DISCUSSION

These results yielded the following conclusion: all ts M mutants exhibit a coordinate increase in association of M with membrane (or viral envelope) and a decrease in association with nucleocapsid. The mechanism for this alteration in phenotype cannot be fully understood until the complete structures of the wt and ts M protein are known. However, one can construct a simple explanation for the coordinate alterations by assuming that two independent binding sites govern the affinity of M for membranes and nucleocapsids, and that the mutation directly affects only the nucleocapsid binding site. The coordinate effect could then be produced simply by mass action:

$$\text{M - Nucleocapsid} \rightleftharpoons \text{M}_{\text{sol}} \rightleftharpoons \text{M - Membrane}.$$

Alternatively, there could be a portion of the M polypeptide that interacts with the membrane only under conditions of reduced interaction with the nucleocapsid. In this case, the two binding sites would not be completely independent.

Results with the ts M mutants demonstrate that the biological function of the M protein can be maintained in at least two different conditions of lipid-protein association; whether these two conditions are statistical or conformational is not addressed by our findings.

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INSERTION OF BACTERIOPHAGE M13 COAT PROTEIN INTO MEMBRANES

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The filamentous bacterial viruses (M13, fd, f1) provide an exceptionally good model system for studying factors that govern the insertion of proteins into membranes. 2,700 copies of the viral B protein are arranged with helical symmetry around the circular single-stranded DNA to form a long filamentous virion so tightly constructed that it is impervious to detergents, urea, proteases, and heating. However, during phage penetration, this tight association is somehow altered to allow insertion of the phage coat protein into the cell membrane and release of the viral DNA. In vitro studies have shown that isolated B protein can be incorporated into synthetic lipid vesicles as the vesicles are formed, and that maximum incorporation occurs at the phase transition temperature of the lipid (1). However, isolated B protein does not insert into preformed lipid bilayers. Apparently, in vivo, the construction of the virion facilitates insertion of the protein into the membrane, but this insertion mechanism has been difficult

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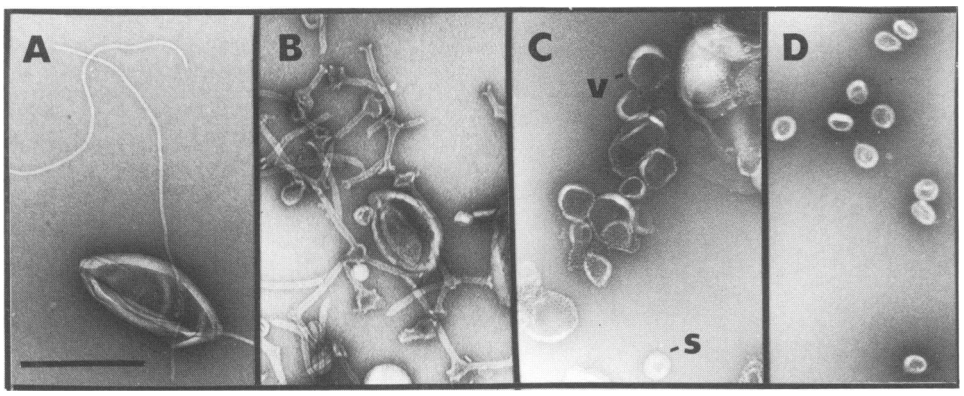

FIGURE 1 *A* Intact filaments with a lipid vesicle. *B* Intact I-forms with DMPC vesicles. *C* Thick walled DLPC vesicles (v) and spheroid-like structures (s). *D* Spheroids. Samples were stained with uranyl acetate. Bar equals 250 nm.

to deduce, especially in light of the extreme stability of the filaments in the presence of lipids or detergents.

Recently, we discovered a remarkable property of filamentous phage that may shed light on the penetration mechanism. We have shown that exposure of filaments to a chloroform-water interface causes a 20-fold contraction of the 900 nm × 5 nm filament into a hollow spherical particle 40 nm Diam (termed spheroid). This highly ordered change results not only in extrusion of two-thirds of the viral DNA (with the origin of replication remaining firmly bound within the particle) but also causes the capsid protein to become susceptible to detergents and proteases (2). We have also described the isolation and characterization of stable virion particles which appear to be intermediates in the transition of filaments to spheroids. These intermediate (I-form) particles are ~ 300 nm × 18 nm, possess a flaired end and a slightly pointed end, and are formed when filaments are exposed to ice-cold chloroform. I-forms contract into spheroids if reexposed to the solvent interface at temperatures above 15°C, but are stable in aqueous environment at temperatures up to 37°C. The contraction appears to involve noncooperative, localized changes in the filament structure (3). We have postulated that penetration of filamentous phage may be a complex event which involves both activation of the virion capsid (to initiate structural rearrangements) and sequential dispersal of the coat protein into the membrane and concomitant injection of the viral DNA. Activation may be accomplished through the binding of the viral adsorption complex (a knob and stem structure located on one end of the virion) to a cellular receptor. Indeed, we have visualized the adsorption complex at the end of the I-form from which the DNA emerges and believe membrane insertion begins at that end. We propose that the chloroform-water interface performs this activation function in vitro and converts the capsid into a lipid soluble form. Thus, these contracted phage may offer a unique opportunity to study the dispersal of highly organized protein structures into membranes. We have begun to examine the interaction of the particles with lipid bilayers.

MATERIALS AND METHODS

Phage were purified as described previously (2). I-forms were prepared by gently vortexing an ice-cold suspension of M13 filaments in a TM buffer (0.01M Tris, 0.001M $MgCl_2$ pH 7.5) with an equal volume of ice-cold chloroform, and collecting the aqueous layer. Lipid vesicles (dilauroylphosphatidylcholine-DLPC, and dimyristoylphosphatidylcholine-DMPC) were the gift of B. Lentz.

RESULTS

I-form particles were used in these studies because they had not reached the end point in capsid rearrangement (the spheroid morphology) and consequently allowed us to use morphological changes as an assay. Filaments and I-forms were mixed with synthetic lipid vesicles (DLPC and DMPC), incubated 60 min at 17°C (between the phase transition temperatures of the two lipids), and examined by electron microscopy (Fig. 1). Apparently unchanged filaments can be seen in close contact with the lipid vesicles. Similarly, intact I-forms were visualized after incubation with DMPC. In contrast, DLPC appeared to have a profound effect. These preparations contained a few distorted I-forms, numerous spheroid-like structures, and many lipid vesicles with thickened walls suggesting protein incorporation. When similar preparations containing ^{3}H-Lys labeled phage were passed through a Sephadex G-150 column, all label emerged in the void volume, indicating that the lipid had not merely disrupted the I-form particles.

DISCUSSION

These results suggest that I-forms, in contrast to filaments, do respond to a lipid environment, and further, that there is some specificity to this response. Experiments are in progress to identify the parameters that govern these effects and to determine whether or not coat protein is indeed inserted into the bilayer.

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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-

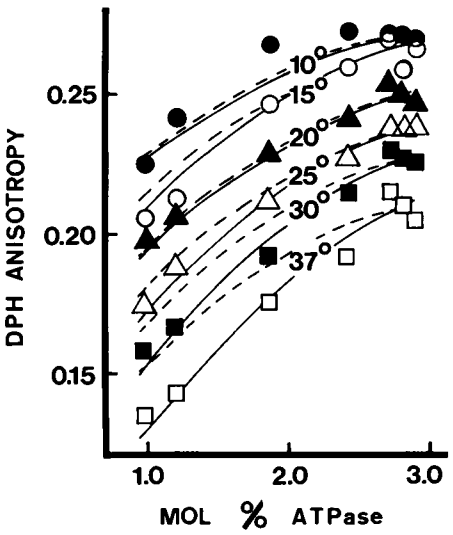

FIGURE 1 Dependence of DPH fluorescence anisotropy on Ca^{2+}-ATPase content of partially delipidated SR membranes. Data at 10°C (○), 25°C (△) and 37°C (□) have been previously reported (3). New data are presented for 15°C (●), 20°C (▲) and 30°C (■). Calculated anisotropies are shown for two-lipid-domain 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 (—).

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