MAIN LECTURES

STRUCTURE AND FUNCTION OF BLOOD CELL MEMBRANES

R.F.A. Zwaal

Biochemical Centre, State University of Limburg
Maastricht, The Netherlands

Membrane structure

Perhaps the most conspicuous structural feature of cell membranes is the bimolecular lipid leaflet, which was first recognized by Gorter and Grendel in 1925 (1). Their findings initiated some four decades of thought on the molecular architecture, culminating in the fluid mosaic membrane model of Singer and Nicolson in 1972 (2). The lipid molecules are thought to be arranged in bilayers with polar headgroups directed outward to the aqueous medium on both sides of the membrane and the hydrocarbon chains pointed towards the centre of the bilayer away from contact with water (Fig.1).

Fig. 1. Schematic representation of proteins interacting with a phospholipid bilayer. The apolar areas of proteins and lipids are represented by heavier lines than those used for the polar areas. Note that hydrophobic domains tend to cluster together away from contact with water.

Membrane proteins are considered to be either peripheral or integral. Peripheral proteins are associated with the membrane predominantly by electrostatic interactions with the polar headgroups of the phospholipids. Integral proteins have a non-polar part of their molecules intercalated in the lipid bilayer or even span the membrane from one side to the other.

Membrane lipids

The lipid fraction of most mammalian plasma membranes is composed of phospholipids, cholesterol, and, to a smaller extent, of glycolipids (usually gangliosides). Slightly more than half of the phospholipid fraction is composed of the choline-containing phospholipids lecithin and sphingomyelin, the remainder consisting of a wide variety of other phospholipids, of which phosphatidylethanolamine and phosphatidylserine are the most abundant. Due to their amphipathic nature, phospholipids spontaneously form concentric multilamellar bilayer structures (liposomes) when suspended in an aqueous environment(3). This is considered to be a consequence of the unfavourable thermodynamic interaction of water with the hydrocarbon chains (4) and also requires that these fatty acid chains be in a liquid-crystalline state (5). The phase transition temperature at which the bilayer changes from a liquid-crystalline to a gel state depends on the length and degree of unsaturation of the apolar side chains and on the nature of the polar headgroup of the phospholipids (6). The presence of cholesterol in the bilayer exerts a condensing effect on the lipids in the liquid-crystalline state and a liquefying effect on lipids in the gel state, thus bringing about an intermediate condition of the bilayer interior(7). At physiological temperature, however, most naturally occurring lipid mixtures are in the liquid-crystalline state which is accompanied by a rapid molecular motion of the hydrocarbon chains and a great lateral mobility of the phospholipid molecules. This motion is thought to be restricted when lipids are associated with membrane proteins. Translocation of phospholipids from one half of the bilayer to the other appears to be a rare event in artificial phospholipid vesicles(8), unless considerable differences in lateral molecular packing are present between the two halves of the bilayer (9). Also, the introduction of membrane proteins may considerably accelerate this process (10), and half-times of only a few hours have been inferred for translocation (flip-flop) rates of lecithin in red cell membranes (11). It has recently been found using ^{31}P -NMR in combination with freeze-fracture electron microscopy that certain lipid classes which are present in biological membranes, i.e. phosphatidylethanolamine and cardiolipin, have a preference for non-bilayer configurations (12). In the presence

of Ca^{2+} , they tend to form cylindrical hexagonal structures or intrabilayer inverted micelles. These non-bilayer lipid structures seem to be abundant in microsomal membranes which are derived from endoplasmic reticulum. Moreover, the presence of considerable amounts of phosphatidylethanolamine in blood cell membranes suggests that transient formation of inverted micellar structures may occur in the plasma membrane during cellular processes that are difficult to reconcile with an inviolate bilayer structure.

Membrane proteins

Membrane proteins come in a wide variety of structures and molecular weights. These include membrane bound enzymes, a variety of glycoproteins and transport proteins, as well as different structural proteins to which often also the cytoskeleton proteins present in microfilaments and microtubules are reckoned. These latter proteins are thought to interact with the membrane via other membrane proteins. Most of the membrane proteins, however, contain structural arrangements that make them suitable to interact with lipids. The best known example is the major sialoglycoprotein of human erythrocytes, which has highly localized sites for interaction with the lipid bilayer (Fig.2). This protein consists of a single polypeptide

Fig.2: Schematic representation of the transmembrane orientation of human glycophorin.

chain of 131 amino acids of known sequence and an average of 16 oligosaccharide chains(13). The carbohydrates are covalently linked to the N-terminal half of the polypeptide chain and are exposed to the external environment of the cell. The C-terminal part of the molecule, which is rich in proline, extends into the cytoplasm, where it may interact with contractile membrane proteins. The two parts of the molecule are connected by a segment of 23 apolar amino acids, presumably extending across the intramembranous domain and anchoring the membrane-spanning protein by hydrophobic interactions.

In model studies this protein exhibits an interaction with lipids, particularly when acidic phospholipids such as phosphatidylserine are present(14). Many other membrane glycoproteins, particularly those of platelet and leucocyte membranes, are thought to have similar structural features and transbilayer orientations. Proteins that do not significantly penetrate the lipid bilayer may have a preference for certain phospholipid head-groups. Erythrocyte cytoskeleton protein spectrin has been shown to exhibit specific interactions with negatively charged phospholipids, such as phosphatidylserine (15).

Membrane asymmetry

There is now compelling evidence that both lipids and proteins are asymmetrically distributed between the two halves of the membrane bilayer(16,17). In erythrocyte and platelet plasma membranes, phosphatidylserine appears to be almost exclusively located on the cytoplasmic surface, the outer monolayer of the membrane being composed of neutral phospholipids, particularly sphingomyelin (18,19). The vectorial arrangement of membrane proteins is more absolute. In general, it appears that in most mammalian plasma membranes the majority of the proteins, including the membrane-bound enzymes, are associated with the cytoplasmic side of the membrane, whereas the carbohydrate moieties are almost exclusively found on the exterior surface of the membrane bound either to glycolipids or to glycoproteins extending across the lipid bilayer (20,21).

Membrane function

Whereas the lipid bilayer forms the structural framework of the membrane, the proteins mediate most of the biochemical functions that membranes perform. These functional aspects include active and facilitated transport of small molecules through the membrane, enzymatic activities involved in maintaining the status quo of the cell and performing its specific functions, binding of hormones to the cell surface and transduction into chemical signals across the membrane, providing an antigenic surface by means of exposed glycoproteins which may also function as receptors for a wide variety of agents that can stimulate the cell to perform a specific response. Moreover, cytoskeleton proteins such as spectrin, actin and tubulin have been shown to be involved in dynamic membrane functions. It should be emphasized, however, that the majority of the membrane processes occur at lipid-water interfaces. Therefore a simple distinction between lipids and proteins in terms of structural and functional components of membranes, respectively, cannot always be main-

tained. Lipids apparently provide the suitable environment, not only for a number of typical membrane processes, but also for related phenomena such as the provision of a catalytic surface for interacting coagulation factors. Nevertheless, the functional significance of lipids and particularly of lipid asymmetry is much less well understood than that of proteins. Two cases have so far been recognized in which lipids may contribute directly in biological control. First, in membrane fusion during exo- and endocytosis the formation of hexagonal structures may be essential (12). The influx of Ca^{2+} into the cytoplasm, being a prerequisite for exocytosis, can produce a lateral phase separation of the cytoplasmically oriented phosphatidylserine, allowing the phosphatidylethanolamine molecules to adopt inverted micellar structures (22). Secondly, the preferential orientation of negatively charged phospholipids, such as phosphatidylserine, at the cytoplasmic surface of blood cells may be of importance in hemostasis. These lipids have "procoagulant activity" due to the fact that they form a catalytic surface for interacting coagulation factors. The outer surface of the erythrocyte membrane is devoid of procoagulant activity, whereas strong clot-promoting activity is present on the inner surface. In view of the similar phospholipid asymmetry in erythrocyte and platelet plasma membranes, it has been postulated that the procoagulant effect of platelet phospholipids is mainly present on the inner surface of the membrane (23). In contrast to other blood cells, however, platelets seem to have the unique property of exposing this activity upon platelet activation. The combined action of collagen and thrombin towards human platelets produces rapid transbilayer movements of phospholipids, leading to an increased exposure of phosphatidylserine at the platelet outer surface. (Fig. 3).

This phospholipid flip-flop is accompanied by a considerable enhancement of platelet prothrombin converting activity, which is one of the key steps in hemostasis.

Membranes and Pathology

The functional role of membrane components can often be inferred from experiments of nature, where structural deviations or alterations in membrane composition are accompanied by certain pathological conditions. In the case of the lipids, alterations in the composition often seem to be a secondary effect, not providing a direct insight into their functional significance. For example, the huge increase of cholesterol in erythrocytes under the conditions of obstructive and hepatocellular liver disease appears to result from fusion of lipoprotein-X with the red-

Fig. 3: Glycerophospholipid composition of the outer monolayer of the platelet plasma membrane before (A) and after (B) activation by the combined action of collagen and thrombin.

cell membrane, which leads to target-cell morphology (24). On the other hand, defects in membrane proteins are often primarily responsible for pathological conditions. Two prominent examples are known for human platelet membranes. Two glycoproteins (presumably similar to erythrocyte glycophorin) are lacking in platelets of patients with Glanzmann's thrombasthenia (25), which is characterized by a defect of platelet aggregation and release induced by ADP or collagen. Platelets of patients with the Bernard-Soulier syndrome lack another membrane glycoprotein (25). This protein is probably equivalent to the receptor for the Von Willebrand factor, which is necessary for adhesion of platelets to the subendothelium. These platelets also fail to interact with thrombin.

Finally, defects in cytoskeleton proteins may affect a number of functions that involve dynamic properties of membranes. Disturbances of chemotaxis, phagocytosis, and lysosomal degranulation can be due to a disruption of the integrity of cytoskeleton-membrane organization. These disturbances explain the failure of bacterial surveillance in, for example, the Chediak-Higashi syndrome (26). Nevertheless, the complexity of dynamic membrane processes still prevents a complete understanding on the molecular level, but rapid progress in the field is made.

References

1. Gorter, E., Grendel, F.: On bimolecular layers of lipoids on the chromocytes of the blood. *J.Exp.Med* 41:439-443, 1925.
2. Singer, S.J., Nicolson, G.L.: The fluid mosaic model of the structure of cell membranes. *Science*, 175:805-837, 1972.
3. Bangham, A.D., Standish, M.M., Watkins, J.C.: Preparation and use of liposomes as models of biological membranes, *J.Mol.Biol.* 13:253-264, 1965
4. Tanford, C.: The hydrophobic effect. New York, Wiley, 1973.
5. Gier, J., de, Mandersloot, J.G., Deenen, L.L.M., van: Lipid composition and permeability of liposomes. *Biochim.Biophys.Acta*, 150:666-675, 1968.
6. Dijck, P.W.M., van, Kruijff, B., de, Deenen, L.L.M., van, Gier, J. de.

- Demel, R.A.: The preference of cholesterol for phosphatidylcholine in mixed phosphatidylcholine-phosphatidylethanolamine bilayers, *Biochim. Biophys. Acta*, 455:576-587, 1976.
7. Demel, R.A., Kruijff, B., de: The function of sterols in membranes. *Biochim. Biophys. Acta*, 457:109-132, 1976.
8. Kornberg, R.D., McConnel, H.M.: Inside-outside transitions of phospholipids in vesicle membranes. *Biochemistry*, 10:1111-1120, 1971.
9. Kruijff, B. de, Wirtz, K.W.A.: Induction of a relatively fast bilayer movement of phosphatidylcholine in vesicle. A ^{13}C NMR study. *Biochim. Biophys. Acta*, 468:318-326, 1977.
10. Kruijff, B. de, Zoelen, E.J.J. van, Deenen, L.L.M. van: Glycophorin facilitates the transbilayer movement of phosphatidylcholine in vesicles. *Biochim. Biophys. Acta*, 509:537-542, 1978.
11. Renooij, W., Golde, L.M.G., van. Zwaal, R.F.A., Deenen, L.L.M. van: Topological asymmetry of phospholipid metabolism in rat erythrocyte membranes. Evidence for flip-flop of lecithin. *Eur. J. Biochem.* 61:53-58, 1976.
12. Cullis, P.R., De Kruijff, B.: Lipid polymorphism and the functional roles of lipids in biological membranes. *Biochim. Biophys. Acta*, 559:399-420, 1979.
13. Tomita, M., Marchesi, V.T.: Amino-acid sequence and oligosaccharide attachment sites of human erythrocyte glycophorin. *Proc. Natl. Acad. Sci. USA*, 72:2964-2968, 1975.
14. Zoelen, E.J.J. van, Zwaal, R.F.A., Reuvers, F.A.M., Demel, R.A., Deenen, L.L.M. van : Evidence for the preferential interaction of glycophorin with negatively charged phospholipids. *Biochim. Biophys. Acta*. 464:482-492, 1977.
15. Mombers, C., de Gier, J., Demel, R.A., van Deenen, L.L.M.: Spectrin-phospholipid interaction. *Biochim. Biophys. Acta.*, 603:52-62, 1980.
16. Zwaal, R.F.A.: Membrane and lipid involvement in blood coagulation *Biochim. Biophys. Acta*, 515:163-205, 1978.
17. Rothman, J.E., Lenard, J.: Membrane asymmetry, *Science* 195:743-753, 1977.
18. Zwaal, R.F.A., Roelofsen, B., Comfurius, P., Deenen, L.L.M., van: Organization of phospholipids in human red cell membranes as detected by the action of various purified phospholipases. *Biochim. Biophys. Acta*, 406: 83- 69, 1975.
19. Chap. H.J., Zwaal, R.F.A., Deenen, L.L.M., van: Action of highly purified phospholipases on blood platelets, Evidence for an asymmetric distribution of phospholipids in the surface membrane *Biochim. Biophys. Acta*, 467:146-164, 1977.
20. Zwaal, R.F.A., Roelofsen, B., Colley, C.M.: Localization of red cell membrane constituents. *Biochim. Biophys. Acta*, 360:159-182, 1973.
21. Bretscher, M.S.: Membrane structure: some general principles. *Science* 181:622-629. 1973.
22. Cullis, P.R., Verkleij, A.J.: Modulation of membrane structure by Ca^{2+} and dibucaine as detected by ^{31}P -NMR. *Biochim. Biophys. Acta* 552:546-551, 1979.
23. Zwaal, R.F.A., Comfurius, P., Deenen, L.L.M., van: Membrane asymmetry and blood coagulation. *Nature*, 268:360-362, 1977.
24. Deenen, L.L.M. van, Gier, J. de, Golde, L.M.G. van, Nauta, I.L.D., Renooij, W., Verkleij, A.J., Zwaal, R.F.A.: Some topological and dynamic aspects of lipids in the erythrocyte membrane. *Nobel Symp.* 34:107-118, 1976.
25. Nurden, A.T., Caen, J.: Membrane glycoproteins and human platelet function. *Br. J. Haematol*, 38:155-160, 1977.
26. Oliver, J.M.: Cell biology of leukocyte abnormalities - Membrane and cytoskeleton function in normal and defective cells. *Am. J. Pathol.*, 93:221-259, 1978.