as well as the heights of the particles and the heights of other smooth areas which do not give a diffraction pattern (1 in Fig. 4).

In our previous study we found that smooth EF pattern areas occur in preparations fractured under LN_2 in alternation with the globular particle areas. We presented reasons in the previous paper for believing that these smooth areas represented the true EF face with the globular particle lattice being an artifactural structure derived from the smooth lattice. We interpret our present findings in the following way. The heights of the particles above the glass in areas like *Y* in Fig. 1 are consistent with the particulate lattice's being the surface particle lattice seen from the back side, i.e., from the side normally covered by the bilayer portion of the membrane. The optical diffraction analysis supports this interpretation in that the filtered images are almost identical to those given by the particle lattice replicated from the external side. The smooth areas that give diffraction patterns (2 in Fig. 4), which lie only slightly higher above the glass than the particle lattice, represent the smooth lattice that we identified in our previous paper as the true external fracture face. We interpret this as a lipid-carbon chain surface. The smooth surfaces lying at a greater height above the glass (1 in Fig. 4), which do not give diffraction patterns, we interpret as the protoplasmic surfaces of unfractured membranes. In the case of Fig. 1, we are dealing with two membranes oriented with their external surfaces toward the glass but superimposed on one another. In the case of the one containing area *Y*, the facture plane originally produced mainly the globular lattice as described in reference (3). There are a few identifiable limited areas of the smooth lattice obscuring the particles. The more elevated membrane superimposed on this one fractured almost completely without forming the globular lattice. Instead it was, after fracture, almost entirely a smooth lattice. This smooth lattice, according to our interpretation, is a lipid-carbon chain surface and hence hydrophobic. In contrast, the previously described (3) globular particle lattice we interpret as being produced by plastic deformation in the lipid bilayer and subsequent decoration. We believe that the large globular particles would have been seen in the present preparation in area *Y* if it had not been washed with water after fracture. Evidently, the structures responsible for the globular particles in unwashed preparations were washed away by water after thawing. This probably means that the seed structure in forming the globular particles is a lipid micelle with polar groups oriented outward produced from the external lipid monolayer by the fracturing process. The shock of fracturing evidently detaches these lipid molecules from the surface protein particles so that they can be washed away. The diagram illustrates the washing procedure and its effect on the fracture faces (Fig. 5).

This work was supported by grant 1 RO1 GM28244 from the National Institutes of Health.

Received for publication 8 May 1981.

REFERENCES

1. Robertson, J. D. 1972. The structure of biological membranes. *Arch. Intern. Med.* 9:202–228.
2. Hicks, R. M., B. Ketterer, and R. C. Warren. 1974. The ultrastructure and chemistry of the luminal plasma membrane of the mammalian urinary bladder: a structure with low permeability to water and ions. *Phil. Trans. R. Soc. Lond. B. Biol. Sci.* 268:23–38.
3. Robertson, J. D., and J. Vergara. 1980. Analysis of the structure of intramembrane particles of the mammalian urinary bladder. *J. Cell Biol.* 86:514–528.

LIPID-DEPENDENT STRUCTURAL CHANGES OF AN AMPHOMORPHIC MEMBRANE PROTEIN

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The coat protein from the filamentous phages fd, f1, and M13 is easily obtained and readily associates with lipids. These protein lipid complexes have been used frequently as model membranes for biophysical studies (1–8). Here we report that the fd coat protein undergoes an $\alpha \rightleftharpoons \beta$ conformational transition that depends on the lipid:protein ratio and on the nature of the lipid tail groups. "Amphomorphic" is proposed as a term for proteins that undergo environmentally mediated $\alpha \rightleftharpoons \beta$ transitions.

RESULTS AND DISCUSSION

Raman spectroscopy (6, 8) and circular dichroism (1, 2) were used to distinguish "50%α" from "50%β". By curve fitting the circular dichroism (CD) and Raman spectra we find no α-helix in the "50% β" structure and little, if any, β structure in the "50% α".

Tanford and co-workers report "50%α" to be the favored conformation in amphiphiles and the β-polymer to

be favored in aqueous buffers (1, 9, 10). Using different procedures, we find both "50% α" and "50% β" to be stable with and without amphiphiles (2, 11, 12, and Table I).

Low amphiphile:protein ratios favor "50% β" over "50% α" (Table I, *A*1, *A*2, *B*2), but the mechanism is unclear. The amphiphiles may shield hydrophobic side chains, engage in charge/charge or hydrogen bonding interactions between the protein and the lipid head group, and serve to bind the protein, thus increasing the local protein concentration.

"50% β" (in a polymeric form, e.g., the β-polymer) results following dialysis of fd coat protein-deoxycholate solutions (10, 13, 14). The time spent during dialysis at low detergent:protein ratios is expected to favor "50% β" (Table 1, *B*2). Thus, the relative stability of "50% α" and "50% β" without amphiphiles cannot be inferred from dialysis experiments.

"50% α" results in egg phosphatidylcholine (PC) and dioleylphosphatidylcholine (DOPC), but "50% β" results in DPPC or DMPC under otherwise identical conditions (11, 12, Table 1, *A*3, *B*2). DPPC and DMPC form wider bilayers than do egg PC and DOPC (15). Possibly "50% β" requires the wider bilayers for stability. Alternatively, DMPC and DPPC contain only saturated fatty acids whereas egg PC and DOPC contain *cis*-unsaturated fatty acids. Possibly "50% β" is unstable in egg PC and DOPC due to poor hydrophobic packing between the unsaturated lipid and protein in the β-form.

Amphomorphic proteins, i.e., those proteins that undergo environment-dependent $\alpha \rightleftharpoons \beta$ transitions, have been described previously (16, 17). In striking similarity to our results (Table I, *A*2, *B*2) the $\beta \rightarrow \alpha$ transition is observed as the SDS:protein ratio is increased (17).

The previous authors pointed out that their proteins have Chou-Fasman $<P_\alpha>$ and $<P_\beta>$ values that are simultaneously high, which may be indicative of proteins that form α or β depending on the environment (16, 17). The fd coat protein fits this concept: $<P_\alpha> = 1.07$ and $<P_\beta> = 1.05$ (18).

It has been pointed out previously that the same hydrophobic residues tend to be found in α-helices or in β-sheets, depending on the packing (e.g., environmental) details (19, 20). Thus, amphomorphism may depend on the presence of clusters of hydrophobic amino acids along the sequence (see also Table I in reference 16). If amphomorphism does indeed depend on hydrophobic clusters, then we expect amphomorphic behavior to be a common property of membrane proteins.

TABLE I
CONFORMATIONAL STABILITY OF THE fd COAT PROTEIN

Conformation	Conditions of stability ($C \leq 1$ mg/ml; $T \leq 50°$)
"50% α"	*A*1. Neutral aqueous buffers*
	*A*2. DMPC, DPPC, and SDS‡ at high amphiphile: protein ratios
	*A*3. DOPC and Egg PC‡ at all lipid protein ratios tested
"50% β"	*B*1. Neutral aqueous buffers (precipitates as a polymer)§
	*B*2. DPPC, DMPC, and SDS at low amphiphile: protein ratios

*Dissociation of the protein from the DNA is accomplished by controlled sonication. Experimental conditions for *A*1, *A*2, *A*3, and *B*2 are identical except for differences in type of lipid or lipid/protein ratio (11, 12). Lipid and protein association were confirmed by sucrose gradient centrifugation.

‡Abbreviations: DMPC, dimyristoyl phosphatidyl choline ($C_{14:0}$); DPPC, dipalmatoyl phosphatidyl choline ($C_{16:0}$); SDS, sodium dodecyl sulfate (C_{12}); DOPC, dioleoyl phosphatidyl choline ($C_{18:1}$); and egg PC, lecithin from egg yolk. Literature values for egg yolk lecithin fatty acid compositions typically are 32% $C_{16:0}$, 10% $C_{18:0}$, a trace of $C_{16:1}$, 48% $C_{18:1}$, and 9% $C_{18:2}$.

§Dissociation of the protein from the DNA is accomplished by detergent solubilization; dialysis then removes the detergent (10, 13, 14).

This research was supported by National Institutes of Health grant GM 25937. Dr. Dunker is an Established Investigator of the American Heart Association.

Received for publication 22 April 1981.

REFERENCES

1. Nozaki, Y., B. K. Chamberlain, R. E. Webster, and C. Tanford. 1976. Evidence for a major conformational change of coat protein in assembly of f1 bacteriophage. *Nature (Lond.)* 259:335–337.
2. Williams, R. W., and A. K. Dunker. 1977. Circular dichroism studies of fd coat protein in membrane vesicles. *J. Biol. Chem.* 252:6253–6255.
3. Hagen, D. S., J. H. Weiner, and B. D. Sykes. 1978. Fluorotyrosyl M13 coat protein: fluorine-19 nuclear magnetic resonance study of the motional properties of an integral membrane protein in phospholipid vesicles. *Biochemistry.* 17:3860–3866.
4. Hagen, D. S., J. H. Weiner, and B. D. Sykes. 1979. Investigation of solvent accessibility of the fluorotyrosyl residues of M13 coat protein in deoxycholate micelles and phospholipid vesicles. *Biochemistry.* 18:2007–2012.
5. Smith, L. M., B. A. Smith, and H. M. McConnell. 1979. Lateral diffusion of M-13 coat protein in model membranes. *Biochemistry.* 18:2256–2259.
6. Dunker, A. K., R. W. Williams, B. P. Gaber, and W. L. Peticolas. 1979. Laser Raman studies of lipid disordering by the B-protein of fd phage. *Biochim. Biophys. Acta.* 553:351–357.
7. Kimelman, D., E. S. Tecoma, P. K. Wolber, B. S. Hudson, W. T. Wickner, and R. D. Simoni. 1979. Protein-lipid interactions. Studies of the M13 coat protein in dimyristoyl phosphatidylcholine vesicles using parinaric acid. *Biochemistry.* 18:5874–5880.
8. Williams, R. W., A. K. Dunker, and W. L. Peticolas. 1980. A new method for determining protein secondary structure by laser Raman spectroscopy applied to fd phage. *Biophys. J.* 32:232–234.
9. Chamberlain, B. K., Y. Nozaki, C. Tanford, and R. E. Webster. 1978. Association of the major coat protein of fd bacteriophage with phospholipid vesicles. *Biochim. Biophys. Acta.* 510:18–37.
10. Nozaki, Y., J. A. Reynolds, and C. Tanford. 1978. Conformational states of a hydrophobic protein. The coat protein of fd bacteriophage. *Biochemistry.* 17:1239–1246.
11. Fodor, S. P. A., and A. K. Dunker. 1981. Lipid tail group dependence of the fd coat protein conformation. *Biophys. J.* 33:6*a*.
12. Fodor, S. P. A., A. K. Dunker, Y. C. Ng, D. Carsten, and R. W.

Williams. 1980. Lipid tail group dependent structure of the fd gene 8 protein. *In* Seventh Biennial Conference on Bacteriophage Assembly. M. S. Dubow, editor. Alan R. Liss, Inc. New York. In press.

13. Makino, S., J. L. Woolford, C. Tanford, and R. E. Webster. 1975. Interaction of deoxycholate and of detergents with the coat protein of the bacteriophage f1. *J. Biol. Chem.* 250:4327–4332.
14. Cavalieri, S. J., D. A. Goldthwait, and K. E. Neet. 1976. The isolation of a dimer of gene 8 protein of bacteriophage fd. *J. Mol. Biol.* 102:713–722.
15. Tardieu, A., V. Luzzati, and F. C. Reman. 1973. Structure and polymorphism of the hydrocarbon chains of lipids: a study of lecithin-water phases. *J. Mol. Biol.* 75:711–733.
16. Rosenblatt, M., N. V. Beaudetti, and G. D. Fasman. Conformational studies of the synthetic precursor-specific region of preproparathyroid hormone. *Proc. Natl. Acad. Sci. U.S.A.* 77:3983–3987.
17. Wu, C. C.-S., K. Ikeda, and J. T. Yang. 1981. Ordered conformation of polypeptides and proteins in acidic dodecyl sulfate solution. *Biochemistry.* 20:566–570.
18. Green, N., and T. Flanagan. 1976. The prediction of the conformation of membrane proteins from the sequence of amino acids. *Biochem. J.* 153:729–732.
19. Lim, V. I. 1974. Structural principles of the globular organization of protein chains: a stereochemical theory of globular protein secondary structure. *J. Mol. Biol.* 88:857–872.
20. Lim, V. I. 1974. Algorithms for prediction of the α-helical and β-structural regions in globular proteins. *J. Mol. Biol.* 88:873–899.