INTERACTION OF INTEGRAL MEMBRANE PROTEINS WITH MULTIVALENT LIGANDS

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Enveloped viruses such as influenza, parainfluenza, and rhabdoviruses assemble by budding at the plasma membrane (1–3). During this process they acquire an envelope which has a lipid bilayer, whose composition reflects that of the host plasma membrane, glycoproteins that form projections or spikes on the surface of the virus particles, and a nonglycosylated M-protein which is associated with the interior surface of the lipid bilayer of the virus particle (1–3). The virion-associated proteins are virus specific. The viral glycoproteins, which are integral membrane proteins, are involved in virus attachment and in penetration of the host cell (1–3). The lipid bilayer of all enveloped viruses examined is more rigid than that of the host plasma membrane from which it is derived (reviewed in reference 4). This suggests that the interaction between envelope-associated proteins and lipids can increase the rigidity of the lipid bilayer. Because enveloped viruses may be viewed as the assembly of specialized regions of plasma membrane, the study of the structure of viral envelopes should be of importance in understanding the organizational principles of the plasma membrane.

RESULTS AND DISCUSSION

To investigate the nature of the interaction of the surface glycoprotein (G-protein) of vesicular stomatitis virus (VSV) with the lipid bilayer, we have reconstituted the G-protein in liposomes of egg yolk lecithin using lysolecithin as the detergent for disrupting the virus particle (5). The extracted protein was added to egg yolk phosphatidylcholine and the lysolecithin was quantitatively removed with bovine serum albumin. In the reconstituted vesicles, the G-protein:lipid ratio is similar to that of intact VSV particles. The G-containing liposomes agglutinate goose erythrocytes and exhibit enhanced binding to cultured cells. From the electron spin resonance (ESR) spectra of a C_5 spin label derivative of stearic acid [2-(3-carboxylpropyl)-4,4-dimethyl-2-tridecyl-3-oxazolidinyloxyl], the insertion of the highly purified G-protein into the lipid bilayer increases the observed hyperfine splitting ($2A'_{zz}$) (cf., Table I) indicating a decrease in the motion of the lipid fatty acyl chains (5). Similar results were obtained using lecithin vesicles reconstituted with G-protein extracted with octylglucoside (6). Cross-linking of the G-protein reconstituted in lipid vesicles with antibody directed against G causes an additional increase in bilayer rigidity (Table I). It is possible that in intact virus particles the M-protein cross-links the viral glycoprotein (5).

The first step in enveloped virus infection of a cell is attachment of the virus particle to the cell surface. ESR spectra of the C_5 spin label incorporated into BHK cells indicate that VSV binding to BHK cells causes an increase in the plasma membrane bilayer rigidity (data summarized in Table II) (5, 7). The ESR spectrum of C_5 spin-labeled intact BHK cells appears to be due to spin label in the plasma membrane (8). VSV-infected cells secrete a water-soluble fragment of the G-protein (G_s) (8–10) which appears to be monovalent. When G_s is added to BHK cells, no change in the ESR spectra of C_5-labeled BHK cells is detected. When anti-G IgG is added to BHK cells pretreated with G_s, an increase in the measured $2A'_{zz}$ of C_5-labeled BHK cells is observed. These data indicate that cross-linking of receptors in the plane of a membrane can cause a structural change in the plasma membrane lipid bilayer. Lyles and Landsberger (12) showed that the adsorption of influenza and parainfluenza viruses to avian erythrocytes causes a structural change in the plasma

TABLE I
EFFECT OF VSV G-PROTEIN INCORPORATION INTO EGG YOLK LECITHIN BILAYERS AS MEASURED BY THE HYPERFINE SPLITTING ($2A'_{zz}$) OF THE C_5 SPIN LABEL

Experiment	$2A'_{zz}$ (experiment) − $2A'_{zz}$ (control)
	(*gauss*)
lecithin vesicles (control)	0.0
G containing lecithin vesicles	1.5
G containing lecithin vesicles (control)	0.0
G containing lecithin vesicles + IgG	1.5

TABLE II
THE EFFECT OF VSV BINDING ON THE STRUCTURE OF BHK-21 PLASMA MEMBRANE LABELED WITH C_5

Experiment	$2A'_{zz}$ (experiment) − $2A'_{zz}$ (control)
	(*gauss*)
BHK	0.0
BHK + VSV	1.3
BHK + G_s	0.0
BHK + G_s + IgG	1.0

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membrane bilayer which involves a cross-linking of the membrane receptors.

These experiments indicate that cross-linking of integral membrane proteins can alter the observed bilayer rigidity. By changing the lipid bilayer structure, cross-linking of specific proteins may be a factor in altering the lateral arrangement of other membrane components.

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PRESSURE EFFECTS ON PROTEIN-LIPID INTERACTIONS

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Biphasic Arrhenius plots of membrane-bound enzymes have been interpreted as phase changes or phase separations in the membrane lipid bilayer (1). However literature reports can also be found where biphasic Arrhenius plots are attributed to conformational changes of the enzyme without interference of lipids (2).

We have made use of the experimental observation that the effect of pressure on protein conformational transition temperatures is much smaller (2–5 K/kbar) than the effect of pressure on the phospholipid phase transitions (20–25 K/kbar), the latter being a true melting phenomenon (3). Our experiments do not exclude the occurrence of protein conformational changes but indicate whether changes in the activity of enzymes are controlled by the physical state of the lipid or whether they are controlled by the protein without the involvement of lipids.

RESULTS AND DISCUSSION

In our experiments, the activity of the enzymes is measured, under steady-state conditions, as a function of pressure. A break is observed at a characteristic pressure. The pressure at which the break occurs is dependent on temperature (dp/dT). The data are represented as pressure effects on breaks in Arrhenius plots (dT/dp).

As a first example, pressure effect on biphasic Arrhenius plots of Azotobacter nitrogenase was studied (4). The temperature at which the break occurs increases with increasing pressure (dT/dp = 20 K/kbar). This is in agreement with the pressure dependence of the transition temperature of several synthetic phospholipids (5). Detergents and phospholipase A remove the break. Reconstitution can be performed only with specific phospholipids. The conclusion is that lipids are involved in the break.

Similar pressure effects were found on the Arrhenius plots of Na-K-ATPase from pig kidney outer medulla when ATP is used as a substrate (dT/dp = 27.7 K/kbar). Interestingly, the *p*-nitrophenylphosphatase activity of the enzyme shows no biphasic Arrhenius plots and likewise no biphasic plots are observed for the activity as a function of pressure (6). This indicates that the activity of the enzyme towards this substrate is not controlled by the lipids.

The sarcoplasmic reticulum Ca-Mg-ATPase is of

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