water is present between the membranes at $|x| \sim 60$ Å than between the membranes at $|x| \sim 0$ Å at this resolution; water again is excluded from the lipid hydrocarbon core regions of these single-membrane profiles.

The difference profile between the unit cell neutron scattering profiles for reconstituted

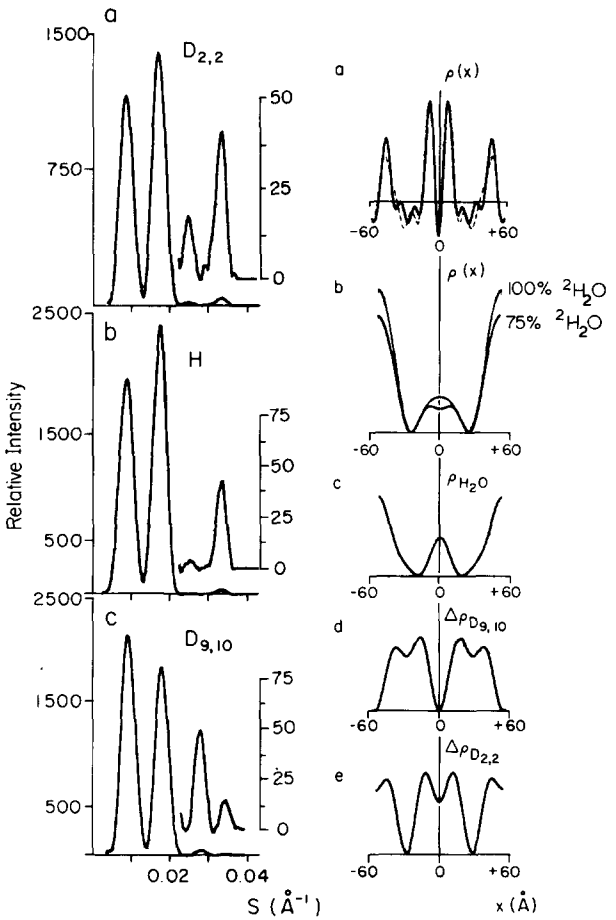

Fig. 2. Appropriately corrected and numerically-smoothed lamellar neutron diffraction from the oriented reconstituted sarcoplasmic reticulum membrane multilayers containing (a) DOPC deuterated at the C-2 position in the fatty-acid chains, (b) protonated DOPC, and (c) DOPC deuterated at the C-9 and C-10 positions in the fatty acid chains. The first four orders of lamellar diffraction are shown; significant changes in the amplitudes of the various reflections depending upon the deuteration sites are readily apparent.

Fig. 3. Summary of the profile structure of the reconstituted sarcoplasmic reticulum membrane. In (a), the unit cell electron density profiles for reconstituted sarcoplasmic reticulum with sarcoplasmic reticulum lipids (dotted line) and the sarcoplasmic reticulum lipid/DOPC mixture (solid line) are shown at -14 Å resolution; the single-membrane profile is contained within $0 \text{ Å} \leqslant |x| \leqslant 60 \text{ Å}$. In (b), the neutron scattering profiles for reconstituted sarcoplasmic reticulum membranes reconstituted with the sarcoplasmic reticulum lipid/DOPC mixture and hydrated with two different $H_2O/^2H_2O$ ratios are shown at approx. 28 Å resolution. Their direct difference provides the unit cell water profile structure shown in (c). The direct difference profiles between the neutron scattering profiles for reconstituted sarcolasmic reticulum membranes reconstituted with sarcoplasmic reticulum lipid/specifically-deuterated sarcoplasmic reticulum membranes containing DOPC specifically deuterated at the C-9 and C-10 positions in the fatty-acid chains and those containing protonated DOPC is shown in Fig. 3d at approx. 28 Å resolution. There are clearly two less-than-fully-resolved deuterium labelling sites evident per single-membrane profile with unequal densities. A model refinement analysis of these labelling sites (see Methods) indicated that these sites occur at $|x| \simeq 17 \pm 1$ Å and $|x| \simeq 3 \pm 1$ Å within the reconstituted sarcoplasmic reticulum single-membrane profile with a density ratio of 1.12, respectively, for these two sites. Similarly, the difference profile for reconstituted sarcoplasmic reticulum membranes and DOPC specifically deuterated at the C-2 position in the fatty-acid chains is shown in Fig. 3e also at approx. 28 Å resolution. Again, two distinct labelling sites per reconstituted sarcoplasmic reticulum single-membrane profile with unequal densities are evident: a model refinement analysis of these labelling sites indicated that these sites occur at $|x| \simeq 11 \pm 1$ Å and $|x| \simeq 49 \pm 1$ Å within the reconstituted sarcoplasmic reticulum single-membrane profile with a density ratio of again 1.12, respectively, for these two sites. Hence, there are 1.12 times more DOPC molecules in the inner monolayer facing $|x| = 0$ Å than there are in the outer monolayer facing $|x| = D/2 - 60$ Å in this asymmetric lipid bilayer within these reconstituted sarcoplasmic reticulum membranes. In addition, the time-average distance between labels at the C-2 and at the C-9 and C-10 positions in the outer monolayer is 1.4-times greater than that in the inner monolayer within these reconstituted sarcoplasmic reticulum membrane profiles. These results are summarized in Table I. Clearly, the lipid bilayer (assuming that DOPC and sarcoplasmic reticulum lipids are similarly distributed in the reconstituted sarcoplasmic reticulum membrane profile) within the reconstituted sarcoplasmic reticulum membrane profile is strongly asymmetric due to the presence of the calcium pump protein.

DOPC versus sarcoplasmic reticulum lipid/protonated DOPC are shown in (d), where the DOPC was deuterated in the C-9 and C-10 positions of the fatty acid chains and similarly in (e) for the C-2 position. The resolution of profiles (b)–(e) is approx. 28 Å.

(C) The separate lipid profile within the reconstituted sarcoplasmic reticulum membrane profile

Using the results described in section (B) above and the procedure described in Methods, the neutron and X-ray scattering profiles of the lipid bilayer within these reconstituted sarcoplasmic reticulum membrane profiles were calculated to resolutions of 28 Å and 14 Å. These calculations assumed that the DOPC and sarcoplasmic reticulum lipids were distributed similarly within the reconstituted sarcoplasmic reticulum membrane profile; the strong similarity between the reconstituted sarcoplasmic reticulum membrane profiles containing only sarcoplasmic reticulum lipids and those containing the 60 : 40 sarcoplasmic reticulum lipid/DOPC mixture strongly supports this assumption (see Fig. 3a). The neutron scattering profile of the lipid bilayer within these reconstituted sarcoplasmic reticulum membranes is shown at 14 Å resolution in Fig. 4a while the corresponding electron density (or X-ray scattering) profile is shown at 28 Å-resolution in Fig. 5a and 14 Å-resolution in Fig. 5c. As can be seen, they reflect the asymmetry in the experimentally determined distributions of deuterium-labelled DOPC within these reconstituted sarcoplasmic reticulum membrane profiles.

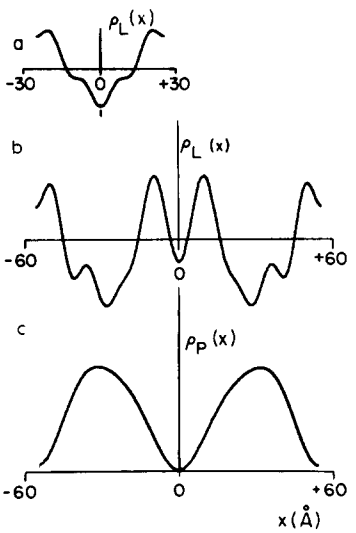

Fig. 4. In (b), the separate neutron scattering profile for the sarcoplasmic reticulum lipid/DOPC bilayer within these reconstituted sarcoplasmic reticulum membranes is shown as calculated at approx. 14 Å resolution. In (c), the separate neutron scattering profile for the calcium pump protein within these reconstituted sarcoplasmic reticulum membranes is shown as calculated by direct difference methods at approx. 28 Å resolution. Note that the single-membrane profile is contained within $0 \text{ Å} \leqslant |x| \leqslant 60$ Å. For the sake of comparison, the neutron scattering profile for the symmetric sarcoplasmic reticulum lipid/DOPC bilayer (in the absence of protein) is shown in (a) placed appropriately over the profiles of (b) and (c).

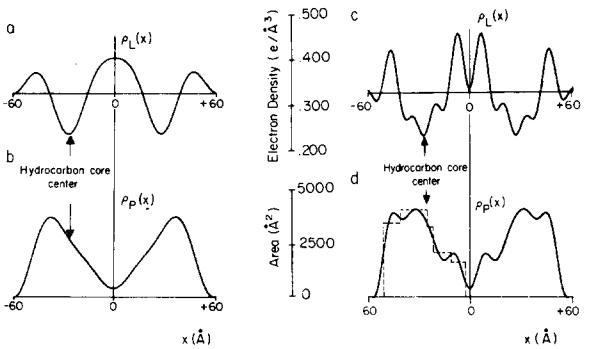

Fig. 5. In (a) and (c), the separate electron density profiles for the sarcoplasmic reticulum lipid/DOPC bilayer within these reconstituted sarcoplasmic reticulum membranes are shown as calculated at approx. 28 Å and approx. 14 Å resolution, respectively. In (b) and (d), the separate electron density profiles for the calcium pump protein within these reconstituted sarcoplasmic reticulum membranes as calculated (see text for details) are shown at approx. 28 Å and 14 Å resolution, respectively. The single-membrane profile is contained within $0 \text{ Å} \leqslant |x| \leqslant 60$ Å. We note that the protein profiles are expressed as area profiles, i.e. the area occupied by the protein as a function of the membrane profile coordinate $|x|$ assuming that the mass density of the protein is independent of $|x|$.

(D) The separate calcium pump protein profile

Following the procedure described in the Methods section, the neutron scattering profile for the calcium pump protein within these reconstituted sarcoplasmic reticulum membrane profiles was calculated to approx. 28 Å resolution as shown in Fig. 4b. The various profiles used in this calculation (namely the reconstituted sarcoplasmic reticulum membrane total neutron scattering profile, the separate lipid neutron-scattering profile and the separate water neutron-scattering profile within these reconstituted sarcoplasmic reticulum membrane profiles) were all at approx. 28 Å resolution. As can be seen from Fig. 4b, the calcium pump protein profile within these reconstituted sarcoplasmic reticulum membranes is clearly asymmetric even at this rather low resolution.

Again following the procedure described in Methods, the electron density (or X-ray scattering) profile for the calcium pump protein within these reconstituted sarcoplasmic reticulum membrane profiles was calculated to both approx. 28 Å resolution (see Fig. 5b) and approx. 14 Å resolution (see Fig. 5d). These calculations utilized all the various profiles at the comparable resolution except that the resolution of the separate water profile was at approx. 28 Å resolution for both cases. The degree of asymmetry in the relatively low-resolution electron density profile for the calcium pump protein (Fig. 5b) agrees favorably with its low-resolution neutron scattering profile (Fig. 4b). The features of this asymmetry are seen more clearly at higher resolution (Fig. 5d).

It is clear from these results that a greater portion of calcium pump protein occurs in that half of the membrane profile facing $|x| = 60$ Å (namely approx. 65%) than occurs in that half facing $|x| = 0$ Å (namely approx. 35%). We note, that the half of the membrane profile facing $|x| =$ 60 Å containing the greater portion of protein contains less lipid and conversely.

(E) Model refinement of only the X-ray data

As previously described, the electron density (or X-ray scattering) profiles for reconstituted sarcoplasmic reticulum membranes over a range of lipid/protein ratios have been calculated [4]. These reconstituted sarcoplasmic reticulum membranes contained only sarcoplasmic reticulum lipids (i.e. no DOPC). These profiles were subjected to a model refinement analysis identical to that described previously for a different reconstituted membrane system [2]. The results of this analysis are not being presented here in detail (see Refs. 3 and 4) because they are virtually identical to those already described above concerning the separate electron density profiles of water, lipid and calcium pump protein within these reconstituted sarcoplasmic reticulum total electron density profiles. However, we note that proper allowance for asymmetry in the distribution of lipid molecules in bilayer profile within these reconstituted sarcoplasmic reticulum membranes as indicated by the neutron diffraction analysis described above was essential for the successful convergence of the refinement analysis to a unique solution.

Discussion

(A) Ca^{2+} pump protein profile in the reconstituted sarcoplasmic reticulum membranes

Both the combined X-ray and neutron diffraction direct difference methods (utilizing critical information obtained from neutron diffraction data and deuterated membrane components) and the model refinement of X-ray diffraction data (utilizing a variation in the reconstituted sarcoplasmic reticulum membrane lipid/protein ratio) have provided virtually identical calcium pump protein profiles for these reconstituted sarcoplasmic reticulum membranes at the higher lipid/protein ratios (see Ref. 4).

The strong asymmetry in the derived calcium pump protein profile clearly indicates that the vectorial distribution of calcium pump protein molecules in these particular reconstituted sarcoplasmic reticulum membrane profiles cannot be symmetrical; this vectorial distribution must be significantly asymmetrical. Our current lack of independent evidence concerning the exact ($\pm 5\%$) degree of asymmetry in this vectorial distribution prevents us from determining the molecular profile for the calcium pump protein from this protein profile (see below). However, this distinct asymmetry in the calcium pump protein vectorial distribution in these particular reconstituted sarcoplasmic reticulum membranes is consistent with their high Ca^{2+} transport activity, namely up to 80% that of isolated sarcoplasmic reticulum membranes as previously shown [6].

We note that the calcium pump protein molecular profile has been derived by us [22,23] utilizing combined X-ray and neutron diffraction direct difference methods and isolated light sarcoplasmic reticulum membranes (in which the calcium pump protein molecules are vectorially distributed in an essentially unidirectional manner in the membrane profile and the protonated sarcoplasmic reticulum lipid molecules were exchanged for biosynthetically perdeuterated sarcoplasmic reticulum lipid molecules). A comparison of this calcium pump protein molecular profile in isolated sarcoplasmic reticulum membranes and the calcium pump protein profile within these reconstituted sarcoplasmic reticulum membranes is shown in Fig. 6. Some similarities exist between the two within the lipid

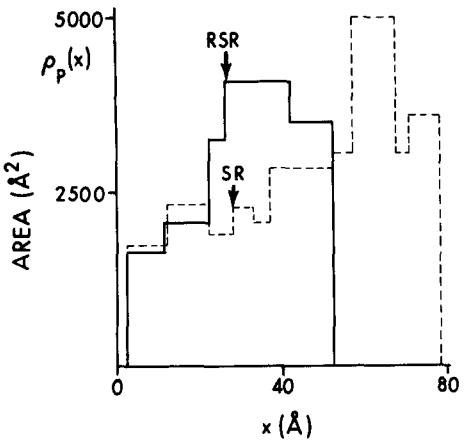

Fig. 6. The separate electron density profile (expressed as an equivalent step-function area profile) for the calcium pump protein within these reconstituted sarcoplasmic reticulum membranes (solid line) is compared with the separate protein profile within isolated light sarcoplasmic reticulum membranes (dotted line) as also obtained via direct difference methods [3,22,23]. These separate protein profiles are shown within their respective single-membrane profiles. The equivalent step-function area profile for reconstituted sarcoplasmic reticulum shown here is taken from Fig. 5d.

bilayer region of the membrane profile (0 Å $\leqslant |x| \leqslant$ 60 Å) while the protein protruding substantially from the extravesicular surface of the sarcoplasmic membrane profile (60 Å $\leqslant |x| \leqslant$ 80 Å) is simply not evident in the reconstituted sarcoplasmic reticulum calcium pump protein profile. We note that MacLennan et al. [21] observed the absence of projections on the extravesicular surface of negatively-stained reconstituted sarcoplasmic reticulum membranes which are readily evident on the extravesicular surface of the negatively-stained isolated sarcoplasmic reticulum membranes. This significant difference must in some way arise from the lack of unidirectionality in the vectorial distribution of calcium pump protein molecules within these reconstituted sarcoplasmic reticulum membrane profiles and/or the absence of non-calcium pump protein in the reconstituted sarcoplasmic reticulum membranes [4,24].

(B) The perturbed lipid bilayer profile of the reconstituted sarcoplasmic reticulum membranes

The lipid bilayer profile within these reconstituted sarcoplasmic reticulum membranes is strongly asymmetrical both in terms of the number of phospholipid molecules and the time-average configurations of the fatty-acid chains in each monolayer of the asymmetric bilayer. This asymmetry must be induced by the asymmetry in the calcium pump protein profile within these reconstituted sarcoplasmic reticulum membranes.

The data summarized in Table I indicate that the time-average configurations of the DOPC fatty-acid chains in the outer monolayer of the reconstituted sarcoplasmic reticulum membrane bilayer (i.e. that facing $|x| = D/2$) are essentially all-*trans* while those in the inner monolayer (i.e., that facing $|x| = 0$ Å) contain a significant number of *gauche* (and/or *anti-gauche*) conformations on average both in the C-2 to C-9/C-10 and C-9/C-10 to C-18 segments of the chains. This result is in strong contrast with that found for the same sarcoplasmic reticulum lipid/DOPC bilayer in the absence of Ca^{2+} pump protein. In this symmetric lipid bilayer, the time-average configuration of the DOPC fatty acid chains is essentially all-*trans* in the C-2 to C-9/C-10 segment while the C-9/C-10 to C-18 segment must possess a significant number of *gauche* (and/or *anti-gauche*) conformations. We note that such asymmetric lipid and protein profiles within the profile structures of other membranes have been reported [5].

The model refinement analysis of neutron diffraction difference data obtained using selectively deuterated lipids can reliably provide the time-average configuration of the lipid molecules within each monolayer of the asymmetric lipid bilayer within these membranes. These results should be carefully considered in the analysis of spectroscopic data sensitive to the details of the intramolecular dynamics of the lipid molecules within such membranes (e.g. ^{2}H-NMR).

(C) Comment on the 'direct decomposition' approach

We have exhaustively analyzed the X-ray and neutron lamellar diffraction data from two totally different reconstituted membrane systems using independently both the 'direct difference' (utilizing combined X-ray and neutron diffraction data together with deuterated membrane components) and 'model refinement' (utilizing only X-ray diffraction data and variation of the membrane lipid/protein ratio) approaches [4,5]. We note that

this 'model refinement' approach may not converge to a unique solution if the distributional asymmetry in the number of lipid molecules in each monolayer of the lipid bilayer within the membrane is not known independently. Hence, the 'direct difference' approach appears to be generally essential to the correct decomposition of the total membrane scattering profile into its separate component profiles. The 'model refinement' approach in the absence of this essential independent information can lead to multiple incorrect solutions!

We further note that either the lipid or protein component may be deuterated in the 'direct difference' approach (see Ref. 5 for details) and that this approach can also be applied to isolated membranes (as opposed to reconstituted membranes) (see Refs. 22 and 23 for details).

Conclusions

(A) The vectorial distribution of calcium pump protein molecules within these highly functional [6] reconstituted sarcoplasmic reticulum membranes must be asymmetric.

(B) The profile structure of the calcium pump protein within these reconstituted sarcoplasmic reticulum membranes is significantly different from that within isolated sarcoplasmic reticulum membranes. This may account for the reported [6] differences in the calcium transport activity between these reconstituted sarcoplasmic reticulum membranes and the isolated sarcoplasmic reticulum membrane.

(C) The presence of calcium pump protein within the lipid bilayer of these reconstituted sarcoplasmic reticulum membranes substantially perturbs each monolayer of the bilayer in a different manner.

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