Chapter 1

Lipid model systems

1.1 Biomembranes

According to the fluid-mosaic-model of Singer and Nicolson (1972), biomembranes consist of a lipid matrix forming a two-dimensional solvent for integral and peripheral proteins. The bimolecular lipid matrix (bilayer) represents a highly selective permeability barrier and is responsible for building compartments within the cell, whereas nearly all other functions of membranes are arranged by specific proteins. Biomembranes are structurally and functionally asymmetric, this means the inner lipid layer differs from the outer with respect to the lipid and protein composition. The main lipid components of biomembranes are phosphoglycerins, which consist of glycerin, two long chain fatty acids and a phosphate residue with an alcohol component, e.g., choline. Fig. 1.1B shows the strucural formula of the phosphoglycerin dipalmitoylphosphatidylcholine. Other lipid compounds are sphingomyeline, glycolipids and sterols.

The most complex system biomembrane is hardly accessible to biophysical methods because it is virtually impossible to experimentally control the great number of parameters involved. The large number of different lipids in natural membranes and the variety of membrane proteins interacting with each other, with the lipids and other components of the cell usually do not allow defined experimental conditions. Thus, we have to simplify the system biomembrane to model systems. This can be done by using simple monolayers and bilayers formed of one or a composition of few defined lipids.

1.2 Monolayer

Monolayers or Langmuir-Blodgett films can be build by spreading amphiphilic molecules on a water-gas or water-alcane interface (Gaines, 1966). Lipid monolayers correspond essentially to one layer of the bimolecular layer of a biomembrane. In this respect the monolayer is a model with less similarity to natural biomembranes compared with bilayers, but its biophysical examination provides information about lipid packing, alignment of the lipid molecules and their surface pressure, which is not accessible at bilayers. With

a Langmuir trough we can characterize monolayers by surface pressure-molecular area isotherms and, if the Langmuir trough is equipped with a Kelvin apparatus, surface potential-molecular area isotherms. The surface potential responds extremely sensitive to small changes of the molecular structure of the lipids (Brockman, 1994). The interaction of surface active molecules, dissolved in the subphase, with lipid molecules can be investigated with these techniques, which is of particular importance in this work. Fig. 1.1A shows monolayers being in different phase states. The gas phase is characterized by large distances and slight interactions between the lipid molecules. In the liquid-expanded state the hydrocarbon chains of the lipids are aligned towards air but free rotating along their bonding axes. In the condensed state the lipid molecules are arranged in their closest packing and the hydrocarbon chains are uniformly aligned (Gaines, 1966; Brockman, 1994).

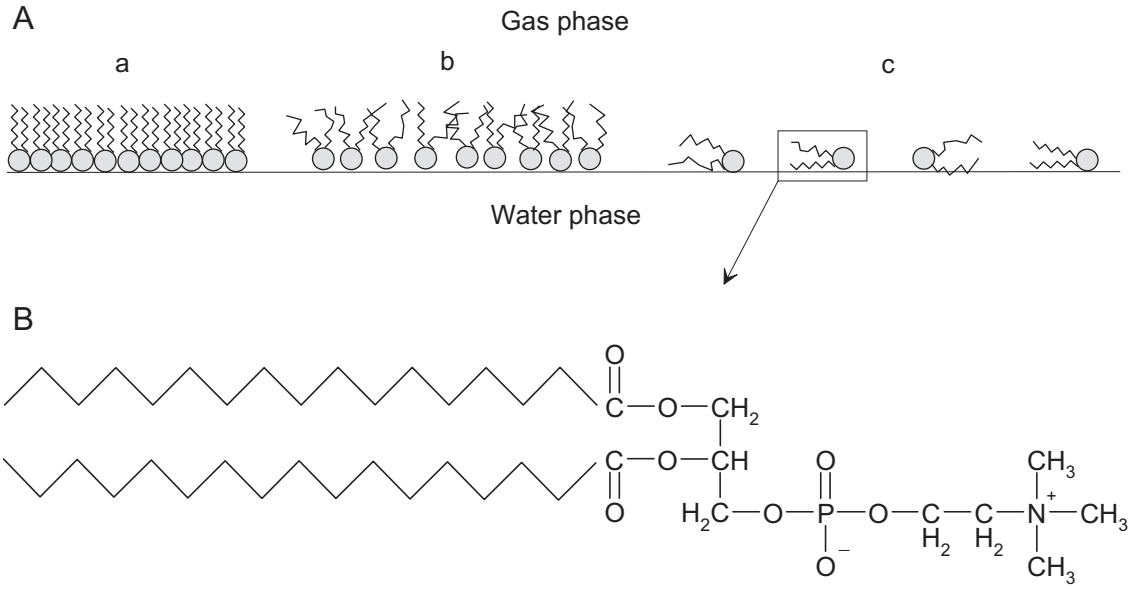

FIGURE 1.1 (A) Schematical representation of monolayers in a the condensed, b the liquid-expanded and c the gas phase state. (B) Structural formula of the molecule dipalmitoylphosphatidylcholine, which is a frequent lipid in biomembranes.

1.3 Bilayer

Bilayers consist of two leaflets of two-dimensional ordered monomolecular layers. These layers, formed of amphiphilic molecules, are arranged in a way that the hydrophobic parts of the molecules are faced each other and the hydrophilic parts are faced the polar solvens, i.e., the water phase (Fig. 1.2). Responsible for the formation and stability of bilayers are mainly hydrophobic forces. However, the close packing of the apolar hydrocarbon chains of lipid bilayers is also favored by van-der-Waals and electrostatic forces and hydrogen bonds between the polar headgroup and water molecules (Gaines, 1966). From a thermodynamic point of view, the bilayer is the most stable energetical form between lipids and water.

Bilayers can be build as planar membranes or vesicles (see below). Black lipid membranes, planar membranes separating two aqueous phases, are particularly suitable for electrical measurements (Benz et al., 1976). Using the charge pulse technique it is possible to estimate the dipole potential of bilayers.

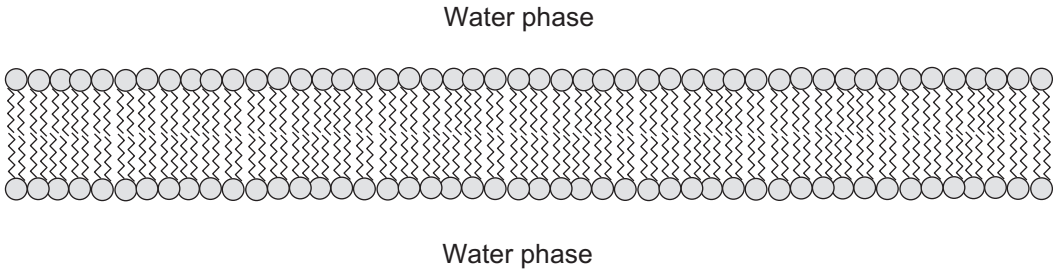

FIGURE 1.2 Schematical representation of a planar lipid bilayer as it can be build as black lipid membranes.

1.4 Vesicles

Vesicles (= Liposomes) (Fig. 1.3) consist essentially of closed spherical bilayers enclosing an aqueous compartment in analogy to cell membranes. Unilamellar vesicles consist of only one bilayer and represents the model most similar to natural membranes of cells. Vesicles are accessible to, e.g., calorimetric and NMR studies, which are suitable to gain insights in structural properties of bilayers, such as phase behavior and dynamics of the lipid molecules (Biltonen and Lichtenberg, 1993). In addition to this are permeability and binding studies the most often used applications for vesicles (Verkman and Solomon, 1980).

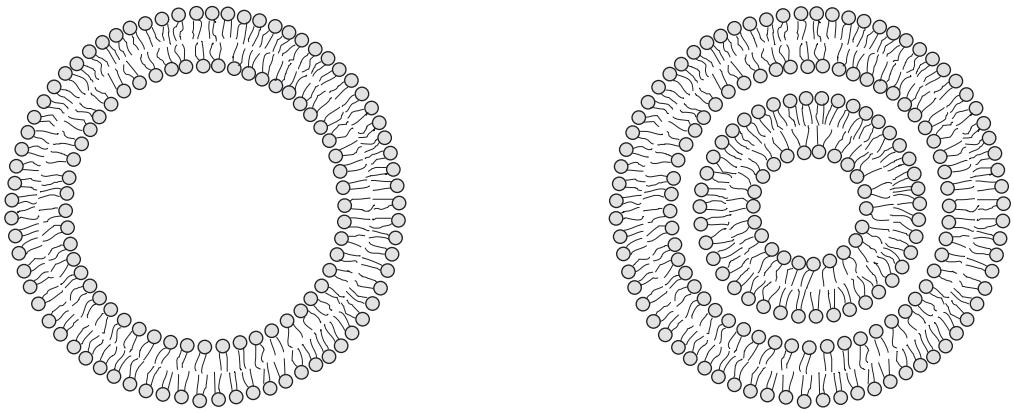

FIGURE 1.3 Schematical representation of an unilamellar vesicle (left figure) and a multilamellar vesicle (right figure) consisting of two bilayers. Note that the ratios of vesicle diameters and bilayer thickness does not reflect the real ratios of size.

1.5 The surface active molecule phloretin

Phloretin (Fig. 4.1) is the classical reversible inhibitor of the hexose transport systems in the human red blood cell membrane (LeFevre, 1961; Andersen, 1976); it also slows the movement of glycerol and urea (Macey and Farmer, 1970). These effects of phloretin would not be of a particular biophysical interest, if the mode of action of phloretin were restricted to the transport systems of cell membranes, this means to membrane proteins. Since all biological membranes contain extensive regions of lipid bilayers, Andersen et al. (1976) decided to investigate the action of phloretin on bimolecular lipid membranes in the hope of finding a simple, physicochemical mechanism responsible for its effects on biomembranes and transport systems. They found that phloretin *increases* carrier-mediated and lipophilic cation conductances and *decreases* carrier-mediated and lipophilic anion conductances of lipid bilayers. Lipid bilayers are essentially impermeable to small ions, whereas larger ions possessing many nonpolar groups, such as dipicrylamine and tetraphenylborate, are much more lipid soluble and therefore partition to a considerable degree to membranes (Läuger, 1972; Benz and Läuger, 1977; Benz, 1988). The permeability of an ion is dependent on its concentration in the membrane phase and therefore changes with a changed partition coefficient. Thus the effect found by Andersen et al. (1976) can be attributed to an increase of the partition coefficient for cations and a decreased partition coefficient for anions. This also means that the electrostatic energy required for the transfer of ions into the membrane interior changed under the influence of phloretin.

One of the factors determining the ion conductance of a lipid membrane is its charge distribution. The membrane interior is positive by several hundred millivolts compared with the surface due to the uniformly aligned lipid dipoles creating a dipole potential (Haydon and Myers, 1973; Hladky and Haydon, 1973; Szabo, 1976; Pickar and Benz, 1978; Flewelling and Hubbell, 1986; Brockman, 1994). In fact, it became clear that the dipole potential of membranes and also monolayers can be lowered by adding phloretin to the aqueous phase. Andersen et al. (1976) proposed a model according to that phloretin, which possesses a large dipole moment, adsorbs to lipid layers in a way that the phloretin dipoles are uniformly aligned creating a dipole potential opposite to that of the lipid molecules. Thus the resulting (positive) dipole potential is reduced, which is responsible for the observed ion conductance change.

1.6 Examination of phloretin and its effects on mono- and bilayer

In this work I have investigated the adsorption behavior of phloretin and its effects on the dipole potential and structural properties of mono- and bilayers. Due to the surface-active property of phloretin I expected interactions with the lipid layers concerning the packing of the lipids and its phase behaviors. Of particular interest was on the one hand

the examination of what kind the adsorption of phloretin to lipids is, this means, whether it can be described by a simple Langmuir adsorption isotherm as it was proposed in literature, or else, is the adsorption related to the electric effects on the lipid layer. On the other hand I was interested in the relation between the effects of phloretin on the lipid packing and the dipole potential reduction.

Phloretin became a model substance to investigate dipole potential effects on lipid layers. However, most of these effects have been observed also with analogous molecules, even though mostly less marked. It is therefore likely that these biophysical effects on membranes are of physiological significance and represent a possibility how molecules can interact with cell membranes.