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LIPID-PROTEIN INTERACTIONS IN SARCOPLASMIC RETICULUM

A DISRUPTED SECONDARY LIPID LAYER SURROUNDS THE Ca^{2+} -ATPASE

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The effect of an intrinsic membrane protein on the molecular structure and dynamics of its surrounding lipid bilayer has been described in terms of a simple two-domain model, the immobilized "boundary" lipid model (1). While this model was originally proposed for membranes containing cytochrome *c* oxidase, it has been applied as well to sarcoplasmic reticulum (SR) membranes containing the Ca^{2+} -ATPase (2). As applied to the SR, this simple model has been criticized for two reasons. First, it could not accurately predict at all temperatures and lipid:protein ratios the extent of protein-induced lipid bilayer order as measured by diphenylhexatriene (DPH) fluorescence anisotropy (3). Second, deuterium spin-resonance probes report enhanced "disorder" for membranes containing the Ca^{2+} -ATPase relative to protein-free lipid bilayers (4). We propose that these observations can be explained by assuming that the Ca^{2+} -ATPase is surrounded by, in addition to "boundary" lipid, a secondary lipid layer with disrupted packing order.

RESULTS AND DISCUSSION

In Fig. 1, we show the dependence of DPH fluorescence anisotropy on the protein content of native and partially delipidated SR membranes, prepared as previously described (3). Earlier, we had attempted to fit these data using the assumption of two independent lipid domains, a boundary layer with 30-35 lipids/protein and the other pure lipid (3). The anisotropies calculated according to this boundary lipid model are shown by dashed curves in Fig. 1. As previously noted (3), the calculated anisotropies failed to reproduce the experimental data at high temperature. A possible reason for this discrepancy is the occurrence of a secondary lipid "solvation" layer around the Ca^{2+} -ATPase with a packing order different from that of bulk lipid. This is a nearly universally accepted concept for aqueous solvation (5). If this model were to apply to the SR membrane, then it would be erroneous to assign DPH

fluorescence properties characteristic of pure lipid to the environment immediately beyond the boundary layer. Indeed, because of the low lipid:protein ratio of SR (~ 100 lipids/ Ca^{2+} -ATPase), we would expect to find in the SR membrane essentially no lipid environment resembling bulk lipid.

To test the secondary solvation layer model, we adjusted the values of DPH fluorescence anisotropy assigned to the nonboundary lipid at each temperature so as to minimize the square deviation between the calculated (3) and observed anisotropies shown in Fig. 1. This procedure accurately reproduced the variation of DPH fluores-

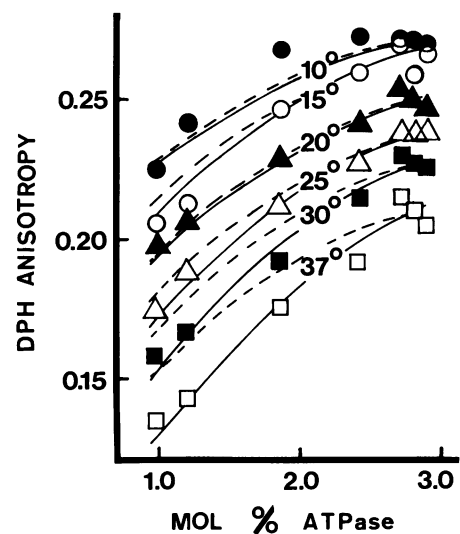


FIGURE 1 Dependence of DPH fluorescence anisotropy on Ca^{2+} -ATPase content of partially delipidated SR membranes. Data at 10°C (O), 25°C (Δ) and 37°C (\square) have been previously reported (3). New data are presented for 15°C (\bullet), 20°C (\blacktriangle) and 30°C (\blacksquare). Calculated anisotropies are shown for two-lipid-domain models in which one domain is assumed to be boundary lipid (34 lipids/ Ca^{2+} -ATPase) and the other to be either bulk lipid (---) or a disrupted secondary lipid layer (—).

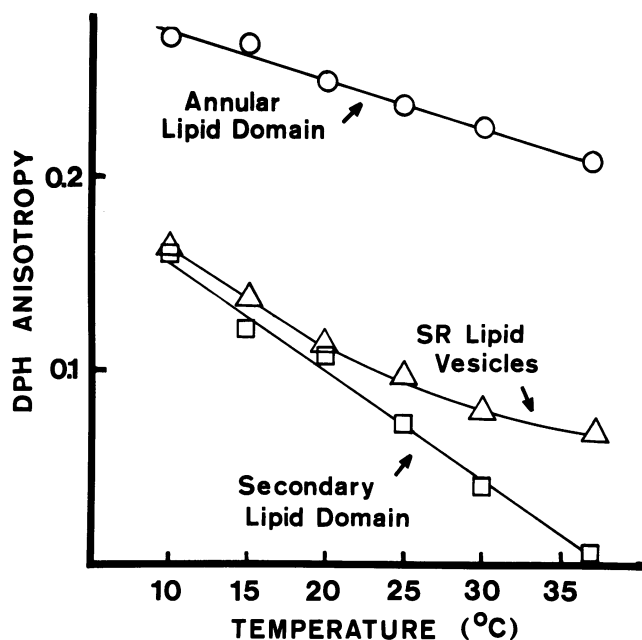


FIGURE 2 Temperature-dependence of the DPH fluorescence anisotropy measured in delipidated membranes for the presumed boundary lipid domain (O) and for multilamellar vesicles prepared from extracted SR lipid (Δ), as well as values calculated for the presumed secondary lipid layer (□).

cence anisotropy, with Ca^{2+} -ATPase content, as shown by the solid curves of Fig. 1. The temperature dependence of fluorescence anisotropy assigned in this way to the secondary lipid layer is shown in Fig. 2. The continuous variation of this parameter with temperature is physically reasonable and enhances the credibility of the model. We note that, in addition to the measured anisotropies in delipidated samples (top curve in Fig. 2), only a single adjustable parameter (the slope of the bottom curve in Fig. 2) is required to describe completely the variation of overall DPH fluorescence anisotropy with both temperature and protein content in the SR membrane.

Fig. 3 *A* illustrates the secondary lipid layer with disrupted packing order that we suggest surrounds the Ca^{2+} -ATPase. This disordered region results from the difference in lipid packing between the region next to the protein surface and the protein-unaffected bilayer far from the protein-lipid interface. Fig. 3 *B* demonstrates the expected variation of membrane packing order between the different lipid layers as suggested by the DPH fluorescence anisotropy values in Fig. 2 (6). The extent of the disrupted region and the severity of the disorder will depend both on the relative cross-sectional areas of the protein and lipids and on the detailed shape of the protein surface. Therefore, the nature of the secondary layer would be expected to be different for all proteins, and perhaps for different conformational states of the same protein. Therefore, we can suggest two possible explanations for the different behavior of the secondary layer above but not below 25°C when compared with pure lipid

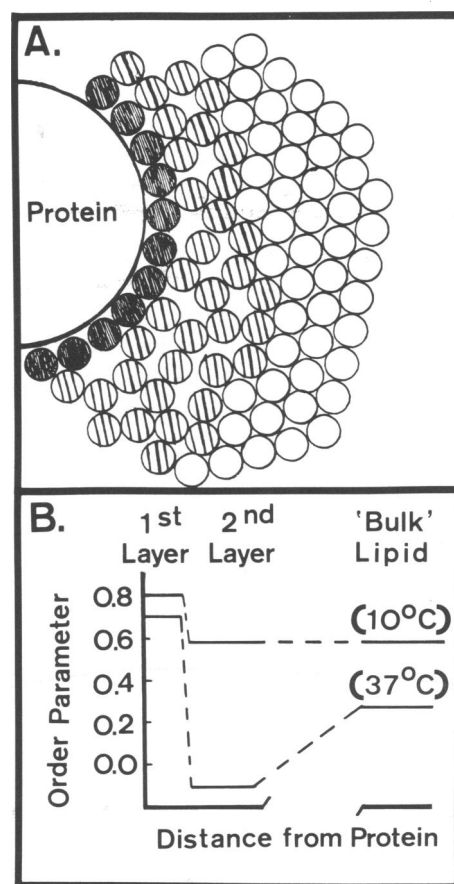


FIGURE 3 *A*. Proposed model for the primary or boundary (shaded), and secondary (striped) lipid layers surrounding the Ca^{2+} -ATPase in SR membrane. A surface view of the membrane is shown. At a distance sufficiently removed from the protein surface, a lipid domain with the packing order of pure lipid bilayer (unshaded) is presumed. *B*. Schematic representation of variation with distance from the protein surface of the approximate lipid acyl chain order parameter derived by the procedure of Jähnig (6) from the DPH fluorescence anisotropy values given in Fig. 2 for 10° and 37°C. This diagram is scaled and positioned to correspond to Fig. 3 *A*.

vesicles (Fig. 2). First, the Ca^{2+} -ATPase is thought to undergo a shift in conformational state at 20–25°C, which could affect the packing in the secondary lipid layer. Alternatively, it is possible that the temperature dependence of the secondary layer reflects an inherent property of the lipid mixture present in native SR membrane.

Finally, we comment on the relationship between our results and previous reports. (a) Regarding deuterium magnetic resonance results (4), the secondary lipid region should contain a variety of different lattice sites, many of which would support lipid molecules tilted substantially relative to the bilayer normal. On the time scale of a deuterium spin-resonance experiment, a probe molecule should reflect the orientational order averaged over both boundary and secondary lattice sites. Therefore, it is not surprising that such probes report a disordering effect of the Ca^{2+} -ATPase on the adjacent lipid bilayer. Due to the

shorter time scale of a fluorescence experiment, each DPH molecule reports orientational order (Fig. 3 *B*) reflecting roughly a single site (6). (b) A recent study using electron-spin-resonance probes has stressed the ability of cytochrome oxidase to "immobilize" lipid structure beyond the primary or boundary layer (7). This apparent disagreement with our results can be resolved by recognizing that the shape of an electron-spin-resonance spectrum is sensitive to the rate of lipid acyl chain motion as well as to the average order in the bilayer. Thus, it may be that lipid molecules within the secondary layer experience slower acyl chain motions than in a bulk lipid bilayer. This would not preclude decreased average order relative to bulk lipid due to a greater extent of motion. (c) The spectra of electron spin probes attached to the Ca^{2+} -ATPase have been found to vary with lipid:protein ratio, despite the constant juxtaposition of the probe to the protein (8). This has been interpreted in terms of protein-rich domain formation, leading to increased protein-protein contacts (9). We agree that the properties of both the primary and secondary lipid layers should be sensitive to protein-protein separation. However, our calculations indicate that this complication is unnecessary to explain our data over the range of protein content considered here. In addition, we find in freeze-fracture electron micrographs of native SR membranes no evidence of particle patches which would indicate protein-rich domains, even at 4°C.¹

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¹Hoechli, B. R. Lentz, and G. Meissner. Unpublished results.

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THE INTERACTION OF MEMBRANE-ACTIVE COMPOUNDS WITH THE SURFACES OF SCHISTOSOMULA AND ADULT *SCHISTOSOMA MANSONI*

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The adult *Schistosoma mansoni* is a multicellular parasite which lives in the portal blood vessels of man, and causes the disease called schistosomiasis (bilharziasis). The organism is covered by a complex pentalaminate surface membrane (Hockley and McClaren, 1973). The schistosomulum, the name given to the growth stage of the parasite formed after penetration through the mammalian skin by the infective larva (cercaria), also has a pentalaminate surface membrane, which changes in ultrastructure and

antigenicity during the migration of the parasite through the tissues of the host (McClaren et al., 1978). These changes are accompanied by a decreased sensitivity of the membrane to the manifestations of the immune response of the host (McClaren, 1980). Changes in the lipid phase of artificial and natural membranes can affect the expression of antigenic determinants (Kinsky, 1978) and their ability to be damaged by immunological mechanisms (Schlager and Ohanian, 1980).