Membrane Lipids and the Conformations of Membrane Proteins

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ABSTRACT The general relations between protein conformation and the optical activity of peptide chromophores are outlined and applied to the analysis of the optical rotatory dispersion and circular dichroism of the plasma membranes of human erythrocytes and Ehrlich ascites carcinoma cells. It is concluded that the proteins of these membranes are "globular" and that they have considerable helical content. The spectroscopic consequences of perturbing the membranes with phospholipase *C*, phospholipase *A*, lysolecithin, and sodium dodecyl sulfate are examined in the light of the effects of these agents upon certain enzymatic and physical properties of the membranes and upon their proton magnetic resonance spectra. The data suggest that the architecture of membrane proteins is strongly dependent upon apolar lipid-protein and/or lipid-sensitive protein-protein interactions.

INTRODUCTION

The problem of assembly and breakdown of cellular membranes has most often been approached on the assumption that lipid-lipid and lipid-protein interactions are the major forces of membrane cohesion. However, it is also possible that large regions of cellular membranes are comprised of protein or lipoprotein lattices, in which the major cohesive forces are between proteins (1–3). These associations between membrane lipids and proteins and between the proteins themselves must be through weak interactions, i.e. ionic interactions, hydrogen bonding, London–Van der Waals forces, and/or "hydrophobic bonding," rather than through covalent linkages, since the lipids of cellular membranes can be separated from the proteins under mild conditions by organic solvents and by detergents (4–7), and the interaction between various membrane proteins can be disrupted by sodium lauryl sulfate.

The interactions between lipids in micellar and smectic systems are fairly well understood and have been often reviewed (8, 9), but the nature of lipid-protein interactions in membranes is only now coming into focus. In the traditional models of membrane structure (10–13) the forces binding proteins to

lipids are considered to be primarily ionic. However, there is no evidence that ion pairing is generally responsible for the *tight* lipid-protein associations in cellular membranes, except for some of the phosphoinositides of myelin, which can be extracted only by acidified organic solvents (14–16). Indeed, neither the acidic phosphatides of *Halobacterium halobium* (17) nor the mixed phospholipids of ascites carcinoma membranes (18) are dissociated noticeably from their membrane proteins by manipulations of ionic strength and pH which disrupt the artificial ionic complexes discussed above. Also, in contrast to the artificial complexes, the phosphatides of these and other membranes are readily separated from the proteins by extraction with 2:1 chloroform:methanol, a solvent which should stabilize ionic interactions. The fact that membrane lipids cannot be readily separated from membrane proteins by ionic manipulations does not necessarily indicate lack of ionic bonding, but rather domination of the lipid-protein associations in membranes by nonpolar interactions.

The role of organic solvents and detergents in the extraction of lipids and solubilization of membrane proteins constitutes strong evidence for hydrophobic bonding between these membrane components. The experimental and theoretical basis for hydrophobic bonding, derived mainly from the study of protein structure, has been fully discussed elsewhere (19) and will be presented only briefly here.

The ultimate spatial disposition assumed by associating groups can be considered to tend toward a minimum energy state for the system. While all component interactions are involved in attaining this energy minimum, apolar interassociations are particularly important, and contribute to cohesive structuring in three ways. The first is by weak (Van der Waals) interactions between apolar residues. The second derives from interaction of these residues with water. Since solvent water molecules tend to become ordered when surrounding an apolar group, the clustering of such residues together is associated with a higher entropy, hence lower energy state of the system as a whole. Conversely, the removal of an apolar chain from such a hydrophobic environment involves both the breaking of Van der Waals bonds and the decrease in entropy imposed on the aqueous solvent system—both energetically unfavorable. The third contribution of apolar associations derives from the low dielectric constant of the hydrophobic environment: hydrogen bonding (interamide and peptide) and ionic interactions are strengthened by the exclusion of water. Klotz (20) views these hydrophobic interactions as dominant in the conformational stability of all biopolymers, and this contention is supported by the theoretical work of Scheraga et al. (19) and by experimental approaches. The latter emphasize the effects of nonaqueous solvents (21), hydrocarbons (22, 23), and fatty acids (24) on proteins and the interactions of nonpolar polypeptides (25). Studies of model polypeptides with nonpolar side chains indicate that in aqueous solvents the α-helix is a

favored conformation for hydrophobic polypeptides. The most striking evidence, however, comes from the X-ray crystallography of myoglobin, hemoglobin, and lysozyme (26–28). It is of particular interest that the functionally highly sensitive and specific association between the α- and β-chains of hemoglobin is also primarily of hydrophobic character (29). The same is true of the very tight binding of hemes (29), a fact that is particularly relevant to protein-lipid interactions in general. It should be noted that the heme-globin interactions profoundly affect the properties of the protein as a whole, the architecture of globin being quite different from that of hemoglobin.

SPECTROSCOPIC STUDIES OF MEMBRANES

It has recently become possible to examine the structure of cellular membranes by spectroscopic techniques. In particular, measurements of the optical rotatory dispersion (ORD)[1] and circular dichroism (CD) in the regions of peptide absorption reflect the architecture of membrane proteins, (1, 2, 30–32)[2] while measurements of proton magnetic resonance (PMR) (33, 34) indicate the physical state of lipids in membranes. These techniques also allow one to follow the structural consequences of various perturbations. I will illustrate this with phospholipase *C*, lysolecithin (phospholipase *A*), and sodium dodecyl sulfate (SDS), representing encroachments of increasing severity. Although many membrane types have been studied, I will restrict my comments to observations made on erythrocyte "ghosts" and on plasma membrane (PM) fragments from Ehrlich ascites carcinoma (EAC).

UNMODIFIED MEMBRANES

Optical Activity

CD and ORD are two closely related manifestations of optical activity. CD, expressed in terms of $[\theta]_\lambda$, the ellipticity per optically active residue at wavelength λ, is proportional to $(\epsilon_L - \epsilon_R)$, the difference in absorbance for left and right circularly polarized light. Optical rotation, expressed here as $[m]_\lambda$, the mean residue rotation at wavelength λ, is proportional to $(n_L - n_R)$, the difference in refractive index for left and right circularly polarized light.

Since the optical activity of peptide bonds depends strongly upon the secondary structure, and perhaps even the tertiary and quaternary structure, of the peptide chain, its measurement can therefore give some clues to protein architecture. This is brought out in Figs. 1 and 2, which also serve as points of reference for the succeeding discussion. In the spectral region of

[1] Abbreviations used: ATPase, adenosine triphosphatase; CD, circular dichroism; EAC, Ehrlich ascites carcinoma; NADH, dihydro nicotinamide adenine dinucleotide; ORD, optical rotatory dispersion; PM, plasma membrane; PMR, proton magnetic resonance; RNA, ribonucleic acid; SDS, sodium dodecyl sulfate.

[2] Gordon, A., D. F. H. Wallach, and J. H. Straus. Data to be published.

peptide absorption, the CD and ORD spectra of polypeptides and proteins are summations of the contributions of the various individual peptide chromophores in the several conformations present. Since infrared spectroscopy has shown that the plasma membranes of human erythrocytes and Ehrlich ascites carcinoma have negligible β-conformation (35–37), this discussion will

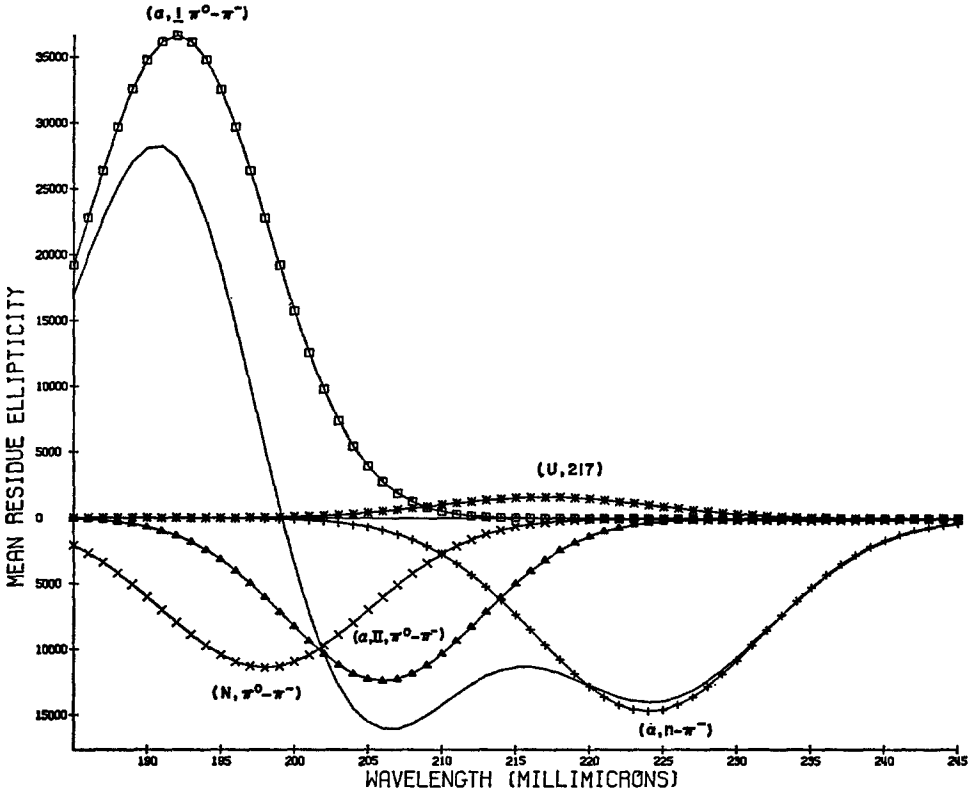

FIGURE 1. Calculated CD spectra of poly-L-glutamic acid and the individual bands contributing to the spectrum. (—), 50% α-helix–50% "unordered" coil.

be limited to mixtures of helix and "unordered" conformation. (This simplification may not be permissible in the case of other membranes.) Fig. 1 represents the CD spectrum of poly-L-glutamic acid with half of its peptide bonds in α-helical conformation and half unordered. The figure also shows computed individual spectral contributions of the various peptide chromophores in the two conformations, namely: (*a*) the large, negative "helical" $n - \pi^-$ band at 223 mμ (α, $n - \pi^-$); (*b*) the small, positive "unordered" band at 217 mμ (U, 217); (*c*) the large, negative, "helical" $\pi^0 - \pi^-$ band, polarized parallel to the helix axis at 206 mμ (α, $\|$, $\pi^0 - \pi^-$); (*d*) the large, negative, "unordered" $\pi^0 - \pi^-$ band at 198 mμ (U, $\pi^0 - \pi^-$); and (*e*)

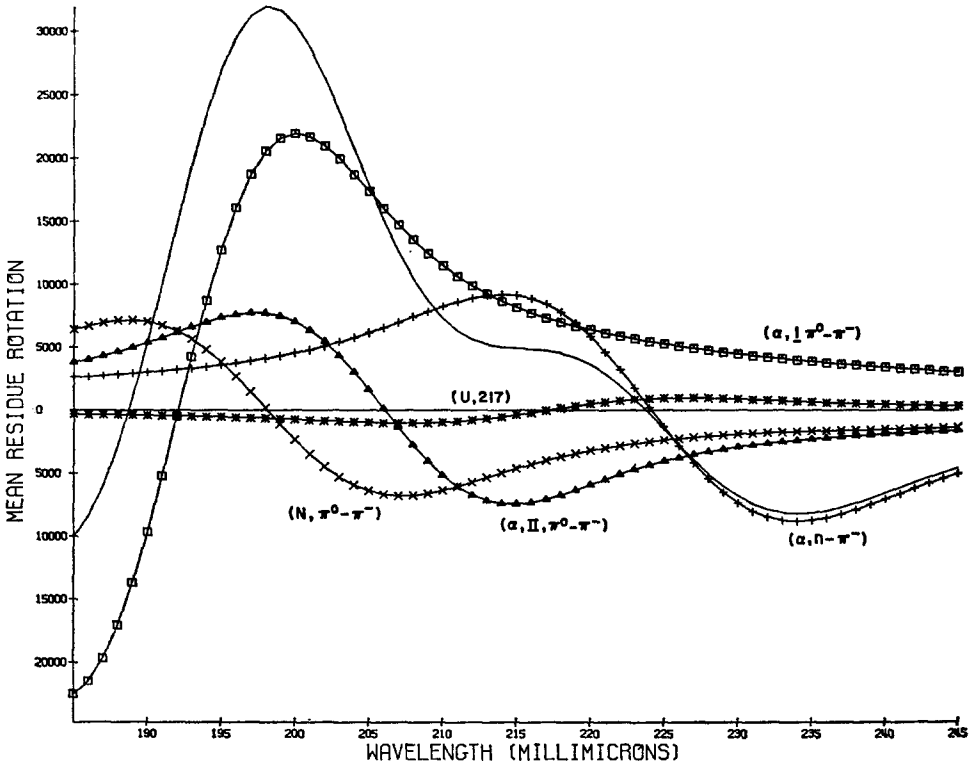

FIGURE 2. Calculated ORD spectra of poly-L-glutamic acid and the individual cotton effects contributing to the spectrum. (—), 50% α-helix–50% "unordered" coil.

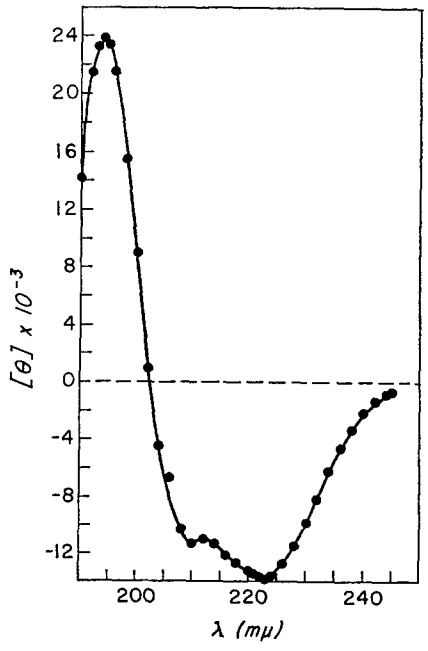

FIGURE 3. Experimental CD spectrum of erythrocyte ghosts. Solvent 0.007 M phosphate, pH 7.4.

the large, positive, "helical" $\pi^0 - \pi^-$ band, polarized perpendicular to the helix axis at 191 mμ (α, $\perp$, $\pi^0 - \pi^-$).[2]

Fig. 2 is the corresponding ORD spectrum. For each CD band there is a symmetric, bimodal ORD curve, centered on the CD band and with the extrema occurring at plus (+) and minus (−) one-half width of the CD band. It is clear that optical rotation occurs well beyond the absorption band and

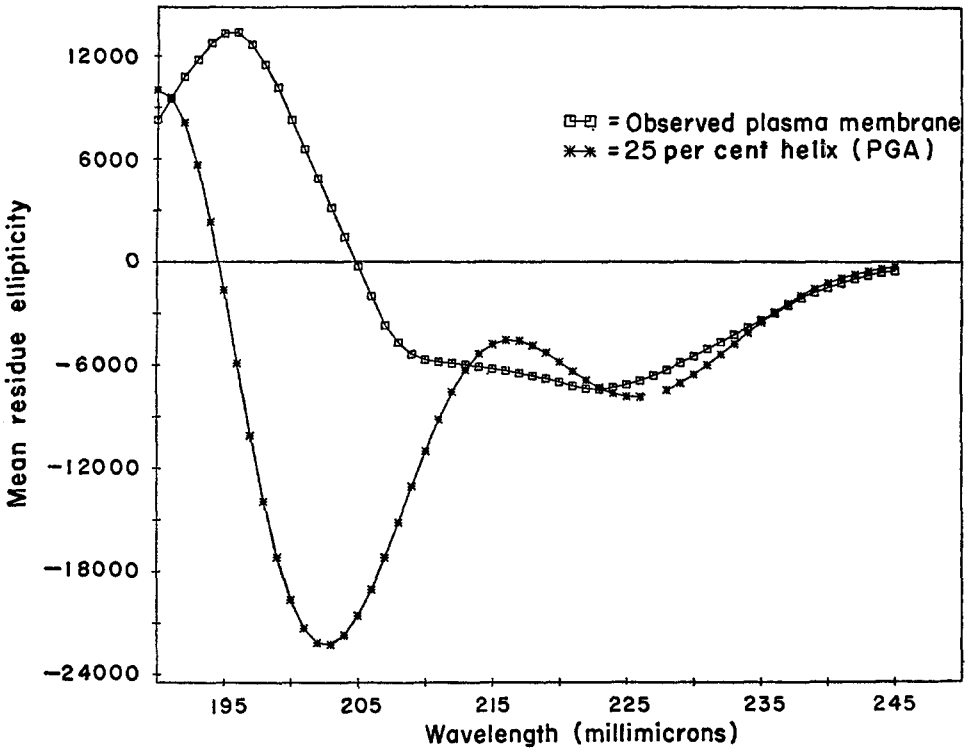

FIGURE 4. Experimental CD spectrum of plasma membrane of Ehrlich ascites carcinoma cells (□), and calculated CD spectrum of poly-L-glutamic acid, 25% α-helix–75% "unordered" coil (*). Solvent 0.001 M Tris-HCl, pH 8.2.

that the position of ORD spectra is very sensitive to the width of the optically active bands. The negative ORD extremum at 233 mμ is due almost entirely to the helical $n - \pi^-$ band at 223 mμ.[2]

The CD spectra of erythrocyte ghosts and PM are presented in Figs. 3 and 4 and are rather similar. Both show a prominent negative band at 223 mμ, indicative of considerable helical conformation. The positive band at 195 mμ also indicates helix, but it is displaced to the red of the position found in synthetic polypeptides. This displacement, which occurs to a somewhat lesser degree in helix-containing globular proteins, is thought to reflect localization of some of the peptide bonds in an apolar environment of high re-

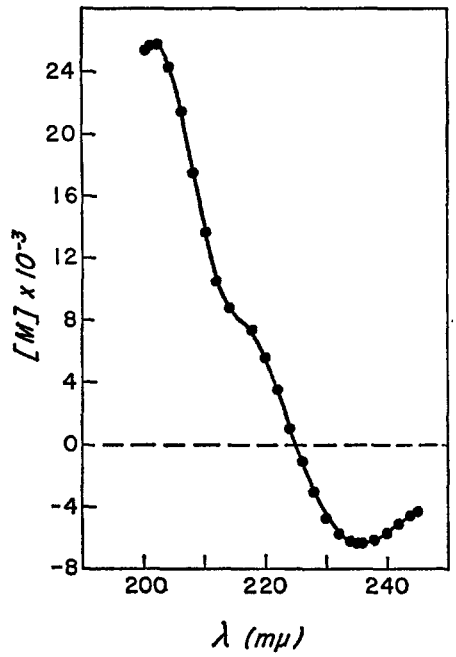

FIGURE 5. Experimental ORD spectrum of erythrocyte ghosts. Solvent 0.007 M phosphate, pH 7.4.

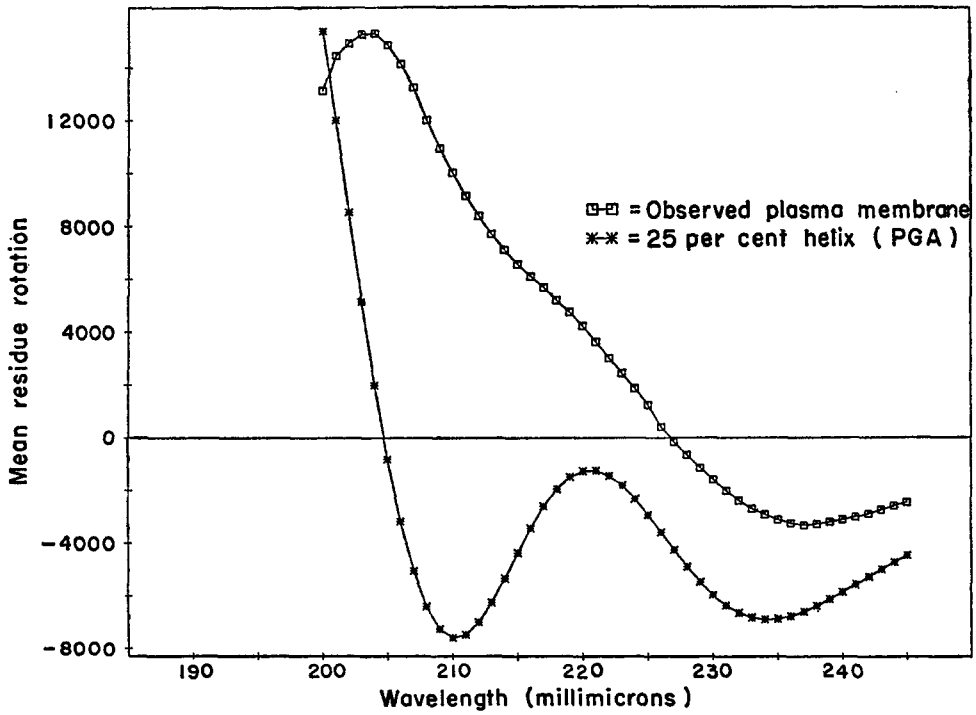

FIGURE 6. Experimental ORD spectrum of plasma membrane of Ehrlich ascites carcinoma cells (□), and calculated ORD spectrum of poly-L-glutamic acid, 25% α-helix–75% "unordered" coli (*). Solvent 0.001 M Tris-HCl, pH 8.2.

fractive index.[3] The intensities of the membrane CD spectra are small compared with those of synthetic polypeptides, but this is only a somewhat more extreme case of what is found in globular proteins.[3] The ORD spectra of ghosts and PM, shown in Figs. 5 and 6, exhibit a phenomenon characteristic

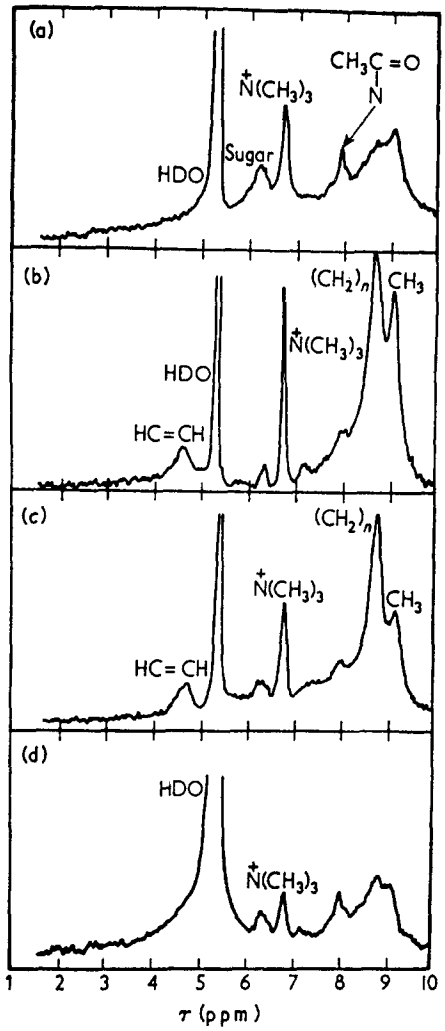

FIGURE 7. The PMR spectrum of a 5% sonicated dispersion in D_2O. (*a*) Erythrocyte membrane fragments; (*b*) total equivalent membrane lipid (phospholipid + cholesterol); (*c*) total equivalent phospholipid; and (*d*) lipid-deficient membrane fragments extracted with ether to remove all cholesterol and about 25% phospholipid (64 scans each). *Reprinted by permission from the J. Mol. Biol., 1968, 31:101.*

of membrane spectra, namely, displacement of the negative extremum several mμ to the red of its position in α-helical polypeptides. This is due to the large width of the helical $n - \pi^-$ band.[2]

Proton Magnetic Resonance (PMR)

High resolution PMR signals represent the rapid segmental motions of spe-

[3] Straus, J. H., A. S. Gordon, and D. F. H. Wallach. An analysis of the optical rotatory dispersion and circular dichroism of globular proteins. Submitted for publication.

cific proton groupings, these motions depending upon the microscopic viscosity of the region in which the proton groups lie. The PMR spectrum of sonicated erythrocyte ghosts in D_2O suspension is shown in Fig. 7 *a* and *d* and should be compared with that of the erythrocyte lipids in Fig. 7 *b* and *c* (33).

Sharp resonance signals occur at 6.3 ppm due to CH_2OC and CH_2OP protons of lipid and/or carbohydrate. The peak at 7.8 ppm is due to $NCOCH_3$ protons of *N*-acetyl groups. The sharp signal at 6.7 ppm represents the $N^+(CH_3)$ protons of lecithin and sphingomyelin. Remarkably, the signals expected from the $(CH_2)_n$ groups (at 8.7 ppm) and the CH=CH groups (at 4.7 ppm) of hydrocarbon chains are lacking in the membrane spectrum, and the CH_3 signal at 9 ppm is weak.

The spectra indicate that: (*a*) the proton groups of membrane carbohydrate lie in a microenvironment of low viscosity, presumably at the membrane:water interface; (*b*) the $N^+(CH_3)_3$ protons have an environment similar to that with lecithin alone in D_2O; and (*c*) the lipid hydrocarbon chains are restricted in their molecular motion. The fact that the PMR spectrum of the extracted lipids (Fig. 7 *b*) shows large, sharp CH_3, $(CH_2)_n$, and CH=CH signals suggests that the restriction of the chain movement within membranes may be due to apolar lipid-protein interactions (33, 34). This conclusion is supported by infrared spectroscopy (37).

PERTURBED MEMBRANES

Phospholipase C

We have followed some of the micromorphologic consequences of the action of phospholipase *C* and it will be profitable to examine these first.[4] Fig. 8 is an electron micrograph of a replica of unfixed erythrocyte membranes "freeze-cleaved" at −196°C in a glycerol-water mixture (38, 39). The cleavage plane reveals large areas of both outer and inner surfaces of "ghosts." Protruding from these surfaces are distinct 80–130 A particles. These are more plentiful on the external surfaces, where they occur in small chains or clusters. Most of the membrane surface area is free of particles and, in high quality replicas, these bare areas exhibit a distinct substructure (40).

Fig. 9 shows the two major morphologic consequences of enzyme action found to date: (*a*) within minutes after initiation of enzyme action the particles associated with the external surfaces redistribute to give large, two-dimensional clusters and leave large portions of the surface denuded; and (*b*) later, the "ghosts" break down into large vesicles and these vesiculate further with time. Vesicles may bud from or into the ghosts and vesicles-within-vesicles are common.

Phospholipase *C* treatment also causes some reduction in the activity of

[4] Weinstein, R. S., and D. F. H. Wallach. Data to be published.

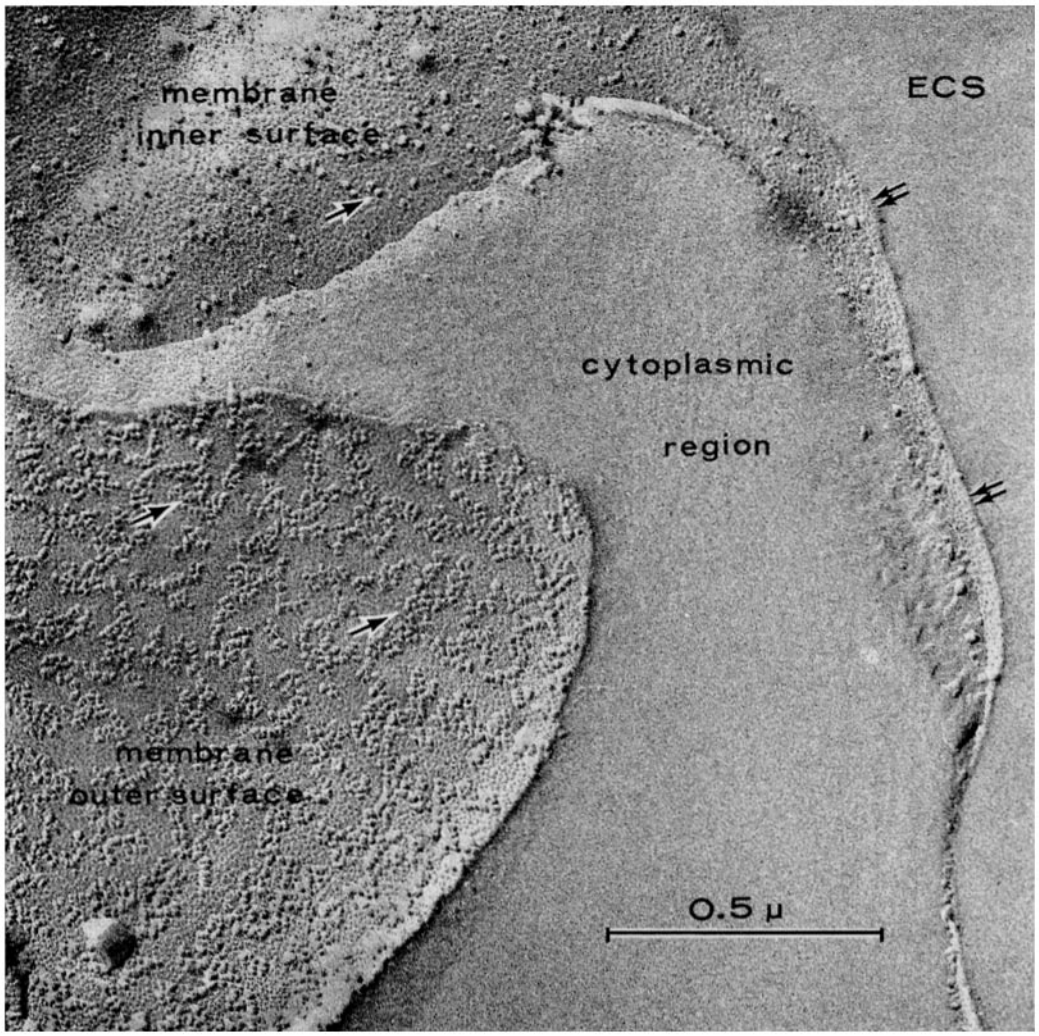

FIGURE 8. Carbon-platinum replica of a freeze-cleaved human red cell ghost membrane. The cleavage plane has been deviated along the membrane's outer surface (lower left) before passing into the empty cytoplasmic region of the ghost. The cleavage plane has then passed along the membrane's inner (juxtacytoplasmic) surface before breaking through the thin membrane (double arrows) and reentering the extracellular space (*ECS*). Outer and inner aspects of the membrane differ with respect to a small protruding particulate component (single arrows) which is more abundant on the outer surface of the membrane than on its inner surface. The figure illustrates the large area of membrane topography that can be examined with the freeze-cleave technique. *Courtesy of Dr. Ronald S. Weinstein of the Massachusetts General Hospital.*

membrane-associated enzymes. Our results to date are summarized in Table I; the ion-sensitive ATPase appears to be most affected. The action of phospholipase *C* results in the release of about two thirds of the lipid phosphorus from erythrocyte ghosts (41). The value for EAC PM is about 40 %. Lecithin, sphingomyelin, and phosphatidyl ethanolamine are attacked, but there is no evidence of peptide cleavage (no increase in $\alpha - NH_2$). There was no enzyme activity in the absence of Ca^{++}. The glyceride and ceramide resulting from

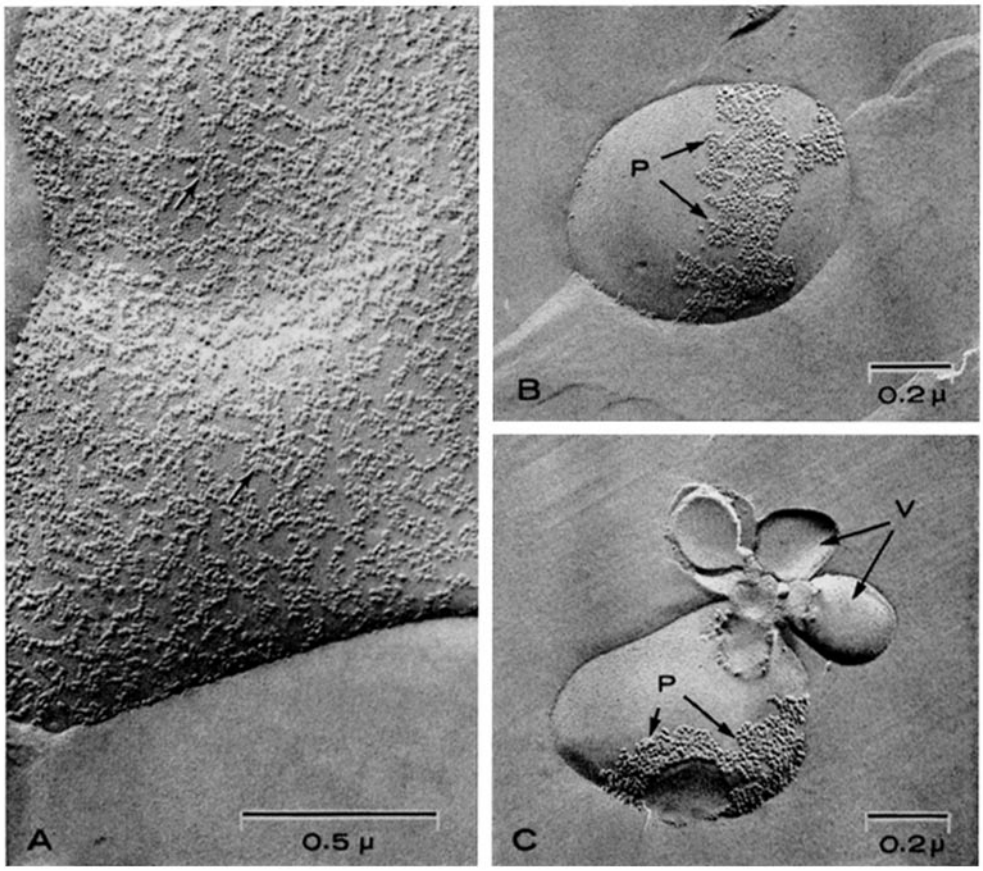

FIGURE 9. Replicas of the outer surfaces of experimentally altered red cell membranes. *A*, On the surface of an intact hemoglobin-free ghost, membrane-associated particles appear aggregated in small clusters and chains (arrows). This ghost membrane is indistinguishable from the membranes of intact red cells. The extracellular glycerol-water (bottom) appears structureless, *B* and *C*. In contrast to the large area of membrane seen in *A*, treatment of membranes with phospholipase *C* in *B* and *C* has caused the membrane to break down into vesicles. Membrane-associated particles (*P*) on the surfaces of individual vesicles migrate toward one region of the surface leaving the remainder of the membrane devoid of particles. In *C* a vesicle is subdividing into smaller vesicles (*V*).

enzyme action, as well as cholesterol and uncleaved phosphatide, remain associated with membrane structures sedimentable at 50,000 rpm (Spinco SW50 rotor, 30 min, 4°C). There is no chemically detectable protein solubilization.

The PMR spectra of enzyme-treated ghosts show loss of the $N^+(CH_3)_3$ signal (33). Significantly, the $(CH_2)_n$ and CH=CH signals are still lacking, indicating continued restriction of fatty acid chain mobility.

The action of phospholipase *C* produces subtle but significant alterations in the CD and ORD spectra of both erythrocyte ghosts and EAC-PM,

TABLE I

EFFECT OF PHOSPHOLIPASE C ON MEMBRANE ENZYMES

Membrane	Enzyme activity (% of control)*		
	Mg^{2+}-ATPase	Na^+-K^+ATPase	NADH-diaphorase
	%	%	%
Untreated membranes	100	100	100
EAC PM	85	56	72
Erythrocyte "ghosts"	78	—	100

* Enzyme treatment as in footnote 2, namely 7 μg enzyme/100 μg membrane protein for 1 hr at 25°C. For "ghosts" buffer was 7 mM phosphate, pH 7.4; and for PM, 1 mM Tris-HCl, pH 8.2. In both cases 1 mM Ca^{++} was used. There was no enzyme activity in the absence of Ca^{2+}. Conditions of assay as in reference 42.

namely: (*a*) a shift of the CD peak (α, $\perp$, $\pi^0 - \pi^-$) and crossover to the *red*; (*b*) broadening of the 223 mμ (α, $n - \pi^-$) band, causing a red-shift of the ORD trough; and (*c*) slight decrease in amplitude. This is illustrated for the case of PM in Figs. 10 and 11. A summary of the band-shifts is given in Table II.

The positions of the $\pi^0 - \pi^-$ bands in membranes, as in certain globular proteins[3] lie to the red of those found for synthetic polypeptides. This suggests that the environment of the average peptide bond in the case of membranes and some globular proteins is less polar and more polarizable than water.[3] The spectral shifts caused by phospholipase *C* indicate that the peptide environment has become even more organophilic. The PMR data cited above are consistent with this view.

In conclusion, the data, though fragmentary, suggest that phospholipase *C*, by removing charged groups from membrane lipids, unbalances the interaction between membrane components in the direction of excessive apolar attraction. The clumping of the membrane-associated particles might be interpreted in this way.

Lysolecithin and Phospholipase A

Most of our biochemical studies on the effects of lysolecithin have been done with EAC PM. Here lysolecithin (1–2 mg/mg membrane protein) causes a 90 % reduction of light scattering, indicating a decrease in the size and/or refractive index of the membranes. 30–35 % of the membrane protein and all of the membrane RNA become nonsedimentable at 50,000 rpm (30 min). The effects of lysolecithin and phospholipase *A* on some enzymes associated

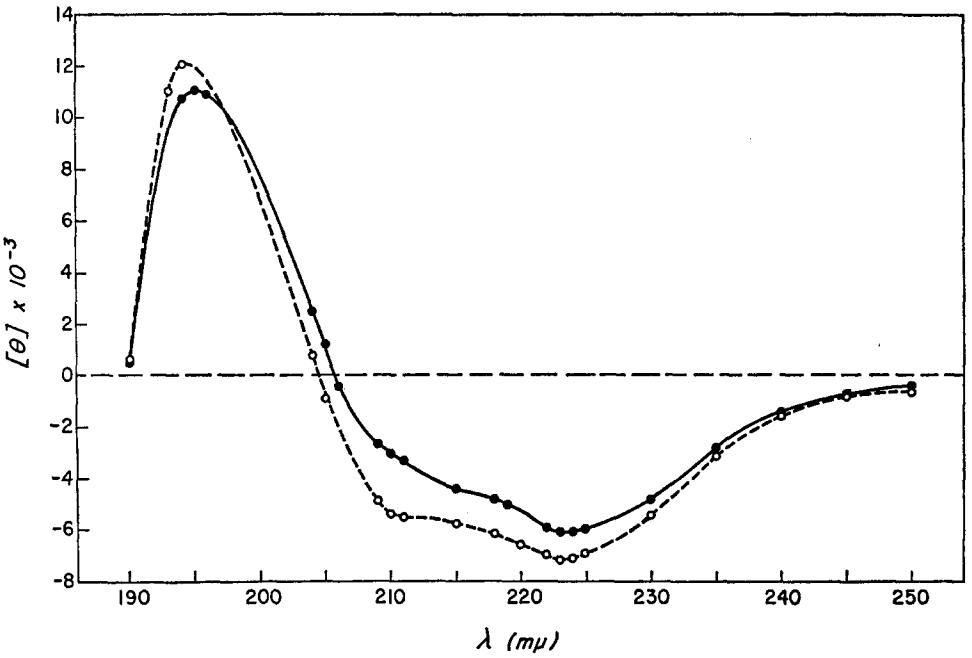

FIGURE 10. Experimental CD spectra of plasma membranes of Ehrlich ascites carcinoma cells (—○—) and plasma membrane after incubation with 1 mM Ca^{+2} and phospholipase *C* (—●—). Buffer 0.001 M Tris-HCl, pH 8.2.

with EAC PM are shown in Table III. It is clear that the NADH oxidase is insensitive and the ion-sensitive ATPase highly sensitive to lysolecithin.

Comparison of the effects of lysolecithin with that of phospholipase *A* is highy instructive. At the high enzyme level, essentially all of the membrane glycerophosphatide is converted into the lyso- form. However, 10 μg membrane protein can yield at best 1.5 μg lysolecithin, which should activate rather than inhibit membrane ATPases. It appears, therefore, that the action of lysolecithin is not merely that of a surface-active agent. This view is supported by the fact that we cannot duplicate the biochemical action of lysolecithin with lysophosphatidyl ethanolamine. The special effects of the en-

zyme are presumably due to its conversion of *membrane-bound* phosphatides into bound lyso- compounds and fatty acids.

PMR spectra of lysolecithin show large $(CH_2)_n$ signals (Fig. 12 *a*). However, a mixture of lysolecithin with erythrocyte ghosts lacks this signal (Figs.

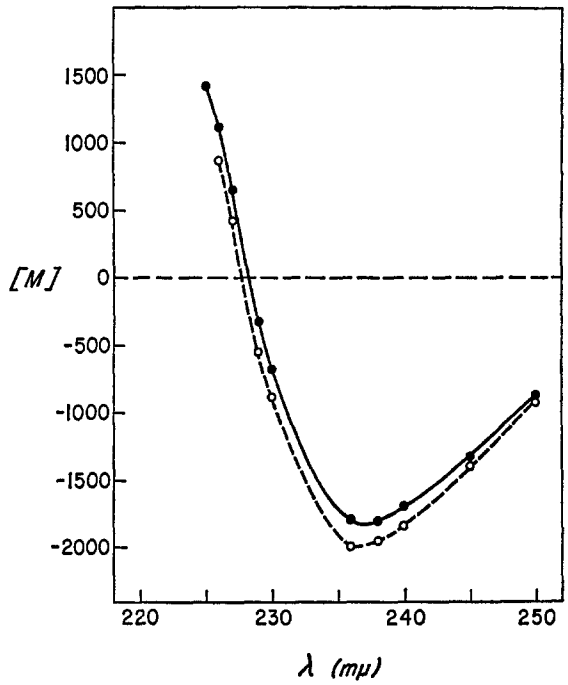

FIGURE 11. Experimental ORD spectra of plasma membranes of Ehrlich ascites carcinoma cells (—○—), and plasma membrane after incubation with 1 mM Ca^{+2} and phospholipase *C* (— ●—). Buffer 0.001 M Tris-HCl, pH 8.2.

TABLE II

EFFECT OF PHOSPHOLIPASE C ON THE OPTICAL ACTIVITY OF ERYTHROCYTE "GHOSTS" AND EHRLICH ASCITES CARCINOMA PLASMA MEMBRANE

	Spectral position					
	CD			ORD		
Membrane	Minimum	Crossover	Maximum	Trough	Crossover	Peak
	mμ	mμ	mμ	mμ	mμ	mμ
"Ghosts":						
Control	223	203.5	194	235	225	202
Enzyme	223	204.5	195	237	226	—
EAC PM:						
Control	223	204.5	195	236	225.5	203.5
Enzyme	223	205.5	196	237	226.5	—

12 *b* and *c*). This is taken to indicate that lysolecithin added to erythrocyte ghosts is bound by apolar interactions with membrane protein (33).

The correlation between the PMR of benzyl alcohol and its hemolytic action is germane here (43) (Fig. 13). As the concentration of benzyl alcohol is increased from 0 to 60 mM, the susceptibility of erythrocytes to osmotic

TABLE III

COMPARISON OF THE EFFECTS OF LYSOLECITHIN AND PHOSPHOLIPASE A ON SOME ENZYMES ASSOCIATED WITH EHRLICH ASCITES CARCINOMA PLASMA MEMBRANE

Conditions	Enzyme activity (% of control)*, ‡		
	Mg^{2+}-ATPase	Na^+-K^+ ATPase	NADH-diaphorase
	%	%	%
Control	100	100	100
+ Lysolecithin:			
5 μg/ml	105	108	—
20 μg/ml	55	42	100
30 μg/ml	46	27	—
50 μg/ml	45	10	100
+ Phospholipase *A*:§			
0.5 μg/ml, 1 hr, 25°C	50	18	—
+ Phospholipase *A*:			
10 μg/ml, 1 hr, 25°C	33	0	96

* Membrane protein—10 μg/ml.

‡ Conditions of assay as in reference 42.

§ Enzyme purified from Naja naja venom as in footnote 2. Enzyme treatment was in 0.01 M Tris-HCl, pH 7.4; 10^{-4} M $CaCl_2$; 5×10^{-5} M EDTA. The enzyme was inactive in full absence of Ca^{2+}.

lysis decreases and the bandwidth ($\Delta\gamma_{1/2}$) of the aromatic proton signals decreases concurrently. This bandwidth change is similar to what is observed with the erythrocyte lipids and is taken to reflect solution of the alcohol in hydrophobic regions. Near 60 mM there is an abrupt increase in bandwidth in the case of erythrocytes and this coincides with hemolysis. No such reversal occurs with a dispersion of membrane lipids. The data are interpreted to indicate that membrane lysis occurs only when a sufficient proportion of lipid-binding sites are saturated with the alcohol.

The effect of lysolecithin on the CD and ORD of membranes, illustrated in Figs. 14 and 15, is as follows: (*a*) a shift to the *blue* of the CD peak and crossover (to 193 mμ and 201 mμ, repectively); (*b*) a marked increase in negative ellipticity near 212 mμ; and (*c*) narrowing of the 223 mμ band as indicated by

the shift of the ORD trough to 233 mμ. The results with erythrocyte ghosts are comparable. The action of phospholipase *A* duplicates the effects of lysolecithin. The blue displacement of the (α, $\perp$, $\pi^0 - \pi^-$) band suggests a shift of helical peptide chromophores into a more polar, less polarizable environment. Also, the fact that the 223 mμ band narrows, without changing in amplitude, indicates that there is a concomitant deviation from and/or decrease of α-helical structure.

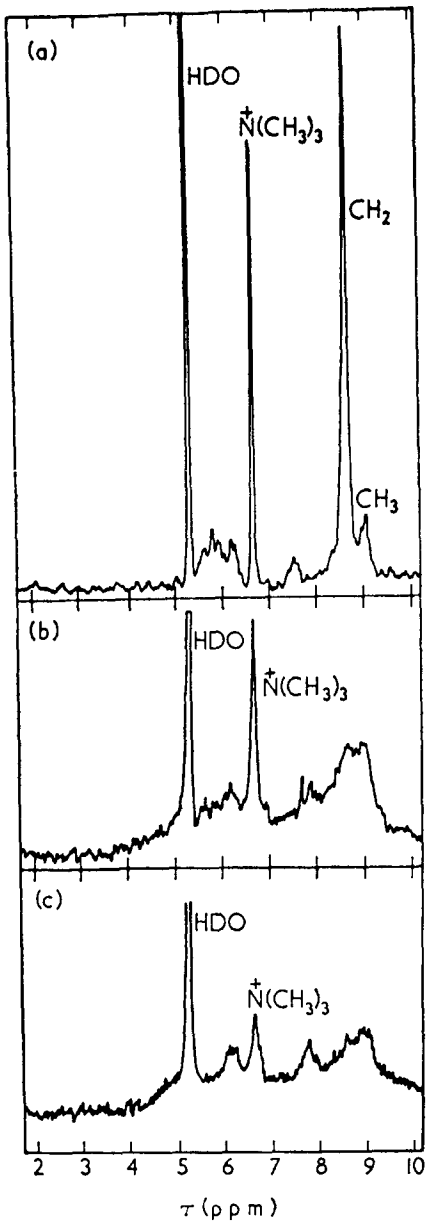

FIGURE 12. The PMR spectrum of ultrasound dispersions in D_2O of (*a*) lysolecithin (2%); (*b*) erythrocyte membrane fragments codispersed with 1% lysolecithin; (*c*) as in (*b*) but with lysolecithin (64 scans). *Reprinted by permission from the J. Mol. Biol., 1968, 31:101.*

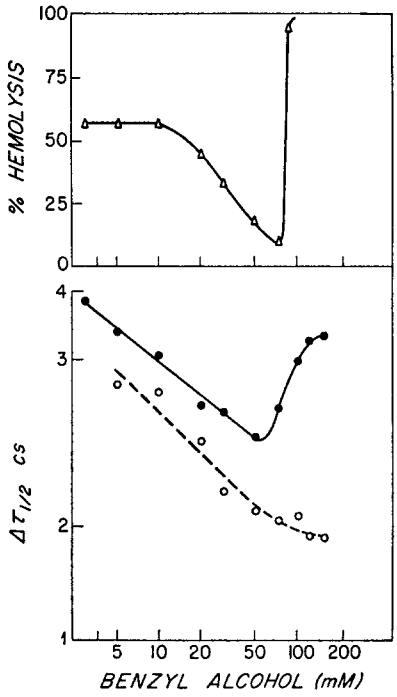

FIGURE 13. *Top panel:* Per cent hemolysis in hypotonic medium as a function of concentration of benzyl alcohol (—△—). *Bottom panel:* Band width of aromatic protons of benzyl alcohol as a function of benzyl alcohol concentration on addition to erythrocyte ghosts (—●—) and on addition to membrane lipids (——○——). Redrawn from Metcalfe et al. (43).

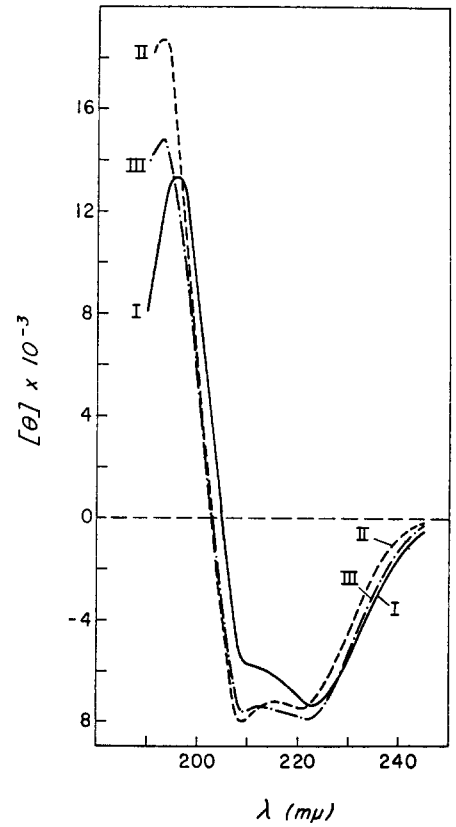

FIGURE 14. Experimental CD spectra of plasma membrane of Ehrlich ascites carcinoma cells (I), plasma membranes plus lysolecithin (II), and plasma membranes after incubation with phospholipase *A* (III). Buffer 0.001 M Tris-HCl, pH 8.2.

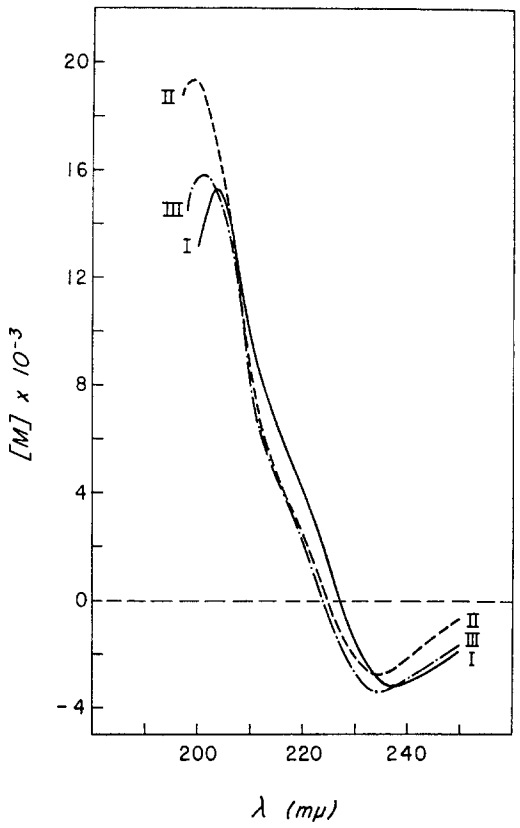

FIGURE 15. Experimental ORD spectra of plasma membranes of Ehrlich ascites carcinoma cells (I), plasma membranes plus lysolecithin (II), and plasma membranes after incubation with phospholipase *A* (III). Buffer 0.001 M Tris-HCl, pH 8.2.

TABLE IV

EFFECTS OF SDS ON ACTIVITIES OF MEMBRANE-ASSOCIATED ENZYMES OF EHRLICH ASCITES CARCINOMA PLASMA MEMBRANE

Conditions		Enzyme activity (% of control)*, ‡		
SDS		Mg^{2+}-ATPase	Na^{+}-K^{+}ATPase	NADH-diaphorse
%	*mg/mg protein*	%	%	%
0	0	100	100	100
0.0005	0.33	104	97	91
0.001	0.67	105	104	65
0.002	1.35	90	124	—
0.005	3.3	10	12	0
0.010	6.7	4	0	0

* Assay as in reference 42.

‡ Protein concentration—15 μg/ml.

To conclude: It appears that lysolecithin binds strongly to membrane proteins through apolar interactions, producing a change in protein conformation. Furthermore, the action of lysolecithin appears to be more than a simple detergent effect.

Sodium Dodecyl Sulfate

At low concentrations SDS stimulates both the Mg^{2+}-ATPase and the Na^+-K^+-ATPase of EAC-PM, the latter to a greater extent (Table IV). As the detergent levels are raised, all membrane-associated enzymes tested become inactivated. Marchesi and Palade (44) report similar data for erythrocyte

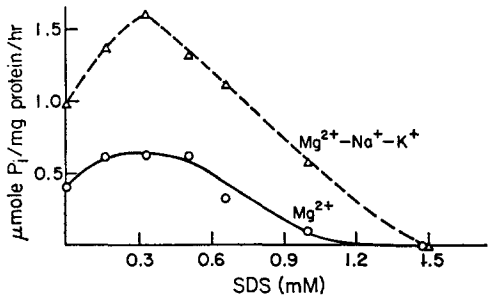

FIGURE 16. Effect of sodium dodecyl sulfate (SDS) on ghost ATPase in the presence of Mg^{2+} and Mg^{2+} $-Na^+$ $-K^+$. Ghosts (1 mg protein/ml) were incubated for 30 min at 37°C in Tris-maleate (pH 7.0), 40 mM; Mg^{2+}:Na^+:K^+, 4:100:20 mM (or Mg^{2+}, 4 mM, alone); and ATP, 4 mM. *Figure reprinted by permission from the J. Cell Biol., 1967, 35:385.*

ghosts, although at higher protein and detergent concentrations (Fig. 16). Their results correlate well with those of Chapman and Kamat (34) on the effect of SDS on the PMR spectra of erythrocyte "ghosts" (Fig. 17). Under conditions where the ghost ATPases are stimulated (0.1 mg SDS/mg protein) the PMR spectrum is as that of untreated "ghosts." At inactivating levels of SDS (0.4–0.8 mg SDS/mg protein) the PMR signals of CH=CH (4.7 ppm) and $(CH_2)_n$ protons are liberated. At the highest SDS levels a resonance band from aromatic amino acids appears, indicating protein denaturation.

For technical reasons one cannot measure the CD and ORD of "ghosts" under the conditions (high protein concentration) employed by Marchesi and Palade (44) and by Chapman and Kamat (34). The effect of SDS on the CD and ORD of "ghosts" (Figs. 18 and 19) was measured at a protein concentration of 50 μg protein per milliliter. At 0.002 %, the results obtained with SDS are similar to those obtained with high levels of lysolecithin and full action of phospholipase *A*. However, in 2 % SDS there is a marked loss in amplitude of the 223 mμ CD band and the ORD trough, a further blue shift

of the CD and ORD crossover and a larger negative CD band at 207 mμ: all indicating a change from helical to random conformation; i.e., denaturation. It is at such high concentrations of SDS that membrane proteins go into solution as SDS-complexes, which can be separated by electrophoresis on acrylamide gels.

In conclusion, it appears that increasing concentrations of SDS effect

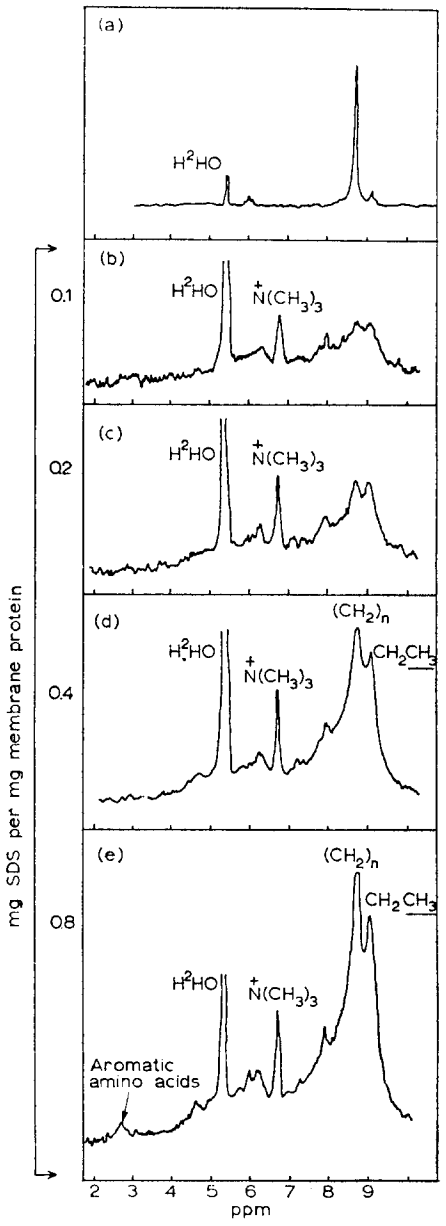

FIGURE 17. The PMR spectra at 40°C of (*a*) a 2% solution of sodium dodecyl sulphate in ^2H_2O (36 scans) and (*b* to *e*) a 5% codispersion of erythrocyte membrane fragments with increasing concentrations of SDS (64 scans). The concentration of SDS present with membrane (*e*) corresponds to the amount present in *a*. *Figure reprinted by permission from Regulatory Functions of Biological Membranes, B. B. A. Library, Vol. 11, Elsevier Publishing Company, Amsterdam, 1968.*

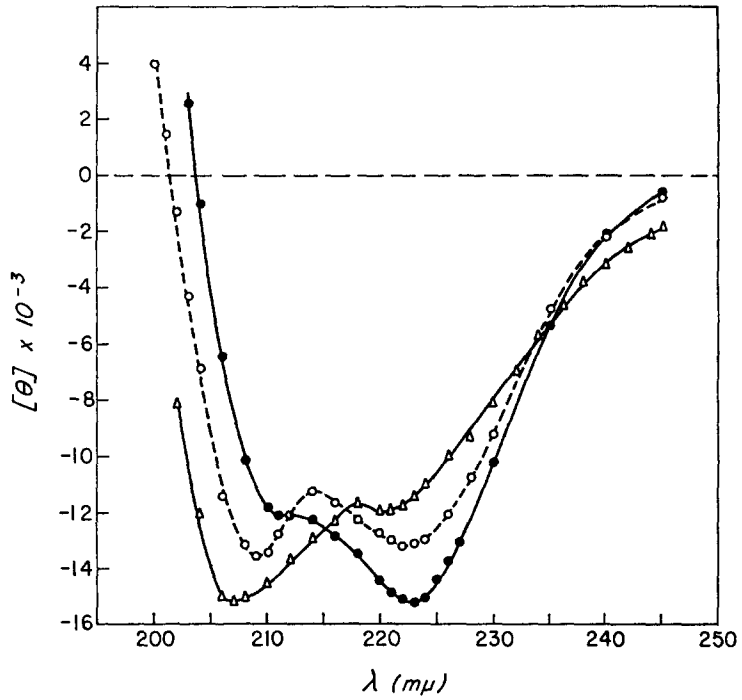

FIGURE 18. Experimental CD spectra of plasma membranes of Ehrlich ascites carcinoma cells (—●—), plasma membranes plus 0.002% SDS (—○—), and plasma membranes plus 2% SDS (—△—). Buffer 0.001 M Tris-HCl, pH 8.2.

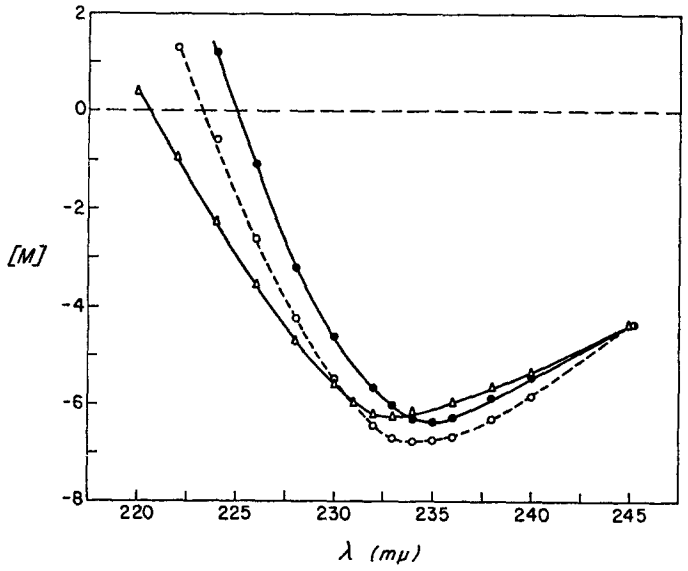

FIGURE 19. Experimental ORD spectra of plasma membranes of Ehrlich ascites carcinoma cells (—●—), plasma membranes plus 0.002% SDS (—○—), and plasma membranes plus 2% SDS (—△—).

progressive denaturation of membrane proteins, with concomitant disruption of the hydrophobic interactions between membrane proteins and lipids.

CONCLUSION

The results presented here indicate that the structure and function of membrane proteins depend heavily upon their association with lipids. Two modes of interaction should be considered, namely: (*a*) the hydrocarbon moieties of membrane lipids create a highly polarizable, apolar solvent environment which is required for the correct folding of peptide chains and the proper spacing of protein subunits; and (*b*) the secondary structure (conformation) of individual polypeptide chains, as well as the tertiary and quaternary associations between these chains depend upon direct molecular interaction with lipid. One cannot at present differentiate between these possibilities, but very likely both are needed for the normal functional architecture of membranes.

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