Finally, we stress that using the method described we have been able to obtain simply and quickly, reliable molecular-motion parameters for the IgG·Gd(III) complex and thereby provide evidence for rapid motion in the Fc portion of the IgG molecule. The proposal of Huber et al. (1976) that the inherently flexible antibody molecules become rigid upon interaction can now be investigated by this method in an appropriate antibody–antigen system. This could be the "trigger" for complement activation and clearly a definitive answer to this must come from a technique monitoring changes in solution—as is the case here.

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References

Burton, D. R., Forsen, S., Karlström, G., Dwek, R. A., McLaughlin, A. C., and Wain-Hobson, S. (1977), *Eur. J. Biochem.*, in press.

Cohn, M., and Leigh, J. S. Jr. (1962), *Nature (London) 193,* 1037–1040.

Dower, S. K., Dwek, R. A., McLaughlin, A. C., Mole, L. E., Press, E. M., and Sunderland, C. A. (1975), *Biochem. J. 149,* 73–82.

Dwek, R. A. (1973), Nuclear Magnetic Resonance in Biochemistry, Applications to Enzyme Systems, Oxford, Clarendon Press.

Eigen, M., and De Maeyer, L. (1963), in Rates and Mechanisms of Reactions, Part II, Freiss, S. L., Lewis, E. S., and Weissberger, A., Ed., Interscience, New York, N.Y., p 1035.

Eisinger, J., Shulman, R. G., and Szymanski, B. M. (1962), *J. Chem. Phys. 36,* 1721–1729.

Hamilton, W. C., and Ibers, J. A. (1968), Hydrogen Bonding in Solids, New York, N.Y., W. A. Benjamin, p 105.

Huber, R., Deisenhofer, J., Colman, P. M., Matsushima, M., and Palm, W. (1976), *Nature (London) 264,* 415–420.

Lanir, A., Gradstajn, S., and Navon, G. (1975), *Biochemistry 14,* 242–248.

Navon, G. (1970), *Chem. Phys. Lett. 7,* 390–394.

Reuben, J. (1971), *Biochemistry 10,* 2834–2838.

Reuben, J. (1975), *J. Chem. Phys. 63,* 5063–5064.

Yguerabide, J., Epstein, H. F., and Stryer, L. (1970), *J. Mol. Biol. 51,* 573–590.

Role of Hydrophobic Forces in Membrane Protein Asymmetry†

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ABSTRACT: M13 virus coat protein is an integral cytoplasmic membrane protein at all stages of viral infection. The pure virus coat protein can also be incorporated into synthetic lecithin vesicles near the lipid-phase transition temperature (T_m), spanning the bilayer with its N terminus exposed on the outside and its C-terminus inside (Wickner, W. (1976), *Proc. Natl. Acad. Sci. U.S.A. 73,* 1159–1163). The assembly of coat protein into vesicles in this asymmetric fashion has a sharp maximum near the phase-transition temperature of the lipid fatty acyl chains. At temperatures well below the T_m, coat protein assembles into lecithin vesicles with both termini exposed on the exterior vesicle surface. In contrast to this important role of lipid physical state, asymmetry of assembly is unaffected by parameters which govern polar interactions such as pH, ionic strength, lipid polar head group, or prior proteolytic removal of either the N or C terminus. It is proposed that the orientation of this membrane protein, and perhaps others, is determined by interactions of the hydrophobic portion of the protein with the lipid hydrocarbon core.

Asymmetric orientation is a characteristic feature of membrane proteins (Singer, 1974). The question of how this is established is at the heart of understanding membrane assembly. Once established, asymmetry is maintained by the low rates of diffusion of protein polar faces across the hydrocarbon core. This fact underscores a second question: How can proteins be assembled into a conformation which spans the bilayer?

The (gene 8) coat protein of the filamentous coliphage M13 offers several advantages for studying these problems. It is composed of fifty amino acid residues (Asbeck et al., 1969; Nakashima and Konigsberg, 1974) with a central hydrophobic region. Both the coat protein from infecting virus and that newly synthesized by the infected cell are integral components of the cytoplasmic membrane (Trenkner et al., 1967; Smilowitz et al., 1972; Marco et al., 1974; Smilowitz, 1974); both coat the progeny DNA as it extrudes through the membrane (Smilowitz, 1974). During infection, coat protein accounts for up to a third of the membrane protein synthesis (Smilowitz et al., 1972) and each coat protein molecule has its acidic N terminus exposed on the membrane outer surface (Wickner, 1975; Wickner, 1976). It has been proposed that the basic C terminus of coat protein is on the inner surface of the cytoplasmic membrane and is involved in conducting DNA through the lipid bilayer (Marvin and Wachtel, 1975).

In a previous communication (Wickner, 1976), coat protein was found to assemble into lecithin vesicles prepared by the cholate-dilution technique of Racker et al. (1975). In these experiments, clear, nonsedimentable solutions of cholate, lipid,

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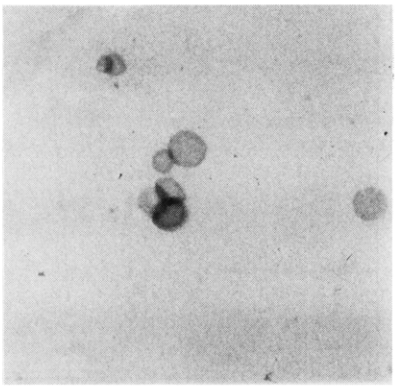

FIGURE 1: Vesicles of M13 coat protein and dilauryllecithin were prepared at 0 °C, as described under Materials and Methods. Copper grids (400 mesh, purchased from Pelco Corp.) were dipped in a 1% solution of polybutene in xylene and air dried on filter paper. They were placed on a carbon film floating on water and, after 5 min, picked from the film with a Saron-wrap drum and set on filter paper to drain. A suspension of phospholipid vesicles (50 μl in 0.1 M NaCl, 0.01 M Tris-HCl, pH 7.5) was applied to the carbon-coated grid surface and, after 1 min, drawn from the grid edge with filter paper. One drop of uranyl acetate (5 mg/ml) was applied to the same surface and, after 30 s, drawn off with filter paper. Samples were visualized in a Phillips 300 electron microscope.

and M13 coat protein are rapidly diluted to below the critical micellar concentration of cholate, causing vesicle formation. When such vesicles were prepared near the melting temperature (T_m[1]), the coat protein spanned the membrane with N termini exposed on the vesicle exterior and the C termini on the interior. When vesicles were prepared well below the T_m, both ends of the bound coat protein were exposed on the exterior vesicle surface. Despite the artificial nature of this assembly reaction, it is novel in showing that membrane protein asymmetry can be established in the absence of (1) other proteins, (2) lipid fatty acyl or polar head group asymmetry, or (3) prior membrane structure. This report examines several of the parameters which control this assembly.

Materials and Methods

Materials. TPCK-trypsin and soy bean trypsin inhibitor were purchased from Worthington, α-chymotrypsin was from Sigma, and lecithins were from Calbiochem. Dipalmitoyl-L-α-phosphophatidylserine was the generous gift of Drs. Beth Luna and Harden McConnell. Growth and isolation of M13 virus, as well as purification of the major (gene 8) coat protein, have been described (Wickner, 1975). Preparation of antiserum to M13 coat protein, affinity purification of the antibody by combination with virus, and isolation of goat antibody to rabbit γ-globulin were by previously published methods (Wickner, 1975; Wickner, 1976).

Lipid vesicles were formed by the cholate-dilution technique of Racker et al. (1975) as applied to the M13 coat protein (Wickner, 1976). Briefly, M13 coat protein (1 mg in 0.5 ml of 0.02 M $NaHCO_3$, pH 8.5, with 5 mg of sodium cholate) and lipid (0.2 ml of 40 mg/ml, suspended in 0.1 M KP, pH 7.0, and sonicated for 1 min at a temperature above the lipid T_m) were mixed at a temperature above the lipid T_m and transferred to the indicated temperature for 30 min. The solution was diluted with 20 ml of 0.1 M KP (pH 7.0) and, after 1 h, vesicles were collected by centrifugation (1 h, 44 000*g* at the indicated temperature).

[1] Abbreviations used are: T_m, the temperature of fatty acyl phase transition; coat protein, the M13 coat protein, product of gene VIII; DLL, dilauryllecithin; DML, dimyristoyllecithin.

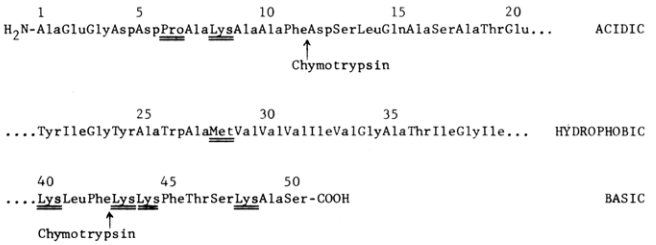

FIGURE 2: The sequence of M13 coat protein (Asbeck et al., 1969; Nakashima and Konigsberg, 1974). The positions of chymotrypsin cleavage (Woolford and Webster, 1975) are indicated with arrows. Underlined amino acids were radioactively labeled in some experiments reported here.

To determine the orientation of radioactive coat protein, vesicles were suspended in buffer (1.0 ml of 0.1 M KP, pH 7.0) and incubated with chymotrypsin (50 μl of 5 mg/ml) for 1 h at 37 °C, and a portion was sedimented in a Beckman airfuge (15 min, 23 °C, 167 000*g*). The supernatant and pellet were mixed with 1 ml each of 0.5% Triton ×100 and assayed for radioactivity.

Results

Electron Microscopy. Vesicles of dilauryllecithin and M13 coat protein, prepared by cholate dilution near the lipid T_m and examined in the electron microscope after mounting on a carbon-coated grid and staining with uranyl acetate, have a uniform, spherical appearance (Figure 1). Vesicles were mixed with phage heads to permit quantitative size measurements. Vesicles of dilauryllecithin prepared without coat protein had a diameter of 780 Å ± 80 Å, whereas those with coat protein had a diameter of 2500 Å ± 500 Å. Similarly, protein-free dimyristoyllecithin vesicles had a diameter of 240 Å ± 60 Å, whereas those with coat protein had a mean diameter of 2200 Å ± 200 Å. Similar effects of hydrophobic proteins on the size of synthetic lipid vesicles have been seen in other systems (Dr. Harden McConnell, personal communication).

Orientation of vesicle-bound coat protein was assayed, as previously described (Wickner, 1976), by its accessibility to chymotrypsin. The acidic N terminus of coat protein (Figure 2) is specifically labeled by growing the virus with [^{3}H]proline, while four of the five lysines are near the basic C terminus. The central hydrophobic region, conveniently labeled with ^{35}S, is protected from chymotrypsin cleavage by apolar interactions with detergent or lipid hydrocarbon chains (Woolford and Webster, 1975). Chymotrypsin cleaves the detergent-solubilized peptide at the positions indicated in Figure 2. It also cleaves both the N and C termini of coat protein bound to lipid vesicles formed well below the lipid T_m (Wickner, 1976). Chymotrypsin releases N-terminal coat protein label from vesicles formed near or at the T_m but does not release C-terminal coat protein label from these vesicles. To show that this was due to the inaccessibility of buried internal C-termini, vesicles were loaded with internal chymotrypsin during preparation (Table I). A clear, nonsedimentable solution of M13 coat protein, cholate, and dilauryllecithin (T_m 0 °C) was diluted with ice-cold buffer containing chymotrypsin. Isolated vesicles bore all of the coat protein, measured by [^{35}S]methionine label, and 93% of the [^{3}H]lysine label. When these vesicles were incubated at 37 °C without further addition, extensive cleavage occurred at the C termini, as measured by subsequent separation of two-thirds of the [^{3}H]lysine label from the ^{35}S peptide core upon gel filtration in the presence of deoxycholate. Control experiments (not shown) indicated that both chymotrypsin and incubation at 37 °C were neces-

TABLE I: Chymotrypsin Cleavage from Within.[a]

	Coat Protein Label	
	^{35}S	[^{3}H]Lys
Vesicles made without chymo	2900	19 730
Chymo-loaded vesicles	2970	15 330
After digestion from within:		
Protein:	2930	6 825
Peptide:	0	10 449

[a] Vesicles of [^{3}H]Lys- and [^{35}S]Met-labeled M13 coat protein and dilauryllecithin were prepared at 0 °C, as described under Materials and Methods. The diluent buffer contained 20 μg of chymotrypsin/ml. Twenty minutes after dilution, vesicles were rapidly harvested by centrifugation (15 min, 44 000g), resuspended in 0.1 M KP (pH 7.0), incubated for 1 h at 37 °C, and then chilled. Two volumes of buffer (1% deoxycholate, 0.02 M Tris-HCl (pH 8.8), 1 mM EDTA), and 3 μg/ml of L-1-tosylamido 2-phenylethyl chloromethyl ketone were added and a portion was filtered through a Sephadex G-150 SF column as previously described (Wickner, 1976). Label was recovered in fractions halfway between the void volume and column volume (protein position) and at the column volume (peptide position). An otherwise identical experiment with ^{35}S- and [^{3}H]Pro-labeled coat protein showed that all of the loss of [^{3}H]Lys label during vesicle preparation was due to cleavage near the N terminus.

TABLE II: The Role of Lipid Polar Head Group in the Orientation of M13 Coat Protein.[a]

Lipid	Label	% Label Released by Chymotrypsin
DPPC	Pro	77
	Lys	40
DPPS	Pro	84
	Lys	41

[a] Vesicles of [^{3}H]Lys- or [^{3}H]Pro-labeled M13 coat protein and dipalmitoylphosphatidylcholine (DPPC) or dipalmitoylphosphatidylserine (DPPS) were prepared at 57 °C and assayed for coat protein orientation, as described under Materials and Methods.

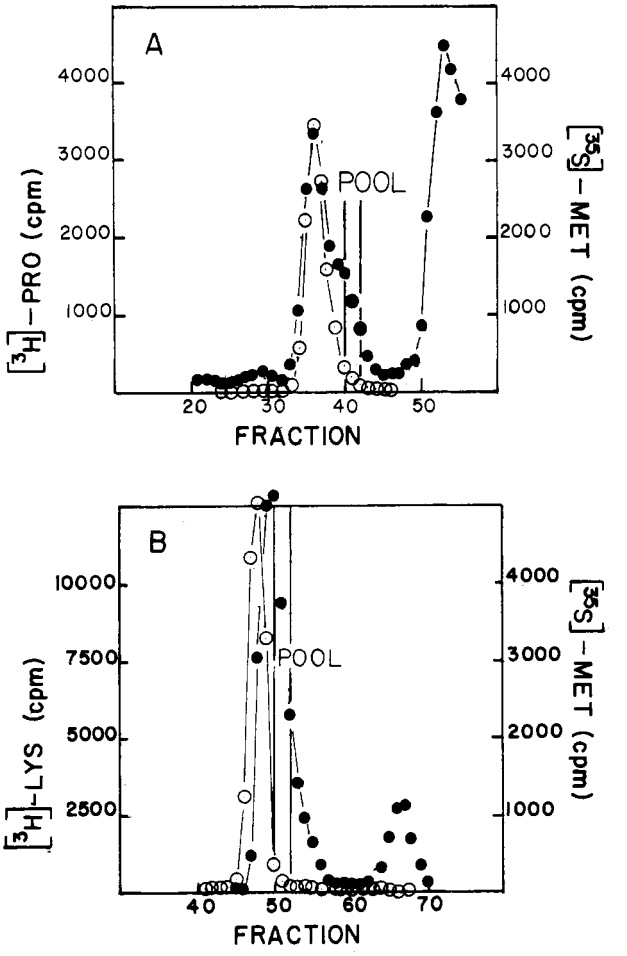

FIGURE 3: Selective proteolysis of M13 coat protein. (A) Chymotrypsin (10 μl of 5 mg/ml) was added to [^{3}H]Pro-labeled M13 coat protein (3.7 mg in 1.0 ml of 5% sodium deoxycholate, 0.01 M $NaHCO_3$, pH 8.5). After digestion for 1 h at 37 °C, 2.8 μl of 5 mg/ml of L-1-tosylamido 2-phenylethyl chloromethyl ketone in methanol was added and the incubation was continued for 10 min. The sample was then chilled to 0 °C, undigested [^{35}S]Met coat protein was added (0.4 mg in 120 μl of 5% sodium deoxycholate, 0.01 M $NaHCO_3$, pH 8.5), and the mixture was filtered at room temperature through a 1.6 × 46 cm G-150 SF column in 1% sodium deoxycholate, 0.02 M Tris-HCl (pH 8.8), 1 mM EDTA. One milliliter fractions were collected and assayed for ^{3}H (●–●) and ^{35}S (○–○); the indicated fractions (40–42) were pooled and vacuum dialyzed against 250 ml of 0.91% sodium cholate–0.02 M $NaHCO_3$ (pH 8.5) to a final volume of 1.3 ml. (B) Trypsin (5 μl of 5 mg/ml) was added to [^{3}H]Lys-labeled M13 coat protein (1.8 mg in 0.5 ml of 5% sodium deoxycholate, 0.01 M $NaHCO_3$, pH 8.5). After digestion for 1 h at 37 °C, soybean trypsin inhibitor was added (10 μl of 5 mg/ml), the sample was chilled to 0 °C, undigested ^{35}S-labeled coat protein was added, and the sample was analyzed by gel filtration, as described above. Fractions 50 to 52 were pooled and vacuum dialyzed as described.

sary for this proteolysis. The failure of external chymotrypsin, added after vesicles are sealed, to cleave the C termini is, therefore, due to the C termini being inaccessible to the enzyme. The C terminus is in contact with the inner aqueous phase, where it can be a substrate for internal chymotrypsin. This experiment, in conjunction with those previously reported (Wickner, 1976), show that coat protein incorporated into vesicles near the lipid T_m spans the bilayer with its N terminus exposed to the outside and the C terminus exposed on the inner membrane face.

Polar interactions were not found to have a dramatic effect on coat protein incorporation into vesicles or on the orientation of the vesicle-bound protein. Neither pH in the range 6.5–9.5 nor ionic strength (up to 0.2 M added potassium phosphate) dramatically affected either incorporation or orientation of coat protein (data not shown). Coat protein incorporated into phosphatidylserine vesicles had the same orientation as in phosphatidylcholine vesicles (Table II), indicating that protein orientation does not rely on specific interactions with the lipid polar-head group. The possible role of the polar ends of the coat protein in orientation was examined as described below.

Selective proteolytic shortening of either the N or C terminus of coat protein was used to examine their role in determining asymmetry. [^{3}H]Proline-labeled coat protein was incubated with chymotrypsin, the reaction was stopped with the protease inhibitor TPCK (L-1-tosylamide 2-phenylethyl cholormethyl ketone), and ^{35}S-labeled undigested coat protein was added. The mixture was analyzed by gel filtration in the presence of deoxycholate (to maintain peptide solubility). The indicated fractions (Figure 3A) had ^{3}H but little or no ^{35}S label; they therefore contained labeled coat protein that was reduced in size, presumably due to cleavage at the C terminus, but which had an intact N terminus. Similarly, limited trypsin digestion of coat protein left the [^{3}H]lysine-labeled C terminus intact under conditions where the N terminus was efficiently

TABLE III: Orientation of Truncated Coat Protein in Dimyristoyllecithin Vesicles.[a]

DML Vesicles With:	% Label Released by Chymotrypsin
[^{3}H]Lys coat protein	25
[^{3}H]Lys peptide 9–50	18
[^{3}H]Pro coat protein	87
[^{3}H]Pro peptide 1–42	83

[a] Vesicles were prepared at 23 °C with [^{3}H]Lys- or [^{3}H]Pro-labeled coat protein and assayed for orientation, as described under Materials and Methods. Where indicated, truncated M13 coat protein, prepared as described in Figures 3A,B, was used.

TABLE IV: The Effect of Coat Protein Concentration on Orientation.[a]

		% Label Solubilized by Chymotrypsin			
		[^{3}H]Pro Coat Protein		[^{3}H]Lys Coat Protein	
Lipid	Temp (°C)	0.5 mg	0.05 mg	0.5 mg	0.05 mg
DLL	0 (Tm)	88	84	27	31
DML	23 (Tm)	85	82	30	32
	0 (Tm-23)	94	94	71	64

[a] Vesicles of dilauryl- or dimyristoyllecithin and [^{3}H]Pro or [^{3}H]Lys-labeled coat protein were prepared and assayed for coat protein orientation as described under Materials and Methods. Either 0.5 mg or 0.005 mg of coat protein was used in vesicle preparation. Results are expressed as the percent of vesicle-bound coat protein label released by chymotrypsin.

removed (Figure 3B). This selective shortening of either the N or C terminus of the M13 coat protein did not affect the orientation with which it assembled into lipid vesicles (Table III).

Coat protein concentration did not affect the orientation of the protein in vesicles (Table IV). In order to understand this result at the level of individual vesicles, populations of vesicles were prepared which differed tenfold in their relative concentrations of coat protein and lipid (Table V). Antibody specific for the exposed coat protein N terminus (Wickner, 1975; Wickner, 1976) was used in conjunction with goat antibody to rabbit γ-globulin to precipitate those vesicles which bore coat protein. Vesicles (measured as lipid phosphorus) were precipitated by antibody in the same ratio to coat protein which they bore in the starting population (Table V), indicating that coat protein is distributed throughout the vesicle population and that specific orientation of coat protein does not depend on a unique protein concentration in each vesicle.

Temperature was found to have a dramatic effect on both the incorporation of protein into vesicles (Figure 4) and on the orientation of incorporated coat protein (Wickner, 1976). When vesicles were prepared at temperatures above the T_m, little coat protein was incorporated. With decreasing temperature, coat protein incorporation increased abruptly near the T_m, then gradually decreased at lower temperatures (Figure 4). This was found for both dilauryllecithin and dimyristoyllecithin vesicles. As reported previously (Wickner, 1976), coat protein incorporated near the T_m spans the

TABLE V: Even Distribution of M13 Coat Protein Throughout the Lipid Vesicle Population.[a]

				Anticoat Precipitate		
Sample	DML (μg)	Coat Protein (μg)	Coat Protein DML	DML	Coat Protein	Coat Protein DML
1	67	19	0.280	38	9.3	0.245
2	143	29	0.200	110	21	0.191
3	431	33	0.077	274	21	0.077
4	752	25	0.033	392	12.7	0.032

[a] ^{35}S-labeled coat protein (40 μg in 33.5 μl of 0.91% sodium cholate) was mixed with: (1) 12 μl of [^{3}H]methyldimyristoyllecithin (120 cpm/μg, 40 mg/ml, prepared by the method of Holloway et al. (1975) and sonicated for 1 min at 23 °C in 0.1 M KP pH 7.0); (2) 67 μl of 0.91% sodium cholate and 36 μl of [^{3}H]methyldimyristoyllecithin; (3) 244 μl of 0.91% sodium cholate and 300 μl of [^{3}H]methyldimyristoyllecithin. After 30 min at 22 °C, the sample was diluted with 30 volumes of 0.1 M KP (pH 7.0). After an additional hour at 22 °C, vesicles were collected by centrifugation (0 °C, 44 000*g*, 60 min) and suspended in 2 ml each of 0.1 M NaCl, 0.05 M Tris-HCl (pH 7.5). Aliquots of vesicles containing 30 μg of lipid were incubated with 0.1 mg of antibody to M13 coat protein (Wickner, 1975) for 15 min at 37 °C, then mixed with an equivalent of goat antibody to rabbit γ-globulin (Wickner, 1976). After 20 min at 37 °C, agglutinated vesicles were collected by centrifugation (0 °C, 90 s, 800*g*) and assayed for ^{3}H and ^{35}S.

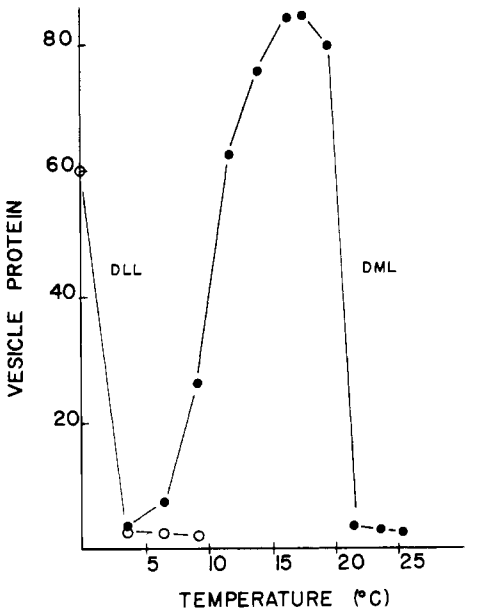

FIGURE 4: The effect of temperature on the incorporation of M13 coat protein into dilauryl-(O–O) or (●–●) dimyristoyllecithin vesicles. Vesicles were prepared with [^{3}H]Lys-labeled coat protein at the indicated temperature, as described under Materials and Methods. After isolation by centrifugation at 30 °C, vesicle-bound radioactivity was measured.

membrane with unique orientation, while protein incorporated well below the T_m had both ends exposed on the outer vesicle surface.

Discussion

Despite the fluid mosaic nature of membranes (Singer and Nicholson, 1972), it is becoming apparent that they have local and long-range elements of restricted structure (Reusch and Burger, 1974; Beining et al., 1975; Yahara and Edelman,

1975). Asymmetric distribution of each protein species across the plane of the membrane appears to be a ubiquitous structural feature (Singer, 1974). The coat protein of coliphage M13 is membrane bound at each stage of infection (Trenkner et al., 1967; Smilowitz et al., 1972; Marco et al., 1974; Smilowitz, 1974), providing a viral probe for studies of asymmetric membrane assembly.

In previous studies, M13 coat protein which entered the cytoplasmic membrane from infecting virus or after synthesis from within the cell was found to share a common orientation. This was consistent with the orientation being determined by properties of the protein and the receptor membrane, rather than by the direction of assembly. This concept has been strengthened by model vesicle studies (Wickner, 1976). Vesicles, prepared from a solution of cholate, coat protein, and dilauryl- or dimyristoyllecithin by the dilution technique of Racker et al. (1975), had coat protein which was asymmetrically bound to the bilayer. Membrane protein asymmetry can therefore be established in the absence of other proteins, of fatty-acyl or polar head-group lipid asymmetry, or of a specific direction of assembly.

In this report, the factors which affect this asymmetric assembly are examined. While the physical state of the nascent hydrophobic phase appears to be crucial to the extent of protein insertion (Figure 4) and the orientation of inserted protein (Wickner, 1976), polar interactions appear to play a small role in these processes. Thus, (1) neither the polar N or C terminus is necessary to protein orientation (Table III), (2) the coat protein orientation is the same in dipalmitoylphosphatidylserine vesicles as in vesicles of phosphatidylcholine, indicating that the charge of the lipid polar head group is not critical (Table II), and (3) ionic strength and pH had little effect.

The nature of the apolar interactions which do govern protein orientation are not known. Despite the similar orientations of membrane-bound coat protein in vivo (Wickner, 1975; Wickner, 1976) and in synthetic vesicles (Wickner, 1976), the physiologic significance of this model system is yet to be determined. Assay of the orientation of coat protein in fatty acid auxotrophs infected with M13 at temperatures which span the lipid phase transition may help to answer this question.

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References

Asbeck, V. F., Beyreuther, K., Köhler, H., von Wettstein, G., and Braunitzer, G. (1969), *Hoppe-Seyler's Z. Physiol. Chem. 350,* 1047.

Beining, P. R., Huff, E., Prescott, B., and Theodore, T. S. (1975), *J. Bacteriol. 121,* 137.

Holloway, P. W., and Katz, J. T. (1975), *J. Biol. Chem. 250,* 9002.

Marco, R., Jazwinski, S. M., and Kornberg, A. (1974), *Virology 62,* 209.

Marvin, D. A., and Wachtel, E. J. (1975), *Nature (London) 253,* 19.

Nakashima, Y., and Konigsberg, W. (1974), *J. Mol. Biol. 88,* 598.

Racker, E., Chien, T.-F., and Kandrach, A. (1975), *FEBS Lett. 57,* 14.

Reusch, V. M., and Burger, M. M. (1974), *J. Biol. Chem. 249,* 5337.

Singer, S. J. (1974), *Annu. Rev. Biochem. 43,* 805.

Singer, S. J., and Nicholson, G. L. (1972), *Science 175,* 720.

Smilowitz, H. (1974), *J. Virol. 13,* 94.

Smilowitz, H., Carson, J., and Robbins, P. W. (1972), *J. Supramol. Struct. 1,* 8.

Trenkner, E., Bonhoeffer, F., and Gierer, A. (1967), *Biochem. Biophys. Res. Commun. 28,* 932.

Wickner, W. (1975), *Proc. Natl. Acad. Sci. U.S.A. 72,* 4749.

Wickner, W. (1976), *Proc. Natl. Acad. Sci. U.S.A. 73,* 1159.

Woolford, J. L., Jr., and Webster, R. E. (1975), *J. Biol. Chem. 250,* 4333.

Yahara, I., and Edelman, G. M. (1975), *Proc. Natl. Acad. Sci. U.S.A. 72,* 1579.