RECENT STUDIES OF LIQUID CRYSTALS OF BIOLOGICAL IMPORTANCE

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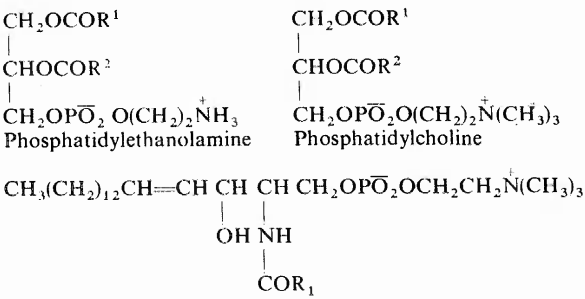
ABSTRACT

Phospholipids and glycolipids are important components of the structure of many cell membranes. They exhibit a melting process associated with the lipid chains at temperatures considerably below the final melting point. In the presence of water they also exhibit lyotropic mesomorphism, and various phases exist. The relevance of this behaviour to the structure and function of cell membranes is discussed.

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 (Scheme I). Mixtures of these lipids occur in biological



Scheme 1. Molecular structures for some phospholipids

membranes[1] and the proportions of these classes vary from one membrane system to another[2]. 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 is in favour of some regions of membranes containing a bilayer structure[3] but there is still uncertainty concerning the position of protein.

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[4]. We also showed that a similar effect[5] occurs with phospholipid molecules, i.e. that phospholipids exhibit thermotropic mesomorphism.

THERMOTROPIC MESOMORPHISM

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[5], then by thermal analysis and has now been studied by a variety of physical techniques[6, 7].

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[8]. 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 cholesteryl acetate which form nematic and cholesteric liquid crystalline phases.

The main conclusions from various studies are that (a) even with the fully saturated phospholipid at room temperature, some molecular motion occurs in the solid. This is evident from the p.m.r. spectra and from the i.r. spectra taken at liquid-nitrogen and at room temperatures. (b) When the phospholipid is heated to a higher temperature, it reaches a transition point, a marked endothermic change occurs and the hydrocarbon chains in the lipid 'melt' and exhibit a very high degree of molecular motion. This is evident both in the appearance of the i.r. spectrum and also in the narrow n.m.r. linewidth. The broad diffuse appearance of the i.r. spectrum is consistent with the chains flexing and twisting and with a 'break-up' of the all-planar *trans* configuration of the chains.

Only one main 'melting of the chains' occurs even when there are *two* different types of chain present in the phospholipid molecule. The transition

temperatures for different phospholipid classes vary even though they contain exactly the same fatty acid residues.

EFFECTS OF WATER

Small amounts of water can have unusual effects upon the mesomorphic behaviour. Thus, the diacylphosphatidylcholines (lecithins) exhibit additional liquid crystalline forms between the first transition temperature and the capillary melting point. The intermediate liquid crystalline form exhibits x-ray spacings consistent with a cubic phase organization. On the other hand, if all the water is removed from the phospholipid, the lipid will no longer exhibit this cubic phase[9].

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 behaviour of the analogous fatty acids becoming lower with increasing unsaturation.

There are a number of important features associated with the transition temperature for the lipid when it is in the presence of water[10]. The first is that the ability to disperse the lipid in water increases markedly above this transition temperature. Only those phospholipids which have transition temperatures when placed in water below or near to room temperature, spontaneously form myelin figures. Fully-saturated phospholipids which have high transition temperatures do not form myelin figures at room temperature. However, if the temperature is raised to the transition point, myelin figures are formed by these phospholipids. In the presence of an excess of water, phospholipids, such as 1,2-dipalmitoylphosphatidylcholine, spontaneously form myelin figures at 42°C.

This ability to form myelin figures with saturated phospholipids has been confirmed by optical- and electron-microscopic investigations. It has been used to attempt to provide information about the situation of the osmium used after osmium tetroxide fixation procedures[11]. N.M.R. and e.s.r. spectroscopic studies[12–14] have been made of these lamellar lecithin systems and the molecular motion of the $N(CH_3)_3$ group and of the different methylene groups along the chain studied. The molecular motion is greatest at the methyl end of the hydrocarbon chains.

A second feature of lipid + water systems is their monolayer behaviour.

Monolayers obtained with phosphatidylcholines are observed to be much more expanded than are the corresponding phosphatidylethanolamines containing the same acyl chains[15]. These results can be compared with the

d.t.a. results discussed earlier[16]. 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. The d.t.a. transition temperatures are higher for the phosphatidylethanolamines than for the corresponding phosphatidylcholines. Phospholipids containing *trans* (elaidoyl) unsaturated chains have higher transition temperatures than those containing *cis* (oleoyl) chains. Phospholipids containing one fully saturated chain and one *trans* unsaturated chain give condensed monolayers similar to those observed with completely saturated phospholipids.

Chapman has recently collaborated with Czech scientists to study n.m.r. spectra of these lipid molecules when in the liquid-crystalline form and when arranged to spin at the 'magic angle'. These results show that at spinning frequencies which are quite slow, marked narrowing occurs. The differing signals narrow at different spinning frequencies.

Phospholipids in the presence of water can also show various types of lyotropic mesomorphism, i.e. they can exhibit different types of liquid crystalline organization[18,19]. Above the transition temperatures when water is present, phospholipids can form different types of phase organization, e.g. some phospholipids give lamellar and hexagonal structures[19,20]. The lecithins appear to exhibit only a lamellar structure over a large range of water concentration[9]. Other phospholipids, such as phosphatidylethanolamines and samples of brain lipid, appear to be able to exist in both hexagonal and lamellar organization, depending upon the concentration in water.

NATURAL MEMBRANES

Lipids in water may show a marked thermal transition associated with a phase change from gel to liquid crystal but the presence of cholesterol can modify or remove this transition.

Recent studies by Steim *et al.*[21] have been with a membrane which does not contain cholesterol. This is the membrane of *Mycoplasma laidlawii*. With this membrane a strong endothermic transition is indeed observed, as in the simple lipid–water systems. The transition temperature is the same as that of the transition of the extracted lipids dispersed in water.

Studies have also been made with membranes which contain cholesterol. e.g. myelin membranes[22]. With wet myelin, thermal transitions are not detectable. In this case the cholesterol and other lipids appear to be organized in a single phase. To maintain the organization of the lipid in myelin a critical amount of water appears to be required. This water is unfreezable at 0°C and may correspond to 'bound' water. On drying the myelin. the cholesterol and other lipids crystallize and precipitate. Endothermic transitions associated with the cholesterol and lipid can then be observed. The total lipid extract in water does *not* show a detectable endothermic transition but the cholesterol-free lipid does. In the absence of cholesterol, part of the myelin lipid is crystalline at body temperature. These results are understandable if there are regions of lipid bilayer present in the membrane with the cholesterol preventing lipid chain crystallization.

The fluidity of membranes may be related to the transition temperatures of their components. Thus, membranes which contain phospholipids having

little unsaturation will have less fluidity than those having phospholipids with much unsaturation. This control of fluidity may then be related to diffusional characteristics of molecules passing into and out of the cell[7]. In line with this concept is a recent study comparing *in vivo* and *in vitro* phase transitions. This study was made with a double mutant of *Escherichia coli* which can incorporate fatty acids with various hydrocarbon structures into its phospholipids[23].

Chapman and Oldfield[24] have pointed out that in some membranes there are lipid chains which are stiff or rigid as well as having fluid regions. Recently membranes containing only rigid lipid chains have also been observed.

REFERENCES

[1] D. Chapman, *Biological Membranes* (Academic Press, 1968).
[2] L. L. M. van Deenen, *Progress in the Chemistry of Fats and other Lipids* (Pergamon Press, 1965), ed. Holman, 8.
[3] M. H. F. Wilkins, A. E. Blaurock and D. M. Engelman, *Nature*, **230**, 72 (1971).
[4] D. Chapman, *J. Chem. Soc.* 784 (1958).
[5] P. Byrne and D. Chapman, *Nature*, **202**, 987 (1964).
[6] R. M. Williams and D. Chapman, *Progress in the Chemistry of Fats and other Lipids*, **11**, 1 (1970).
[7] D. Chapman, P. Byrne and G. G. Shipley, *Proc. Roy. Soc. A*, **290**, 115 (1966).
[8] D. Chapman and D. T. Collin, *Nature*, **206**, 189 (1965).
[9] D. Chapman, R. M. Williams and B. D. Ladbroke, *Chem. Phys. Lipids*, **1**, 445 (1967).
[10] M. C. Phillips, R. M. Williams and D. Chapman, *Chem. Phys. Lipids*, **3**, 234 (1970).
[11] D. Chapman and D. J. Fluck, *J. Biophys. Biochem. Cytol.* **30**, 1 (1966).
[12] Z. Veksli, N. J. Salsbury and D. Chapman, *Biochem. Biophys. Acta*, **183**, 434 (1969).
[13] W. L. Hubbel and H. M. McConnell, *Proc. Nat. Acad. Sci., Wash.* **64**, 20 (1969).
[14] E. Oldfield, J. Marsden and D. Chapman, *Chem. Phys. Lipids* **7**, 1 (1971).
[15] L. L. M. van Deenen, U. M. T. Houtsmuller, G. H. de Haas and E. Mulder, *J. Pharmac. Pharmacol.* **14**, 429 (1962).
[16] D. Chapman, N. F. Owens and D. A. Walker, *Biochem. Biophys. Acta*, **120**, 148 (1966).
[17] D. Chapman, E. Oldfield, D. Doskocilova and B. Schneider, *FEBS Letters*, **25**, 261 (1972).
[18] V. Luzatti, in *Biological Membranes*, ed. D. Chapman, p. 103 (1968).
[19] V. Luzatti and F. Husson, *J. Cell. Biol.*, **12**, 207 (1962).
[20] T. Gulik-Krzywicki, E. Riv-as and V. Luzatti, *J. Mol. Biol.* **27**, 303 (1967).
[21] J. Steim, M. E. Tourtellote, J. C. Reinert, R. D. McElhaney and R. L. Radar, *Proc. Nat. Acad. Sci., Wash.* **63**, 104 (1969).
[22] B. D. Ladbrooke, T. J. Jenkinson, V. B. Kamat and D. Chapman, *Biochim. Biophys. Acta.*, **164**, 101 (1968).
[23] P. Overath, H. U. Schairer and W. Stoffel, *Proc. Nat. Acad. Sci., Wash.* **67**, 606 (1970).
[24] E. Oldfield and D. Chapman *FEBS Letters*, **23**, 255 (1972).
[25] K. R. Naqvi *et al.*