RED CELL MEMBRANE PROTEINS IN MONOLAYER AND MULTILAYER STRUCTURES

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Applications of the principles and techniques of surface chemistry have often proven useful in the study of membrane structure and in attempts to reconstitute natural membranes from their components. Our early studies dealt primarily with membrane lipids, but in the last few years we have been examining the surface properties of proteins extracted from cell membranes in monolayer and multilayer structures, and interacted with lipids. These studies have enabled us to draw some conclusions about the amount of protein in membrane structures, the effect of proteins on membrane properties, and the importance of membrane thickness as a factor in the optimization of different physical properties.

RESULTS AND DISCUSSION

We have used two different preparations from red cell membranes, the extrinsic proteins, spectrin and actin (gel bands 1, 2, and 5), and a mixture of intrinsic proteins (gel bands 3, 4, 6, and 7). Because the extrinsic proteins are present only on the cytoplasmic face of the membrane we were able to perform a Gorter-Grendel type of monolayer experiment (1) to estimate the in vivo thickness of spectrin and actin as approximately two layers (~ 35 Å). In monolayer studies of the intrinsic protein mixtures we found that the surface properties correlated with the average molecular weight of proteins in the mixture of spread molecules (2). This relation is useful for interpreting variations in membrane elasticity as a function of composition.

We have also studied the properties of membrane protein multilayers (3–7). For the extrinsic proteins, five surface properties (yield, shear resistance, potential, viscosity and elastic modulus) show extrema or saturation effects at 50–80 Å thickness (8). (Data for three of these properties are shown in Fig. 1.) The magnitudes of the rheological properties of the extrinsic protein films are close to the analogous properties of the entire red cell membrane, and thus may account for these properties in the intact cell. Recalculating these data as bulk properties, the viscosity and elastic modulus become independent of thickness after ~ 80 Å, while the bulk residual yield decreases (and the diffusion coefficient of ions through the layer increases) with thickness after one monolayer (3). The data of Fig. 2 indicate that the first monolayer in

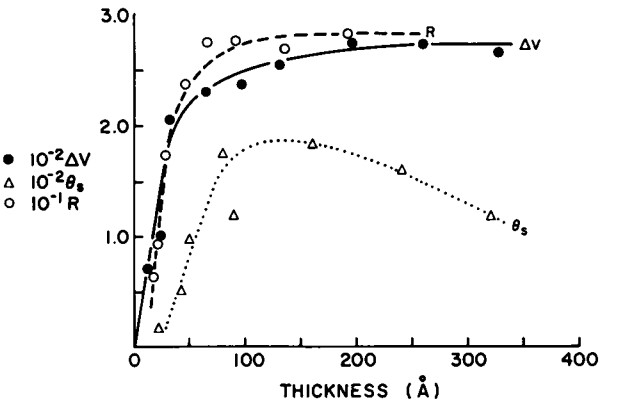

FIGURE 1 Three surface properties of spectrin-actin multilayers as a function of the thickness of the film (in Ångstroms). The surface potential, ΔV, in millivolts (●); the surface yield, θ_s, in dynes per centimeter (Δ); and the shear resistance, R, in arbitrary units (O). All properties are at 25°C and on 0.1 M NaCl. Reproduced with permission from *J. Colloid Interface Sci.* 1980. 75:435–440.

contact with the aqueous subphase forms a much tighter and stronger structure than subsequent layers. Multilayer films of the intrinsic proteins show similar properties, except that they have weaker interactions.

Many of the physical properties of all the multilayer

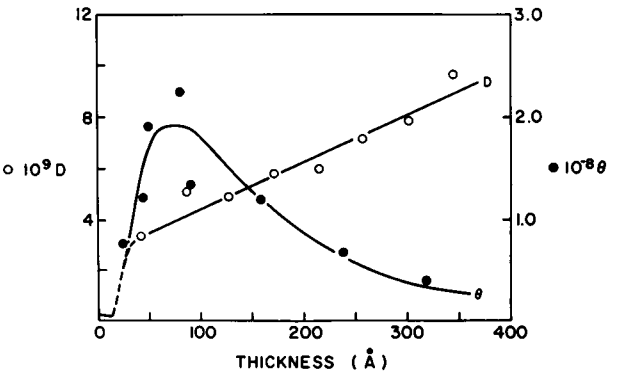

FIGURE 2 Two bulk properties of spectrin-actin multilayers as a function of the thickness of the film (in Ångstroms). The diffusivity, D, in square centimeters per second (O) and the yield, θ, in dynes per square centimeter (●). Below 20 Å the straight line indicates the range of D values obtained by another technique, and the dashed line joins the two sets of measurements of the diffusivity of films to ions. Reproduced with permission from *J. Colloid Interface Sci.* 1980. 75:435–440.

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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