films studied become independent of thickness in the size range of the natural membrane (9), suggesting that the membrane thickness may be the smallest dimension in the transition from surface to bulk properties where physical properties become stable and one achieves a maximum strength while at the same time not causing too great a diffusion barrier.

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NMR STUDIES OF THE INTERACTION OF LIPIDS WITH GLYCOPHORIN *A* IN SMALL UNILAMELLAR VESICLES

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Interactions between proteins and lipids are obviously important for maintenance of the structural integrity of membranes and for the proper functioning of many membrane proteins. A variety of techniques have been employed, and a variety of lipid-protein systems studied in an effort to elucidate the molecular details of those interactions (1). Among the many possible ways of characterizing lipid-protein interaction in these reconstituted systems, spectroscopic measurements offer an advantage in that they reflect interaction at a very fundamental level and in many cases do so with minimal physical or chemical modification. Nuclear magnetic resonance (NMR), in particular, ^{31}P, ^{13}C, and ^{19}F NMR, remains a popular tool. In most cases, increases in line widths or decreases in spin-spin or spin-lattice relaxation times are indicative of motional restriction on interaction with protein. In a few cases, chemical shift changes are indicative of interaction.

RESULTS AND DISCUSSION

Here we have used ^{31}P NMR, ^{19}F NMR, and ^{13}C NMR to provide evidence for a range of specificities of interaction of glycophorin *A* (GPA) with different lipids in reconstituted unilamellar vesicles. Such specificities are not unexpected and have been proposed by a number of authors (2–7). Previous evidence has, however, been based on chemical modification and co-isolation studies which can be interpreted in other ways. Broadening of NMR lines in homogeneous systems thus provides direct evidence of protein-lipid association.

The strongest evidence of preferential association comes from the broadening of the resolvable phosphatidylserine (PS) ^{31}P resonance in PS-phosphatidylcholine (PC) mixtures. At least a portion of this resonance could be assigned to PS on the inner half of the vesicle bilayer and its broadening thus supports the notion that GPA is incorporated in a transbilayer fashion.

^{19}F data from lipid hydrocarbon chains substituted at the 6 position support a preferential interaction with PS, not only at the headgroup level but also at the hydrocarbon chain level. However, only a portion of the signal from ^{19}F-labeled PS is broadened. Drawing an analogy with data showing resolution of PC molecules on the inner and outer halves of bilayers obtained by Longmuir and Dahlquist (8), the portion broadened may be associated with PS on the inner half of the bilayer. We know that GPA is incorporated asymmetrically and this finding would provide supportive evidence for the postulate of Bretscher (9) that the positively charged Arg and Lys enriched C-terminus of GPA may interact through electrostatic forces with the negatively charged PS on the cytoplasmic side of the erythrocyte membrane.

^{31}P NMR and ^{13}C NMR also support a slight preference in interaction of GPA with PE over PC. It would seem logical that any distinction between PE and PC would have to be made at the head group. However, differences in ^{31}P spectra are small, and the largest differ-

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ence in perturbation of ^{13}C NMR spectra does not occur at the carbonyl but at unsaturated sites. Although a localized hydrocarbon interaction is possible, it is also possible that perturbations by GPA to the inner half of the bilayer are greater and that this difference results in preferential broadening of PE simply because of its preferential location on the inner half.

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WHAT IS A SUCCESSFUL RECONSTITUTION OF A MEMBRANE GLYCOPROTEIN THAT LACKS AN ENZYMATIC ACTIVITY?

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Sindbis virus is an enveloped virus composed of two surface glycoproteins associated with a lipid bilayer, a nucleocapsid of RNA, and a core protein. The life cycle of the virus is undoubtedly very similar to that of Semliki Forest virus and includes events that occur at two pH's (1). Adsorption to host cells is observed at neutral pH (1). In contrast, at pH below 6.7, Sindbis and Semliki Forest viruses adsorb to lipid vesicles containing cholesterol (2) and to red cells (3), cause hemagglutination and, in some cases, lead to hemolysis (3). During infection, intact viruses are internalized by cell membranes and transported into lysozomal vesicles. The low pH functions of a viral coat protein are activated by the pH inside the lysozomes (pH 5–6) so that the viral membrane fuses with that of the lysozome to release uncoated nucleocapsids into the cell cytoplasm. It has been postulated that one of the Semliki Forest virus glycoproteins, E1, is responsible for hemagglutination and fusion activities while the other, E2, exhibits the ability to bind to host cell surfaces at neutral pH (1, 3).

The mechanisms of the varied functions of the Sindbis virus coat proteins can be studied by using reconstituted membranes in which such parameters as lipid composition and lipid:protein ratio are manipulated and with which the functions of the viral coat can be separated from infection since the RNA core is absent. However, because no enzymatic activities are known to be associated with the Sindbis coat proteins, a question arises as to what constitutes a functional assay for successful reconstitution of the membrane glycoproteins.

RESULTS AND DISCUSSION

We have previously reported reconstitution of egg lecithin membranes containing the E1 and E2 glycoproteins of Sindbis virus (4). We demonstrated that the reconstituted structures resembled native viral membranes in lipid:protein ratio and in size, that the reconstituted membranes retained solutes such as 6-carboxyfluorescein (6-CF) and that the 6-CF-loaded membranes were internalized by macrophages. However, the uptake of the reconstituted membranes is difficult to compare quantitatively with that of the native virus. As a more precise test of functional reconstitution, we compare here hemagglutination by intact Sindbis virus and reconstituted membranes containing E1 and E2. When the results are expressed in terms of the number of virus particles per erythrocyte, it is apparent that this is an extremely sensitive assay because as