

LIQUID CRYSTALS AND CELL MEMBRANES

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Abstract - Recent studies of liquid crystalline systems particularly those related to biomembrane structures are discussed. This includes studies of phospholipid molecules, their phase behaviour, their crystallisation characteristics in the presence of polypeptides and proteins and their fluidity. A new method of modulating this phospholipid fluidity by means of homogeneous catalysts is described. The potential for the application of this catalytic method for the study of cell membranes is pointed out.

INTRODUCTION

The topic of liquid crystals has been particularly lively and active over the last ten years. There has of course been considerable interest in the nematic, smectic and cholesteric liquid crystals particularly for their technological potential as electrical devices and temperature measuring systems. At the same time there has been also a separate theme of great interest, This has been in the so-called lyotropic liquid crystals. The interest in this case has been mainly from the view point of understanding biological membrane structure. (There has also been much work on detergency and also on medical applications e.g. studies of bile salts and gall stone formation.) In this paper we will summarise briefly some of our own recent interests in the area associated with liquid crystals and cell membranes.

CELL MEMBRANES

There are many different cell membranes. The electron microscope has revealed that not only are there plasma membranes which separate the cell from its environment but that there are also membranes associated with each of the different organelles located within the cell. Thus there are mitochondrial membranes, nuclear membranes, endoplasmic reticulum membranes etc. The various membranes carry out a variety of functions as well as controlling permeability processes, e.g. they organise the chlorophyll molecules in the chloroplast, the visual pigments in the retinal membranes and various enzyme systems in the mitochondrial systems. Membranes have also been associated with various diseases, e.g. multiple sclerosis, muscular dystrophy and cancer.

Cell membranes are composed of lipids, proteins and sometimes cholesterol. They exist in an aqueous environment. The lipids are the molecules whose physical properties link the topic of cell membranes to that of liquid crystalline behaviour and liquid crystals.

PHOSPHOLIPIDS, PHASE TRANSITIONS AND FLUIDITY CHARACTERISTICS

Phospholipids and glycolipids are important molecules in the construction of cell membranes, and include a variety of classes such as the lecithins (phosphatidylcholines), phosphatidylethanolamines, phosphatidylserines and sphingomyelins (Fig. 1). Mixtures of these lipids

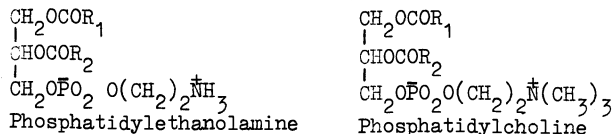


Fig. 1. Molecular structures for some phospholipids

occur in biological membranes and the proportions of these classes vary from one membrane system to another. A range of fatty acids is usually found associated with each class of lipid. These fatty acids vary in chain length and unsaturation. Stearic, palmitic, myristic are common among the saturated acids, and oleic acid is common amongst the unsaturated acids. Unlike simple soaps which contain one fatty acid, the phospholipids contain two fatty acids. The idea that membranes contain bilayers of lipid had been a view held for about 40 years. Recent evidence supports the idea that some regions of membranes containing a bilayer structure is a general phenomenon.

Our own studies of phospholipid molecules began following an earlier study of simple soap systems. Using infra-red spectroscopy we showed a marked change in the spectrum of sodium stearate corresponding to a change from a spectrum characteristic of a crystal to a spectrum typical of a liquid some 200°C below the final melting point of the soap (1). We also showed that a similar effect occurs with phospholipid molecules, i.e. that phospholipids exhibit thermotropic mesomorphism (2).

The capillary melting points of a number of pure phospholipids have been determined and are quite high, e.g. the value for the diacyl-phosphatidylethanolamines is about 200°C, while for the phosphatidylcholines these melting points are 230°C.

In addition to the capillary melting point, other phase changes occur with phospholipids at lower temperatures. Thus, when a pure phospholipid, dimyristoylphosphatidylethanolamine, containing two fully saturated chains, is heated from room temperature to the capillary melting point, a number of thermotropic phase changes (i.e. phase changes caused by the effect of heat) occur. This was first shown by infra-red spectroscopic techniques, then by thermal analysis and has now been studied by a variety of physical techniques (2). Above this temperature the spectrum loses all the fine structure and detail which were present at lower temperatures and becomes similar to the spectrum obtained with a phospholipid dissolved in a solvent such as chloroform.

Differential thermal analysis (d.t.a) shows that a marked endothermic transition (absorption of heat) occurs at this transition temperature. An additional heat change occurs at -135°C and only a small heat change is involved near the capillary melting point of the lipid. This behaviour is similar to that which occurs with liquid crystals, such as p-azoxyanisole or cholesterol acetate which form nematic and cholesteric liquid crystalline phases.

When phospholipids are examined in increasing amounts of water, the various physical techniques, such as microscopy, n.m.r. spectroscopy or differential thermal analysis, show that as the amount of water increases, the marked endothermic transition temperature for a given phospholipid falls. It does not fall indefinitely, but reaches a limiting value independent of the water concentration. We can understand this if we regard the effect of water as leading first to a 'loosening' of the ionic structure of the phospholipid crystals. This, in turn, affects the whole crystal structure and a reduction, up to a certain limit, of the dispersion forces between the hydrocarbon chains. Large amounts of energy are still required to counteract the dispersion forces between the chains and quite high temperatures are still required to cause the chains to melt. These limiting transition temperatures parallel the melting point of the analogous fatty acids becoming lower with increasing unsaturation (3).

As the water penetrates the structure, multi-bilayer sheets of phospholipids are produced. Each sheet is approximately the length of two molecules in width (δ_L). Below the phase transition temperature the lipid chains can be tilted or vertical to the plane of the bilayer. Above this transition temperature the chains are "melted" exhibiting rotational isomerism of the methylene groups. The distance δ_A separating the bilayer sheets can vary dependent upon the amount of water which penetrates the structure (see Fig. 2).

Monolayers obtained with phosphatidylcholines are observed to be much more expanded than are the corresponding phosphatidylethanolamines containing the same acyl chains. These results can be compared with the d.t.a. results discussed earlier. A high transition temperature for liquid crystal formation is, in general, correlated with a condensed-type monolayer and a low transition temperature with an expanded film (4).

Many studies by many authors of the physical properties of these lipid molecules have provided detailed information about the dynamic properties of the bilayer matrix of cell membrane structure (5). Thus the endothermic phase transition associated with the melting of the lipid chains and the increased lateral diffusion characteristics above this

temperature are also seen with cell membrane systems.

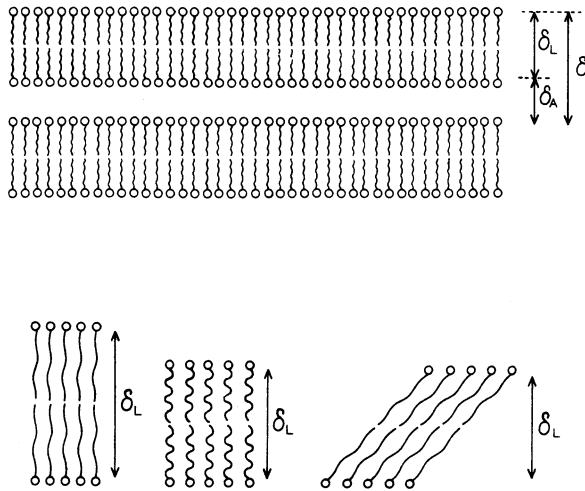


Fig. 2. Sheets of phospholipid bilayers separated by water. The chains within the sheets can be vertical, tilted or in a melted condition.

When some cell membranes are lowered in temperature, lipid crystallisation leading to protein segregation and aggregation takes place (6). Recent studies of channel polypeptides in mixed-lipid lamellar systems (different lipid chain length) show that, as the higher melting lipid crystallises within the layer, the polypeptides are squeezed into the remaining fluid lipid (7). A "zone refining" process occurs with a segregation of polypeptide due to lipid crystallisation taking place. A diagram simulating the process considered to occur in a membrane system, when the membrane is cooled, is shown in Fig. 3.

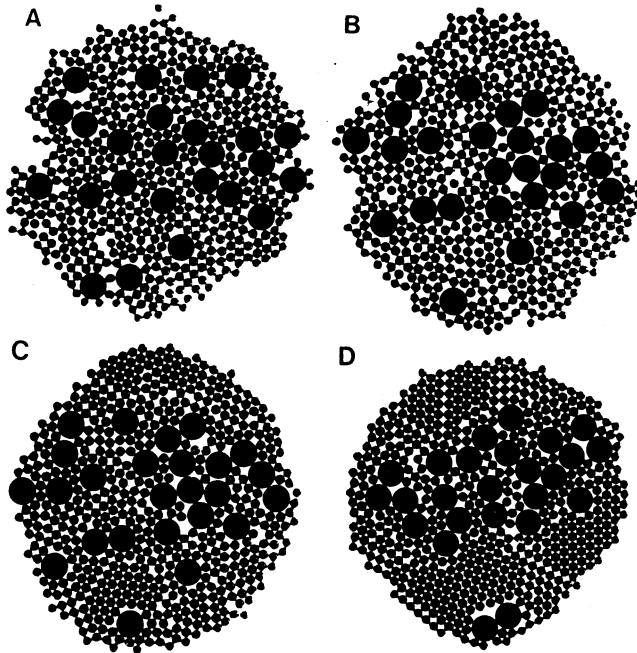


Fig. 3. A simulation of the process which can occur in some cell membranes when the temperature is lowered. (A) A random arrangement of lipids and proteins in the fluid state within the plane of the lipid bilayer. (B→D) Crystallisation of the lipid causes an exclusion of proteins from the lipid regions so that a segregation of these components occurs.

We see the "proteins" first in a random arrangement (A) and then forced to aggregate or cluster as the lipid crystallisation proceeds (B→D). The reverse of this situation can occur

upon heating so that as the lipid begins to melt this increases lipid diffusion and breaks up the clusters or aggregates of proteins (D→A). If the proteins are enzymes a break or discontinuity can be observed in the Arrhenius plot of activity with temperature corresponding to this melting of the crystalline lipid (D→C) and the return to the fluid random condition (A) where the enzyme can exhibit its more normal conformational potential (8).

From studies of these lipid liquid crystal structures a useful concept, the fluidity of the lipid matrix, has been derived (9). The fluidity of the lipid matrix of cell membranes has been shown to be important for providing the environment for protein organisation, movement and function. Various cellular processes have been linked with this fluidity characteristic, including protein rotation, transport processes, protein aggregation, malignant transformation etc. (10). The modulation of membrane fluidity has been considered and discussed with reference to molecules such as cholesterol, polypeptides and proteins (11). Their effects on membrane fluidity have been related to the inhibition of lipid chain mobility and lipid diffusion characteristics.

LIQUID CRYSTALS, CATALYSIS AND CELL MEMBRANES

It is now well known that the presence of a *cis* double bond in the hydrocarbon chain of phospholipid molecules can considerably affect the lipid phase transition temperature of bilayer structures. An increase in the number of double bonds in the chain causes a lowering of the lipid transition temperature. (The transition temperature itself provides a reference for the fluidity characteristics since the lipid undergoes a change from a rigid relatively immobile condition to a fluid structure when heated above this temperature.) Our recent approach to the modulation of membrane fluidity has been to reduce the unsaturated double bonds of the fatty acyl residues associated with membrane lipids *in situ* by means of homogeneous catalytic hydrogenation (12). This has been carried out first with the multi-layer assemblies of the phospholipid molecules in water which are shown in Fig. 2.

Our recent results show, that, by incorporating a homogeneous rhodium triphenylphosphine catalyst into the lipid bilayers, the hydrogenation of the double bonds of the lipids in bilayer structures in water can be accomplished. The studies also show that there is selectivity for this process, so that for example the linolenic fatty acid residues are all hydrogenated before the linoleic residues. There is also a selectivity between lipid bilayers which contain little or no cholesterol compared with bilayers containing appreciable amounts of cholesterol. This indicates that the technique may be selective for particular fatty acid residues and for membranes which have little cholesterol present. Both water-insoluble and water-soluble catalysts have been used successfully. The present method using homogeneous catalysis has the advantage of being able to change membrane fluidity at a constant temperature without altering the individual genotype. It also provides the selectivity for studying the function of particular fatty acids associated with the membrane lipids, e.g. the polyunsaturated acids such as arachidonic, linolenic and linoleic acids present in many cell membranes and the highly unsaturated long-chain acids such as docosahexaenoic acid found to occur in certain membranes like nerve axons and retinal thylakoid membranes.

We can instance two potential applications: (a) Studies of platelet adhesiveness. When blood platelets are stimulated by a variety of agents, the polyunsaturated fatty acids are released from the membrane and converted into prostaglandin endoperoxides. Some of the endoperoxide derived from arachidonic acid is used to synthesise thromboxane which has recently been shown to act as a potent platelet aggregating factor. The selective hydrogenation of the arachidonic acid present in the membranes could enable studies to be made of this aggregation process.

(b) Studies of lymphocytes. The microviscosity of lipid layers of normal lymphocytes is said to be almost twice that of malignant transformed lymphoma cells from rats, mice and humans. An analogous difference has also been observed in human lymphocytes from normal and chronic lymphatic leukaemic patients where it was shown that, by increasing the cholesterol content of the lymphoma cell and hence decreasing the membrane fluidity, the replication rate of these cells is reduced. The hydrogenation of malignant transformed lymphocytes could provide an alternative method for affecting the membrane fluidity and relating this to cell replication processes.

The successful application of the homogeneous catalyst method for the modulation and selective hydrogenation (and deuteration) of natural cell membranes as distinct from model biomembranes will depend upon the successful incorporation of catalysts into natural membranes where competition from membrane proteins and serum proteins occur for the catalyst. Our preliminary experiments show that satisfactory hydrogenation of certain biomembrane structures can be accomplished. We can already envisage a number of improvements that can be made to catalyst design for future applications. These consist of: (a) The design of simpler and smaller catalyst molecules which have low toxicity to living organisms. (b) The

use of alternatives to hydrogen gas (certain hydrogen donor molecules are known to act as a source of hydrogen in many catalytic hydrogenation processes).

The method of hydrogenating the lipid double bonds in membranes and thereby modulating membrane fluidity by using homogeneous catalyst may have considerable use in cell biology. The possibility of incorporating catalysts into other liquid crystal systems so as to carry out reactions other than hydrogenation may also have considerable value. Studies of liquid crystals and cell membranes will continue to be exciting and productive in the years ahead.

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