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Functional Components of Surface Membranes: Potential Targets for Pharmacological Manipulation

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I. Introduction

Surface membranes represent an important and, as yet, largely untapped focus for the pharmacological manipulation of cell functions. Progress in this area has been slow, probably in large part due to the real or apparent complexity of the membranes. The purpose of this review is to highlight some of the newer developments in our understanding of the organization of mammalian cell surfaces, with a special focus on the molecular architecture of the human red cell membrane. The philosophy behind this review is based on the premise that surface membranes serve as permeability barriers for different cell types, but each cell type possesses common elements that work together to maintain a balance in the interchange between the cell's internal milieu and its immediate environment. Since different cells have specific needs we shall also assume that variations in the exchange balance that are characteristic of different cell types may be achieved by subtle variations in the function of components that are otherwise common to all cell types. If these assumptions are valid, it is also likely that potentially toxic factors or pharmacologically-active agents may exert their effects on different cells either by directly modifying specific functional components or by affecting the interactions between multiunit systems.

During the past two decades, when progress in unraveling the complexities of cell membranes has been most rapid, experimental studies have focused on three major areas: 1) organization and dynamics of the lipid bilayer; 2) properties of membrane proteins; and 3) isolation and characterization of "functional" proteins.

II. Amphipathic Lipid Matrix

The basic structural framework of a typical plasma membrane consists of a double layer of phospholipid molecules that form an *amphipathic lipid matrix*. This lipid film has a relatively homogeneous hydrophobic interior sandwiched between two polar surfaces. The individual molecules of these films are in constant motion and are able to shift their positions within the plane of the membrane, yet the membrane is relatively stable and does not undergo spontaneous vesiculations or fusion reactions unless conditions are appropriate. Although water apparently moves across with relative ease, this amphipathic lipid matrix is a barrier to most charged polar molecules, yet its hydrophobic interior seems accessible to lipophilic molecules. This lipid matrix also seems uniquely designed to accommodate the lipophilic portions of protein molecules.

Since the early 1930s, membranologists have debated heatedly whether the phospholipid components of cell membranes were really organized in the form of a lipid bilayer. The classic studies of Gorter and Grendel indicated that red blood cell membranes contained twice as many lipid mol-

ecules as were needed to form a single monolayer of lipid around the cell, and one of the simplest interpretations of this finding was to assume that the lipids were distributed uniformly over the surface in the form of a simple bilayer. Later studies indicated that artificial lipid membranes were also most stable in a bilayer configuration. However, many imaginative alternative models were proposed to explain how lipid molecules might be organized at cell surfaces, and for years it was not possible to conclude unequivocally that the bilayer form was the only plausible structure for the lipid part of intact cell membranes.

Early electron microscopic studies, which we might now refer to as "classic," showed that all cell surface membranes contained a "railroad track" image, called the "unit membrane," and it seemed likely that this structure could best be explained if the cell surface were organized as a lipid bilayer. Nonelectron microscopists were often reluctant to accept the "unit membrane" image as being conclusive, particularly after several investigators discovered that this image could be retained in certain membrane systems after the phospholipid molecules had been extracted with organic solvents. Although the latter studies did not rule out the possibility that the lipid bilayer was responsible for the unit membrane image in intact cell membranes, they certainly raised some questions as to whether the unit membrane image necessarily reflected an underlying lipid bilayer.

Electron microscopists interested in cell membrane structure were rescued from this quandary by the application of newly developed freeze-cleavage techniques to the study of cell membranes. Branton discovered that cell membranes could be cleaved down the middle of the lipid bilayer during the freeze-cleavage process (32), and from this he reasoned that the most likely orientation of lipid molecules was indeed in a lipid bilayer as originally proposed. Although Branton's interpretation was hotly contested, further experimental studies supported his original claim completely. In addition to providing some additional support in favor of a lipid bilayer model, freeze-cleavage studies of cell membranes revealed previously unsuspected structures buried within the hydrophobic regions of the lipid bilayer. These structures are now generally referred to as intramembrane particles (IMP) and are thought to represent the intramembranous portions of integral membrane proteins (16).

Our understanding of the molecular anatomy of membrane lipids took a great leap forward when electron spin resonance and nuclear magnetic resonance techniques were applied to cell membranes and model lipid systems (1). These studies confirmed once again the basic bilayer organization of most membrane lipids, but they also revealed an extraordinary degree of dynamism of the membrane lipids. Individual phospholipid molecules were found to flex their hydrocarbon chains rapidly in place and were found to move rapidly within the plane of the membrane. In some cases phospholipid molecules on one side of the lipid bilayer were found to flip across to the opposing half, but the degree and speed of this flip-flop is still in question.

Certain chemical labeling reagents, which were originally used to map the topography of membrane proteins (3), were also found to label different types of phospholipid molecules depending upon whether the reagents were applied to whole cells or permeable membranes. These findings suggested that phospholipids were distributed asymmetrically across the lipid bilayer. Phosphatidylethanolamine and phosphatidylserine were found to be concentrated along the inner surface of the lipid bilayer of both the human red cell membrane and platelet membranes, in contrast to sphingomyelin, which was located predominantly on the external leaflet of the bilayer. These initial findings have been confirmed by more detailed and quantitative phospholipase digestion experiments (5, 35), and we are now left with the interesting and somewhat enigmatic finding that

highly mobile phospholipid molecules are constrained within specific leaflets of the lipid bilayer. How and why lipid asymmetry is maintained are two burning issues yet to be resolved.

III. Properties of Membrane Proteins

It is probably not an exaggeration to say that the development of sodium dodecylsulfate-polyacrylamide gel electrophoresis (SDS-PAGE) was the one most significant technical advance that opened up the study of membrane proteins. SDS-PAGE is still the best and in many cases the only reliable way to analyze the number of polypeptide chains present in any given membrane fraction. Although this technique is not without its artifacts it provides us with a very simple and highly reproducible way to analyze and separate polypeptide chains of membranes on the basis of molecular size. Human red cell membranes contain at least 10 major polypeptide chains when analyzed by this technique. These polypeptides include the protein called "spectrin," which consists of two polypeptide chains in the 250,000 class. SDS gels have also been invaluable in the study of surface membrane architecture. By combining specific probes with SDS gel analysis it has been possible to show that the bulk of the polypeptide chains of red cell membrane proteins are located on the inside of the lipid bilayer. This result has been obtained by exposing intact red cells to nonpenetrating chemical reagents or enzymes that can act on specific parts of membrane proteins and then determining, by SDS gel electrophoresis, which peptide or peptides were labeled. One can also show by simple variations of this approach that some membrane proteins are exposed to both the external and the internal surfaces of the cell, and thereby establish that portions of their polypeptide chains extend completely across the lipid bilayer. Since polypeptide chains can be analyzed for their sugar content and radioactivity by simply staining or counting SDS gel slices, it has been possible to determine which polypeptides are glycosylated and which are phosphorylated. One can also attempt to determine which polypeptides are next to each other by incubating membrane fractions with reversible crosslinking reagents that react with free amino groups (33). By analyzing the products of such incubations before and after reduction one can do a nearest-neighbor type analysis of membrane proteins and attempt to establish functional associations between the proteins.

A great deal of effort has also been expended in attempts to isolate in "water-soluble" form some of the membrane proteins, so that they can be analyzed by classic protein chemical techniques. There have been many attempts to discover a "universal solvent" for all membrane proteins but this has proved to be elusive.

The most impressive gains in our understanding of the molecular and chemical properties of membrane proteins by way of the isolation route have been achieved through studies of red cell membrane proteins. Specific proteins have been isolated on the basis of their differential solubility in buffers of varying pH and salt composition (16). By these means approximately half the proteins associated with human red cell ghosts can be extracted in aqueous buffers. These procedures have not proved to be universally applicable to all cell membranes, possibly because of various technical difficulties. The two high molecular weight polypeptides called spectrin can be extracted by simply immersing red cell ghost membranes in low ionic strength buffers at alkaline pH and incubating at 37°C for short periods. The success of this technique is based on the relatively loose binding of spectrin to the ghost membrane and the fact that red cell ghosts are probably relatively free of contaminating proteolytic activities. If the latter are not controlled, the extracted proteins can be degraded rapidly. In fact, early attempts to reproduce the extraction of erythrocyte spectrin were often negative, largely due to contaminating proteolytic activity from white blood cells.

A sizable fraction of the protein of red cell membranes cannot be extracted from the lipid by exposing membranes to aqueous buffers of different types as described above, and more drastic methods employing detergents are needed to isolate these elements free from lipid. The proteins that are most intimately associated with the lipid including those that have a transmembrane orientation can only be extracted from membranes by this approach. This experience has led many investigators to postulate that membrane proteins are of two major types, peripheral or easily extractable and integral or tightly associated with lipids of the membrane. This generalization is useful from an operational point of view in terms of designing experiments to study membrane proteins, but it may be less valuable in terms of our understanding of the functions of these proteins.

Solubilizing and purifying membrane proteins is, unfortunately, only the first step in the battle. Human erythrocyte spectrin has been available in reasonably pure form for some years, yet it is almost embarrassing to point out that we know essentially nothing of its primary or secondary structure. The large size of spectrin complicates attempts to subfractionate it into simple peptides that are needed to carry out detailed primary structure studies. Although red blood cell membranes are relatively abundant, and it is easy to prepare large quantities of spectrin, it is worth pointing out that extraordinarily large quantities of spectrin are needed to begin such studies since the polypeptide chains are approximately 10 times larger than most standard proteins. For this reason most investigators are attempting to study spectrin from the point of view of specific functional peptides, either those that are chemically modified such as during phosphorylation or those that may be involved in the binding of spectrin to the inner surface of the membrane.

The study of the integral membrane proteins also presents special problems. Band 3 is the major integral protein of the red cell membrane and is thought to be involved in creating a channel for anion movements across the lipid bilayer (22). This protein is now also available in reasonably pure form and studies on its primary structure have been underway for some years. It has been established with reasonable certainty that band 3 has three functional and chemical domains that include an external segment that is glycosylated, a relatively large intramembranous piece that contains many hydrophobic amino acids, and a bulky cytoplasmic segment made up of approximately 400 amino acids. This molecule is also mammoth by ordinary protein chemistry standards and is a trial to sequence, but its study is further complicated by the fact that many peptides generated from it are insoluble under the usual conditions of peptide isolation (27). The insolubility of peptides derived from integral membrane proteins has been a great stumbling block in determining the structure and possible conformation of peptides involved in forming intramembranous domains. One major transmembrane glycoprotein of the human red cell has been isolated, characterized, and sequenced in its entirety and the characteristics of this molecule are described in detail below.

IV. The Glycophorins

Human red blood cell membranes contain a number of different sialoglycopeptides that can be isolated in water-soluble form by a variety of different procedures (16). Although the exact molecular composition of each of the sialoglycopeptides has not been determined, the major component, which comprises approximately 75% of the total, has been isolated and studied in some detail. This molecule, called glycophorin A (17, 29, 30), is composed of a single polypeptide chain that contains approximately 60% carbohydrate by weight. The peptide-bound carbohydrate exists in the form of 16 oligosaccharides, of which 15 are of the tetrasaccharide type (28), and the 16th is a larger more complex moiety attached to an asparagine residue (15).

The polypeptide portion of glycophorin A comprises approximately 40% of the total mass of the molecule and is made up of 131 amino acids. The distribution of different amino acids is interesting in that there is a very high concentration of threonine and serine residues at the amino terminal end of the polypeptide chain. Many of these are glycosylated by a tetrasaccharide moiety attached to the hydroxyl groups of either the threonine or the serine residues. A simple inspection of the amino acid sequence of glycophorin A (shown in fig. 1) shows that there is a striking concentration of nonpolar amino acids located roughly midway between the amino terminal third of the polypeptide chain and the carboxy terminal third. On the basis of results from a variety of labeling studies we have provisionally suggested that the amino acids extending from residue 71 through residue 90 may be the segment of glycophorin A that is buried within the lipid bilayer of the membrane. The evidence in support of this idea is described below.

Since all the glycosylated amino acids are concentrated at the amino terminal end of the molecule, this segment has been operationally designated the receptor domain. This idea is based on the premise that the sugar moieties, perhaps in concert with the adjacent polypeptide segments, may serve as receptor sites or antigenic groups. In this regard several investigators have demonstrated that this part of the glycophorin molecule binds to specific lectins. Recent studies also indicate that the multiple amino acids at positions 1 and 5 are responsible for the MN blood group determinants (7, 9, 34). It is likely that other blood group determinants and possibly other biological receptors may eventually be found on the glycosylated end of the glycophorin A molecule.

The amino acid sequence of the C-terminal segment of glycophorin A is also noteworthy in that this portion of the polypeptide contains a large number of charged amino acids with a peculiar clustering of acidic residues at the very end of the chain. This part of the chain contains a substantial number of prolines that probably play a major role in determining its conformation. The C-terminal segment also contains 5

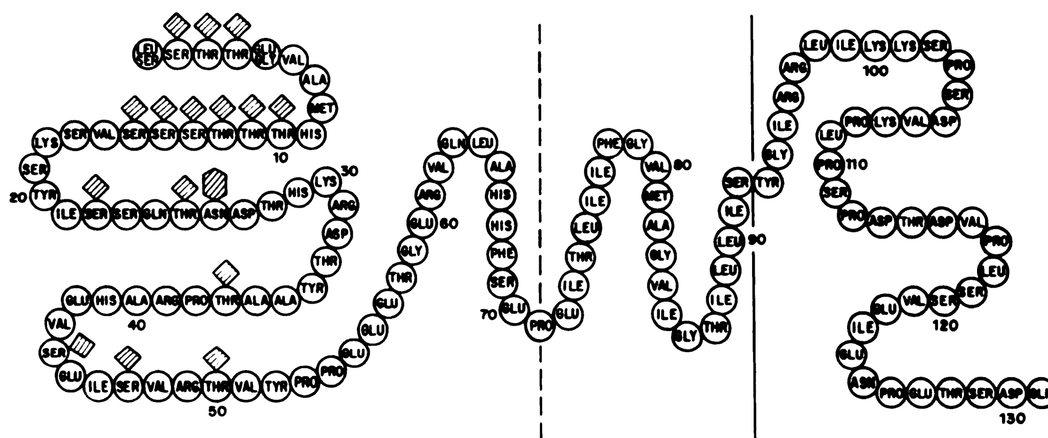

FIG. 1. The amino acids of glycophorin A are arranged in this diagram to approximate the positions they might have if the glycophorin molecule were to be arranged perpendicularly to the lipid bilayer of the membrane. The limits of the bilayer are defined by the two vertical lines. The solid vertical line, which passes between residues 92 and 93, should be the approximate location of the polar groups of the inner half of the phospholipid bilayer. This assignment is based on the results of enzymatic iodination of tyrosine 93 and the distribution of ferritin-antibody conjugates directed against antigenic determinants defined by residues 102 to 118. Since we do not have comparable data with regard to the amount of the N-terminal end of glycophorin A which is buried within the lipid bilayer, we can only guess at the location of this outer lamella of the bilayer relative to the glycophorin molecule; hence, the outer edge of the bilayer is defined by the dashed vertical line.

basic amino acids, 3 lysines and 2 arginines, which are clustered together relatively near the hydrophobic region of the molecule. In fact, if glycophorin A is transmembrane and has residues 71 to 90 buried in the hydrocarbon region of the lipid bilayer as we postulate, then the basic amino acids would be immediately contiguous to the polar groups of the phospholipid molecules along the inner layer of the membrane. The distribution of such residues has interesting implications, particularly since arginine residues have been found to play an important role in the binding of anions such as phosphoesters to the active sites of certain enzymes (19). It is conceivable that arginine residues are playing a similar role in binding to the phosphoryl or other acidic groups of the membrane phospholipids.

The 22-amino acid stretch of peptide that connects the glycosylated end of the glycophorin A molecule with its C-terminal end is composed solely of nonpolar amino acids, and it is logical to suggest that this segment interacts directly with the lipids of the membrane. This peptide, which can be produced by tryptic digestion of the soluble glycophorin A molecule, is insoluble in aqueous buffers but can be solubilized in both nonionic and ionic detergents (12). Under the latter conditions the peptide has a helical conformation (23). It is interesting that this segment of polypeptide contains almost all of the helical content of the intact glycophorin A molecule. We suspect that this hydrophobic portion may be in a helical conformation when the glycophorin molecules are embedded within the intact red cell membrane. Although there is no direct evidence to support this suggestion there are some intriguing experiments concerning subunit associations between glycophorin A molecules that are consistent with this idea (11). They are summarized below.

A. Glycophorin A Spans the Lipid Bilayer of the Intact Red Cell Membrane

There is a considerable amount of experimental data in support of the idea that glycophorin A and probably other membrane glycoproteins are in a transmembrane configuration. The first suggestion that this might be the case was provided by attempts to radiolabel membrane proteins by applying specific chemical probes or enzymes to intact red blood cells as mentioned above. Bretscher (3) showed that parts of the polypeptide chain of the glycophorin A molecule were not labeled when intact red blood cells were exposed to a labeling reagent, but they could be labeled if the radioactive reagent were added to ghost membranes instead. It was reasoned that the additional labeling was achieved because the permeable ghost membranes allowed the reagent to penetrate inside the lipid barrier of the membrane. Thus Bretscher concluded that part of the polypeptide chain of the glycophorin molecule extended outside the red cell membrane and another part was located either within the membrane or in the cytoplasmic compartment of the cell. This experiment was repeated in several laboratories using an enzymatic labeling procedure, lactoperoxidase iodination, with comparable results. Since lactoperoxidase is thought to selectively iodinate tyrosine residues it should, in theory, be the perfect probe for analyzing the orientation of glycophorin A, since the latter has four tyrosines, three of which are located in the glycosylated portion of the polypeptide chain while the fourth is close to the C-terminal end. Thus, three tyrosines should be labeled when intact cells are incubated with lactoperoxidase while the fourth, located at position 93, should not be labeled unless the permeability of the membrane is broken. Although several investigators have reported the expected results, others were not able to reproduce these findings.

Because of the latter negative findings some investigators have suggested that such labeling studies are ambiguous and can be complicated by any one of a number of technical artifacts. Membrane proteins might rearrange, perhaps in subtle ways, as a result of osmotic lysis. Chemical modifi-

cations of the proteins that could result from the labeling procedures themselves might in turn lead to rearrangement of the proteins or changes in membrane permeability or both. In either case such changes could block or modify the reactivity of certain exposed proteins or perhaps even expose other proteins that are normally not external to the lipid bilayer.

Further evidence in support of Bretscher's hypothesis has been provided by the application of recently developed immunocytochemical techniques to this question (6). Antibodies were prepared against a C-terminal peptide obtained from the glycophorin A molecule by cyanogen bromide cleavage, and ferritin-conjugates of these were prepared. These antibodies, which were purified by immunoadsorption, were found to be directed against a 17-amino acid peptide fragment (residues 102 to 118) of the glycophorin A molecule. Ferritin-antibody conjugates of these sera localized exclusively to sites that were distributed uniformly along the inner surfaces of frozen sections of intact red blood cell membranes. Antibody staining was not seen on sections prepared from red blood cells from other animal species, consistent with the immunochemical findings, nor were they found on sections of human red blood cells that were pretreated with unconjugated blocking antiserum. Thus these results establish with reasonable certainty that glycophorin A has a transmembrane orientation in intact human erythrocytes.

B. Glycophorin A May Exist in Multimeric Forms

When crude sialoglycoprotein fractions obtained from human red blood cell membranes are analyzed by the standard Fairbanks SDS-gel electrophoretic system at least three forms can be demonstrated by periodic acid-Schiff (PAS) staining (8). By convention the three most prominent bands have been labeled PAS 1, 2, and 3. Heavily loaded gels also contain a fourth band which appears midway between PAS 1 and 2 and, as an afterthought, has been labeled PAS 4. Up until recently the bands designated PAS 1, 2, and 3 were thought to be distinct sialoglycopeptides with apparent molecular weights of 83,000, 45,000, and 25,000 (8). To further complicate this matter when the same sialoglycopeptide fractions are analyzed by other SDS-gel techniques, significantly different banding patterns can be obtained. Although the precise identification of each of the PAS bands has not been achieved, the simplest explanation for these findings is that there are at least two (and possibly more) distinct sialoglycopeptides, some of which have more than one electrophoretic form. For example, glycophorin A, whose sequence is illustrated in figure 1, migrates in both the PAS 1 and PAS 2 positions. This was not evident when the sialoglycopeptides were first analyzed because only trace amounts of the PAS band 2 are seen when sialoglycopeptides are electrophoresed in the SDS-phosphate system. The relationships between the PAS 1 and 2 forms became evident after Marton and Garvin (18) discovered that heating sialoglycopeptide preparations in the presence of SDS results in the conversion of the PAS 1 form to the PAS 2 form. This observation suggested that either PAS 1 was a multimeric form of PAS 2 or that PAS 2 was created by cleavage of some conformational rearrangement of the PAS 1 form. Recent studies from this laboratory indicate that PAS 1 may be a dimeric form of PAS 2, which is formed by noncovalent associations between glycophorin A molecules that resist dissociation even in the presence of high concentrations of sodium dodecyl sulfate. The conditions necessary for monomer-dimer conversion have been investigated in some detail, and it appears that the site of association between glycophorin A monomers is the hydrophobic segment of the polypeptide chain (11).

In order to explore these complex and sometimes confusing relationships between the different PAS staining bands, it was essential to purify at least one of the components to chemical homogeneity. In the case of glycophorin A this was achieved by

fractionating crude sialoglycopeptide mixtures by gel filtration in the presence of Ammonyx-LO detergent (10). This fractionation procedure also revealed the existence of a second sialoglycopeptide, provisionally labeled glycophorin B, which is similar in many ways to glycophorin A but has distinct chemical differences. The presence of glycophorin B and probably other "glycophorins" has certainly contributed to the confusion described above, since purified glycophorin B migrates in both the PAS 2 and PAS 3 regions. It is also possible to generate a large number of additional high molecular weight forms by exposing glycophorin B to organic solvents. Thus, in the final analysis, detailed chemical studies of each individual polypeptide will be necessary before we will be in a position to unravel the complexity generated by the sialoglycopeptides.

The factors that determine the dimeric form of glycophorin A are interesting and may have implications for the physiological functioning of glycophorin A molecules. On the basis of competitive experiments using purified peptides to compete with the monomer-dimer transitions, we have suggested that the glycophorin A dimer is resistant to dissociation by detergents because of strong noncovalent associations between the hydrophobic segments of the individual polypeptide chains. Since there is evidence that this segment of the polypeptide chain may be in a helical conformation, it is conceivable that there are strong helix-helix interactions that are stabilized by detergents in vitro and perhaps by lipids when the molecules are arranged in the intact membrane. Although this idea is rather speculative, there are some interesting experiments concerning the role of methionine residues in the glycophorin A molecule that offer some intriguing support for this view.

Glycophorin A has two methionine residues, one located at position 5 in the glycosylated region and the other at position 81 within the hydrophobic domain. Each of these methionines can be alkylated selectively under appropriate conditions (25), and when methionine 81, located in the hydrophobic domain, is chemically modified, the capacity of the glycophorins to form stable dimers is greatly diminished. We have also found that the electrophoretic mobility of glycophorin A on SDS-gels can be modified if the glycoprotein is oxidized with hydrogen peroxide under conditions that convert methionine to methionine sulphoxide (26). This change in mobility is completely reversed when the oxidized glycoprotein is reduced by mercaptoethanol. This observation is particularly intriguing since red blood cells and their membranes are also sensitive to oxidative stress. It is conceivable that some of the experiments showing oxidative changes in red cell membranes that were previously thought to be due to SH-SS type changes might be the result of changes of methionine residues within the hydrophobic domains of the membrane proteins.

C. Some Glycophorin A Molecules Are Phosphorylated

Human erythrocyte glycophorin A is phosphorylated when intact red cells are incubated with radioactive inorganic phosphate or when ghost membranes are incubated with ATP (24). The phosphorylated portion of the molecule has been isolated and found to be derived from the C-terminal end, providing further evidence that this end of glycophorin is located in the cytoplasmic compartment of the red cell accessible to endogenous protein kinases. We have also found, somewhat to our surprise, that only one glycophorin molecule out of every hundred appears to be phosphorylated when intact red cells are incubated in inorganic phosphate. This relatively small degree of phosphorylation was not due to an excessively active protein phosphatase, but rather seemed to be due to selective phosphorylation of a relatively few sites. Some preliminary attempts to increase or decrease the level of phosphorylation of glycophorin A were inconclusive.

Glycophorin A isolated by the LIS-

phenol procedure also contains a significant amount of polyphosphoinositide (2) and some preparations also show substantial amounts of phosphatidyl serine (31). We have found that the polyphosphoinositide is bound noncovalently to the hydrophobic segment of the glycophorin molecule. The site of binding of phosphatidyl serine to glycophorin A molecules has not yet been established, but it would be intriguing if this lipid were also associated in some way with the C-terminal segment, particularly since phosphatidyl serine is thought to be concentrated in the inner half of the lipid bilayer. The asymmetric distribution of membrane lipids might influence in some way the orientation of membrane proteins, but it is perhaps likely that the asymmetric orientation of the proteins is a determining factor in the distribution of lipids across the bilayer. The clustering of charged amino acids at both ends of the hydrophobic segment of glycophorin A illustrated in figure 1 could conceivably play some role in maintaining choline-containing lipids on the external leaflet and phosphatidyl serine on the inner leaflet.

V. Isolation and Characterization of "Functional" Proteins

The attempts to solubilize and analyze the membrane proteins described above were primarily concerned with the preparation of pure polypeptides that could be used for structural analysis. The methods used to purify these molecules were often too drastic to expect functional properties to be preserved, particularly in the case of the integral proteins. Strong detergents or potentially denaturing agents were needed to strip the proteins from the lipid matrix and subsequently purify them to homogeneity. Other investigators, more intent upon preserving function than achieving chemical homogeneity, have developed other approaches to the isolation of membrane proteins using relatively mild nonionic detergents. This approach has been used successfully to isolate macromolecular complexes that may be involved in hormone reception (13). This approach has also been used to attempt to isolate the major anion channel protein, band 3, and the protein or proteins having a glucose carrier function (14, 20, 21).

A critical element in the successful isolation of functional components is the availability of appropriate functional assays to follow the isolation and purification procedures and provide some basis for focusing on a particular protein component. In the case of band 3, the putative anion channel, specific chemical probes have been used to inhibit anion transport while also labeling the involved peptides (4). These experiments have established with reasonable certainty that the polypeptide or polypeptides that make up the band 3 component of the red cell membrane are probably the major elements involved in anion movements across the membrane (22). Through the use of these probes it has been possible to identify membrane fractions that are enriched for the anion channel protein or specifically radiolabel the polypeptide components and thereby facilitate their isolation using more conventional methods. Band 3 isolated in the presence of sodium dodecyl sulfate is generally thought to be inactivated; however, band 3 complexes have been prepared in nonionic detergents that are more or less homogeneous and also seem to retain the capacity to facilitate anion transport across artificial liposomes (21, 20). Obviously a critical element in the evaluation of the success of this approach is the ability to reconstitute functional units back into membrane-bound vesicles so that the transport function can be analyzed, since there is no simple way to determine the functional capacity of "channel-forming" proteins. Thus the ability to prepare stable, single-walled, liposomal vesicles is an essential factor in the success of this approach.

The use of a relatively gentle nonionic detergent to solubilize "functional" proteins is not without its pitfalls, however. Although such isolated proteins usually retain their functional activity after detergent

treatment, their subsequent purification has proved to be more difficult than was anticipated. Further purification is hampered by the fact that nonionic detergent complexes are frequently heterogeneous and attempts to achieve polypeptide homogeneity have almost invariably resulted in loss of function. Thus the mild detergent solubilization procedure has been a useful way to isolate enriched fractions of membrane proteins containing specific functional sites, but it has been more difficult to pinpoint the precise proteins involved in the function under investigation.

VI. Summary

This short survey of three major developments in membrane studies should serve to convince pharmacologists that biochemical approaches to the study of cell membranes have finally borne fruit. A reasonable model of cell membrane architecture is now in hand, and it should be possible to use these findings to design and interpret new pharmacological approaches to the manipulation of cells via their surface membranes.

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