Biochimica et Biophysica Acta 905 (1987) 162–172
Elsevier

BBA 73786

Phase equilibria in the phosphatidylcholine-cholesterol system

J. Hjort Ipsen [a], G. Karlström [b], O.G. Mouritsen [a], H. Wennerström [b] and M.J. Zuckermann [c]

[a] *Department of Structural Properties of Materials, The Technical University of Denmark, Lyngby (Denmark)* and [b] *Divisions of Physical Chemistry I and Theoretical Chemistry, Chemical Center, University of Lund, Lund (Sweden)* and [c] *Department of Physics, McGill University, Montreal, Quebec (Canada)*

(Received 5 August 1987)

Key words: Phosphatidylcholine bilayer; Cholesterol; Phase equilibrium; Thermodynamics; Statistical mechanics

A thermodynamic and a microscopic interaction model are proposed to describe the phase equilibria in the phosphatidylcholine-cholesterol system. The model calculations allow for a solid phase with conformationally ordered acyl chains and liquid phases with conformationally ordered as well as disordered chains. The resulting phase diagram is in excellent agreement with the experimental phase diagram for dipalmitoylphosphatidylcholine bilayers with cholesterol as determined by a recent NMR and calorimetry study. It is thus demonstrated that the phase behaviour of phosphatidylcholine-cholesterol mixtures can be rationalized using only a few basic assumptions: (i) Cholesterol interacts favourably with phosphatidylcholine chains in an extended conformation, (ii) the main transition of pure phosphatidylcholine bilayers takes place in terms of translational variables as well acyl-chain conformational variables, and (iii) cholesterol disturbes the translational order in the crystalline (gel) state of phosphatidylcholine. These results suggest that the occurrence of specific phosphatidylcholine-cholesterol complexes is not implied by the experimental thermodynamic data.

1. Introduction

The effect of cholesterol on phosphatidylcholine bilayers is a longstanding problem in the physical chemistry of model membranes [1–3]. A large number of experimental investigations have been devoted to this problem and their results have been used to answer three basic questions. These are: (i) What is the effect of cholesterol on the main phase transition of pure phosphatidylcholine bilayers? The main phase transition at T_m is the transition which takes the bilayer from a crystalline solid (gel) phase with conformationally ordered acyl chains to a liquid (liquid crystalline) phase with conformationally disordered acyl chains [4]. (ii) What is the effect of cholesterol on the rotational and translational motions of the phosphatidylcholine molecules in the liquid crystalline state? (iii) What is the effect of cholesterol on the conformation of the acyl chains of the phosphatidylcholine molecules? The experimental techniques used to answer these questions include among others calorimetry [5–9], magnetic resonance spectroscopy [10–17], fluorescence depolarization [18–20], infrared and Raman spectroscopy [21–25], neutron- and X-ray scattering [26–28], electron microscopy [26,29–31], and micromechanics [32].

From these experimental studies a general con-

Correspondence: O.G. Mouritsen, Department of Structural Properties of Materials, The Technical University of Denmark, Building 307, DK-2800 Lyngby, Denmark.

sensus has been reached concerning the basic answers to these questions. Addition of cholesterol leads to a more conformationally ordered state of the acyl chains of the phosphatidylcholine molecules [10–17]. Within a given phase, cholesterol has only a small effect on the molecular translational and rotational motions [33–35]. At low cholesterol concentrations a slight broadening of the main phase transition occurs whereas at higher concentrations the liquid phase of the pure bilayer is dramatically stabilized. These results were only obtained with considerable effort and the determination of the phosphatidylcholine (PC)-cholesterol phase diagram in excess water turned out to be a particularly elusive problem. Recently Vist and Davis [16,17] presented a careful study of the d_{62}-dipalmitoyl-PC-cholesterol multi-bilayer system using both deuterium nuclear magnetic resonance (NMR) and calorimetry. The system was therefore characterized on both the molecular and the thermodynamic level. The study was performed in the temperature range of 20–60 °C and from 0 to 30 mol% cholesterol. Analysis of the data from both of these experiments plus results from electron paramagnetic resonance (EPR) measurements [10] leads to the phase diagram of Fig. 1. Sections of the phase diagram are in agreement with data obtained by several other techniques [29,32] as also shown in Fig. 1.

In Fig. 1 and in the text below we have used a notation for the phases which describes both the two-dimensional translational order and the chain-conformational order occurring in the phases: The gel phase is referred to as the solid-ordered (so) phase, where s, for solid, refers to the crystalline order and o, for ordered, to the average acyl-chain conformation. Similarly, the liquid-crystalline phase at low cholesterol concentration is denoted liquid-disordered (ld) whereas at high cholesterol concentration there is a liquid-ordered (lo) phase. The latter phase has not previously been characterized as such. The nomenclature is motivated by the fact that several independent experimental studies have demonstrated the simultaneous occurrence of a liquid-crystalline phase and high acyl-chain order. Micromechanical studies [32] show that the bilayer at high cholesterol concentrations behaves as a liquid with no surface shear rigidity but a greatly reduced

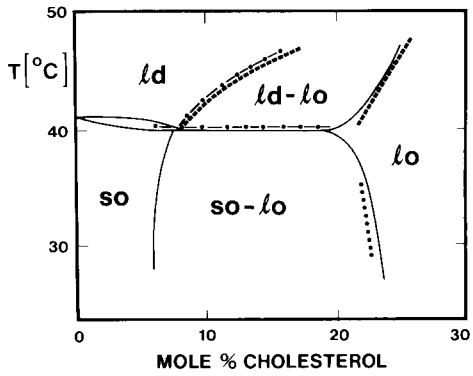

Fig. 1. Experimental phase diagram for the DPPC-cholesterol system as determined by NMR spectroscopy and differential scanning calorimetry (———) [16,17], EPR spectroscopy (- - - - - -) [10], freeze-fracture (· · · · · ·) [29], and micromechanics (· – · – ·) (Needham, D. and Evans, E., unpublished data). Note that the NMR and calorimetry studies were carried out on d_{62}-DPPC for which T_m is about 38 °C. The experimental data have been scaled accordingly to facilitate the comparison within a single figure.

membrane-area compressibility. The NMR measurements show that the deuterium order parameters approach the value 0.5 which is typical of an all-*trans* rotating chain [16,17]. The NMR spectra also show that diffusion is fast enough to average dipolar couplings even at very low temperatures [36]. A fast translational diffusion in this concentration range is also demonstrated by the photobleaching fluorescence studies [34].

The phase diagram of Fig. 1 shows several remarkable features which indicate that cholesterol interacts with phosphatidylcholine in an unusual way. Normally a solid is not as good a solvent as a liquid and the addition of an impurity results in a sizable freezing-point depression. The two-dimensional solid and liquid phosphatidylcholine phases do not initially respond in this way to cholesterol, which according to the phase diagram is almost as soluble in the solid ordered as the liquid disordered phase at low concentrations. However, the situation completely changes for high cholesterol concentrations, where the liquid phase of the bilayer is stable down to temperatures substantially lower than T_m. Therefore, in the high concentration regime, it is clear that cholesterol strongly favours the liquid phase over the solid phase in contrast to the low-concentration behaviour. There

is a third feature of the phase diagram which deserves attention. In a concentration range of about 10% to 20% cholesterol at temperatures above T_m, there are indications of a phase separation where two liquid phases of the bilayer coexist. In the following two sections, the high-cholesterol liquid phase will be described in terms of a liquid ordered phase.

These three remarkable features of the phase diagram of Fig. 1 indicate that there are very specific phosphatidylcholine-cholesterol interactions. In this paper we propose a thermodynamic model which describes the liquid phases of the bilayer on the level of regular solution theory (Bragg-Williams approximation) while the crystalline nature of the solid ordered phase is characterized phenomenologically. A related microscopic model for the phosphatidylcholine-cholesterol system is then presented and solved in the mean-field approximation. The microscopic model allows for an interpretation of the nature of the various phases.

2. Thermodynamic model

The simplest way to describe a non-ideal liquid mixture is to introduce an interaction term of the form $w_{AB}X_AX_B$ into the free energy of the mixture. Here w_{AB} is the interaction parameter and and X_A and X_B are composition variables. This approximation is the basis of regular solution theory [37] and corresponds to the Bragg-Williams approximation in the theory of lattice gases. It is also used in the Flory-Huggins theory of polymer solutions. Clearly the behaviour of phosphatidylcholine-cholesterol bilayers cannot be described by a model where an interaction term of this type is solely responsible for the deviation from ideality.

The extensive spectroscopic evidence that cholesterol causes a straightening of disordered acyl chains implies that the conformational nature of the acyl chains must be taken into account in the expression for the free energy. The acyl chains can take on a number of conformations and we divide them into two classes from which two average conformational states are constructed. The first state is the conformationally ordered (o) state, in which the chains are essentially straight with a low internal energy and a low degeneracy. The second state is the conformationally disordered (d) state, in which the chains have several rotameric defects (*gauche* bonds) and therefore a high internal energy and entropy (degeneracy). We now include this two-state model in the formalism and note that it is in line with previous microscopic models for phosphatidylcholine mono- and bilayers [38,39]. The remainder of this section is devoted to the construction of an expression for the free energy which allows for the determination of the phase behaviour of phosphatidylcholine-cholesterol bilayers in terms of separate labels for the two conformational states (o, d) of the acyl chains and the crystalline (s) and liquid (l) character of the phases.

In the framework of regular solution theory the binary phosphatidylcholine-cholesterol system acquires the characteristics of a ternary system because of the presence of the two conformational states of the phosphatidylcholine molecules. The difference is that, at a given temperature and binary composition, the relative amounts of the two conformational states are dependent intensive variables whose equilibrium values are determined by minimising the free energy. The free energy for the liquid lamellar system is written as follows for the combined model:

$$\begin{aligned} G(n_L, n_C, T) = {} & n_L\mu_L^\theta(l, T) + n_C\mu_C^\theta(l, T) \\ & + (n_L + n_C)[\tfrac{1}{2}w_{dd}p_d^2X_L^2 + w_{od}p_op_dX_L^2 \\ & + w_{Cd}p_dX_LX_C + w_{Co}p_oX_LX_C] \\ & + RT(n_L + n_C)[p_dX_L(S_o - S_d) \\ & + p_oX_L\ln(p_oX_L) + p_dX_L\ln(p_dX_L) \\ & + X_C\ln X_C] \end{aligned} \tag{1}$$

Here the first two terms represent the free energies of the phosphatidylcholine and cholesterol in their respective liquid (l) standard states. For PC we have chosen the system with only straight chains as the reference system. The first parentheses contain four binary interaction terms. The first term is a correction for the difference in interaction between the pure disordered state (d) and the reference state (o). The other three terms are the

pair interactions for a ternary system. The second parentheses contain the entropy contributions. Again the first term accounts for the difference between the d- and o-states of the PC while the other terms are due to an assumed ideal mixing. The relative populations of the two PC states are denoted by p_o and p_d and their sum is equal to unity. For given values of the variables n_L, n_C, and T and the parameters w_{dd}, w_{od}, w_{Cd}, w_{Co}, and $S_o - S_d$, the values of p_o and p_d are determined by minimizing the free energy i.e.

$$\frac{\partial G}{\partial p_o} = \frac{\partial G}{\partial p_d} = 0; \qquad p_o + p_d = 1 \tag{2}$$

The free energy expression in Eqn. 1 contains five parameters and one would therefore expect a rich thermodynamic behaviour. However, liquids are either miscible or immiscible and this feature can be described by simple regular solution theory. The model of Eqn. 1 allows for two new features. The position of the miscibility gap and the critical point can be strongly asymmetric with respect to the concentration of the two components. This was recently shown by one of us [40] for a chemically different system with a free energy expression similar to Eqn. 1. Furthermore, the cooperativity in the interactions gives a phase transition of pure PC bilayers.

Although there are five parameters in Eqn. 1 each one of them has physically reasonable values only within a certain range. The degeneracy of the d-state should lie in the range 10^3–10^5 [39]. The interaction parameter w_{dd} should approximately match this degeneracy so that the d-state is the dominant state at ambient temperatures. In addition, strong interactions should not a priori be expected between any two components i.e. pair interactions should be of the order of a few $k_B T$ if pure components are used for the reference state.

Fig. 2 shows a phase diagram which was calculated with a reasonable choice of parameters. The striking feature is the miscibility gap which at high temperatures terminates in a critical point, whereas at low temperatures the end-point is at a phase transition of the pure phosphatidylcholine system. With the choice of parameters used to obtain Fig. 2, cholesterol and PC in the o-state will form an ideal mixture. This explains the miscibility at low

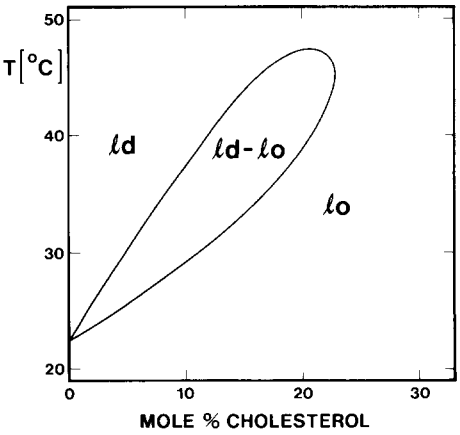

Fig. 2. Phase diagram for the thermodynamic model of the phosphatidylcholine-cholesterol system allowing for liquid phases only. The model parameters used are $w_{dd} = 33.93$ kJ/mol, $w_{od} = 22.31$ kJ/mol, $w_{Cd} = 22.31$ kJ/mol, $w_{Co} = 0$ kJ/mol, and $S_o - S_d = -R \ln 1000 = -57.43$ kJ/mol per K.

temperatures. Above the main phase transition, cholesterol tries to make the PC conformationally ordered (which it prefers) and this gives rise to the two-phase region with predominantly d-states in the PC-rich phase and predominantly o-states in the cholesterol-rich phase.

A more complete phase diagram requires the introduction of the solid conformationally ordered (so) phase. A description of this phase involves not only the local molecular conformations but also effects due to the long-range packing arrangements of the molecules. An analysis of the so-phase on the molecular level would therefore be equivalent to an approximate theory of melting. This is dealt with in the next section. In the present section, the solid phase is described phenomenologically whilst bearing in mind that the theory of solid solutions is considerably more difficult to formulate than a theory of liquid solutions. The free energy of the solid phosphatidylcholine phase may now be written in the following form:

$$G_s = n_L \mu_L^\theta(s, T) + n_C \mu_C^\theta(s, T) + RT(n_L + n_C)(X_L \ln X_L + X_C \ln X_C) \tag{3}$$

where we use the so-phase of pure PC as the standard state for both components. The interaction terms have been omitted under the tacit as-

sumption that the cholesterol content of the solid phase will always be sufficiently small so as to allow the entropy term to dominate the chemical potentials. The parameters in Eqn. 3 are the standard chemical potentials μ^{θ}. For the phosphatidylcholine, $\mu_L^{\theta}(s, T)$ determines the temperature T_m of the main phase transition while $\mu_C^{\theta}(s, T)$ is crucial for the phase behaviour at temperatures near T_m. Eqn. 3 describes the energy of an additional (solid) phase which can interfer with the phase equilibria of the liquid phases in Fig. 2. By minimising the free energy allowing for varying amounts of the different phases we arrive at the phase diagram of Fig. 3. This phase diagram has the salient features of the phase diagram obtained from experiment and we have therefore arrived at a preliminary interpretation of Fig. 1. A discussion of the results of the present section is presented in Section 4 together with those from the parallel microscopic model of the next section.

3. Microscopic model

Understanding the phase equilibria in the phosphatidylcholine-cholesterol system on the molecular level requires a microscopic model which minimally accounts for: (i) the main phase transition in pure phosphatidylcholine bilayers as it proceeds in terms of acyl-chain degrees of freedom as well as translational degrees of freedom, and (ii) the specific interactions between cholesterol and these two sets of phosphatidylcholine degrees of freedom. Recently, a model was proposed for pure PC monolayer and bilayer transitions which incorporates both chain melting and two-dimensional crystallization [41–43]. We have for the present purpose generalized this model to apply to a two-component system by adding specific microscopic phosphatidylcholine–cholesterol interactions.

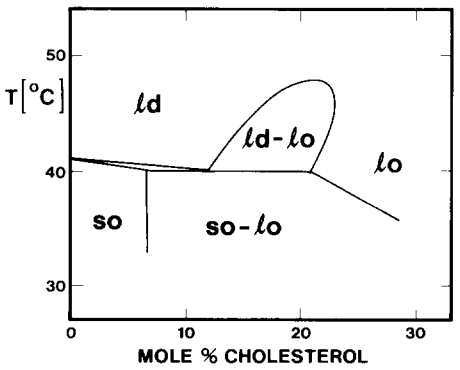

Fig. 3. Phase diagram for the thermodynamic model of the phosphatidylcholine-cholesterol system allowing for solid and liquid phases. The model parameters used are the same as in Fig. 2. $\mu_L^{\theta} = -1.3307$ kJ/mol and $\mu_C^{\theta} = 3.9322$ kJ/mol.

We first describe the microscopic model of the pure phosphatidylcholine system. Within this model, the PC bilayer is considered as two monolayers which are independent of each other. An internal lateral pressure is applied to the monolayer to model the forces required for bilayer stability. Each monolayer is represented by a triangular lattice model where each site is occupied by either a PC or a cholesterol molecule. We then adopt the two-state of Doniach [38] to describe the interactions between the PC molecules. The two-state model accounts for the conformational states of the acyl chains, the all-*trans* conformationally ordered state (o) and the highly excited, conformationally disordered state (d). The degeneracy of the o-state is unity and that of the d-state is of the order of 10^5. The interactions among the PC molecules are characterized by a strength parameter J_0 and they extend to nearest neighbours only. The two-state model has a first-order phase transition driven by the difference in internal entropy of the two chain states and the cooperativity arising from the nearest-neighbour interactions. The transition takes the system from a phase with conformationally ordered (o) chains to the conformationally disordered (d) phase. The interaction parameter values and the internal conformational energies of the two-state model are well-known from previous applications to pure PC bilayer melting [44]. Solidification (or crystallization) in terms of the translational degrees of freedom many in a very approximate way be accounted for by using the Potts model [42]. This model describes the fact that solid phosphatidylcholine domains in the conformationally ordered phase may be formed in a large number of crystal orientations in the two-dimensional plane of the lamellae. Within the Potts model, each PC molecule in the o-state is assigned a socalled Potts variable which is an integer between 1 and Q, where Q is a large number designating the different crystalline

orientations. When an acyl chain goes from the o-state to the d-state it looses its Potts variable, and visa versa, reflecting the fact that only orientationally ordered chains may enter a solid phase. The Potts model now assigns an energy J_P to the grain boundary which may be formed between two different solid domains, such that neighbouring o-state acyl chains interact with an energy $J_P > 0$ if they carry different Potts variables and zero otherwise. The Potts model leads to a first-order phase transition from a Potts-ordered (s) to a Potts-disordered (l) phase. The phase diagram in terms of temperature and J_P/J_o of this pure phosphatidylcholine bilayer model may be described as follows [41,43]: At large values of J_P/J_o, the transitions in terms of the two sets of variables occur simultaneously and they take the system directly from the low-temperature so-phase to the high-temperature ld-phase. At lower values of J_P/J_o, the two transitions decouple and give rise to an intermediate phase, the lo-phase.

The conceptually new feature is now the effect of cholesterol on the two sets of phosphatidylcholine degrees of freedom. Apart from specific interactions between the cholesterol molecule and the two different PC chain states favouring a straightening of the chains, we associate cholesterol with the capacity of a 'crystal-breaker': Whenever a PC molecule has cholesterol as a nearest neighbour, the Potts interaction between the PC molecule and other phosphatidylcholines are weakened. In this way we account quantitatively for cholesterol's disturbance of solid phases. It is anticipated that the effect of cholesterol's crystal-breaking property will be to reduce the effective value of J_P/J_o in a pure PC model and thus, at higher cholesterol concentrations, to promote the stability of the intermediate lo-phase.

The precise mathematical formulation of the complete model is rather involved and it is given in the Appendix. Due to the complexity of the microscopic model, we have restricted ourselves for the present to a derivation of its phase behavior within the mean-field approximation. The level of this approximation is the same as that of the thermodynamic model of Section 2. Even a mean-field calculation is far from trivial for our model and the details of the procedure are also presented in the Appendix.

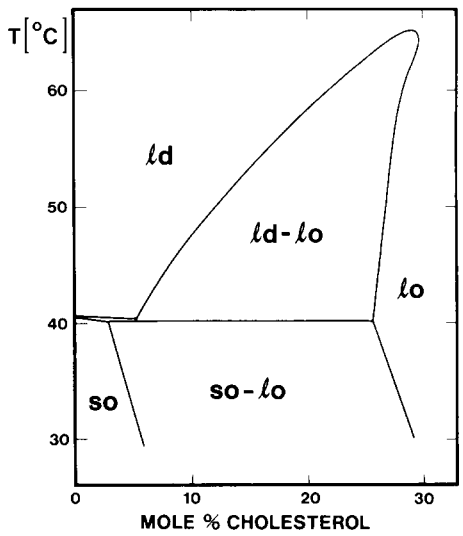

Fig. 4. Phase diagram for the microscopic model of the phosphatidylcholine-cholesterol system. The model parameters are pertinent for DPPC.

We then turn to a description of the results for the phase equilibria derived from the microscopic model. Firstly, by suppressing the effects of the Potts variables ($J_P = 0$ and $Q = 1$) we obtain a phase diagram similar to that in Fig. 2 with a miscibility gap between the lo- and ld-phases. Secondly, for the full model we arrive, for reasonable values of the model parameters (cf. Appendix), at phase diagrams of the type displayed in Fig. 4. It is noted that this diagram has precisely the same topology as the experimental diagram of Fig. 1 and the diagram in Fig. 3 calculated from the thermodynamic model. In terms of the variables of the microscopic model, one then immediately arrives at an interpretation of the various phases of the phosphatidylcholine-cholesterol system.

4. Discussion

In the previous two sections we have presented two parallel models, one thermodynamic and the other microscopic, for the purpose of generating phase diagrams for the phosphatidylcholine-cholesterol system. Before presenting our conclusions in the light of the numerical results, it is important to understand how the models relate to each other and to the experimental picture for the systems under investigation.

First of all, both models represent the conformational states of the acyl chains in terms of a two-state model. The low-energy or conformationally ordered (o) state in this model is taken to be equivalent to an essentially straight chain. This state then becomes the dominant state in both the so- and lo-phases. The measurement of the NMR order parameter [16,17] shows that more conformational disorder is induced by cholesterol in the so-phase. This effect is described by our theory in terms of an admixture of the o- and d-states in the lo-phase (see Appendix). In this case the models presented in this work are minimal models which possess the essentials for the understanding of the phase diagram of Fig. 1 and which can be regarded as foundations on which more refined models can be built. Some possible refinements are: (i) The inclusion of more conformational states and steric effects in the formalism [22,45]. (ii) The use of an appropriate theory of two-dimensional melting. The Potts description of the solid-liquid phase transition is only approximate and does not account for long-range order due to molecular tilt (orientational order) [41,43]. (iii) A description of the ripple phase of the pure PC bilayers and its modification by cholesterol. This awaits the construction of a satisfactory theory. In this context we note that Copeland and McConnell [29] have made extensive electron microscopy studies of the system in the concentration range where the phase diagram of Fig. 1 shows the so-lo phase separation with a solid in equilibrium with a cholesterol-rich phase. The conclusion of these studies is that the system shows a superstructure with alternating ribbons of solid and liquid regions. Neither the model studies nor the experimental NMR studies address this question of the possibility of a superstructure which is effectively a single-phase behaviour. In fact, all these studies are compatible with the occurrence of a superstructure.

The thermodynamic model of Section 2 is basically a phenomenological model. It is important to note that the microscopic model is also phenomenological in the sense that its parameters are not derived from first principles and that the Hamiltonian (see Appendix) operates on a two-dimensional lattice, whereas a phosphatidylcholine bilayer is a three-dimensional entity. However, the advantage of the microscopic model is that we are not limited to the Bragg-Williams or mean-field approximation used to analyse the phase diagrams but can use computer-simulation methods to study the effect of density fluctuations on the phase behaviour [44].

Previous theoretical investigations for phosphatidylcholine-cholesterol bilayers can be divided into (a) models which propose the formation of long-lived complexes [46,47] and (b) models which are based on pair-interactions in a dense two-dimensional system of PC molecules. The theory closest to our microscopic model is due to Pink et al. [22] who analyse data from their own Raman measurements for phosphatidylcholine-cholesterol systems. They use a lattice model with ten conformational states and show that their model is compatible with a miscibility gap between a phosphatidylcholine-rich and a cholesterol-rich phase. Our model is basically an extension of their model having the added feature that the nature of the solid and liquid phases is treated separately from the conformational degrees of freedom. This crucial feature enables us to understand the experimental phase diagram of Fig. 1. The other theories of type (b) include theories by Marčelja [48], Jänig [49], and O'Leary [50]. While these theories have considerably merit in their own right, none of them allows for phase separation.

5. Conclusions

The model calculations presented in this paper reproduce all three of the unusual features of the experimental phase diagram of the phosphatidylcholine-cholesterol system. Moreover, they make it possible to extract a qualitative explanation of the phase behaviour. The nearly-equal affinity of cholesterol for the pure solid and liquid phosphatidylcholine phases as demonstrated by the phase behaviour at low cholesterol contents is a remarkable consequence of two competing effects. Cholesterol disturbs the translational order in the solid phase and this gives an unfavourable free-energy contribution for solvation in the solid. However, probably due to the two-dimensional character of the system, the effect is smaller than in a corresponding three-dimensional system with

short-range crystalline order. This general effect is in the case of cholesterol nearly balanced by the specific character of the short-range phosphatidylcholine–cholesterol interaction which promotes conformationlly ordered phosphatidylcholine chains next to cholesterol. Thus, cholesterol effectively acts so as to decouple PC-chain conformational order from PC-chain crystalline order, but it is a coincidence of nature that this decoupling only affects the phase behaviour at higher cholesterol concentrations. However, at these higher concentrations the consequences of the decoupling are very dramatic and lead to a highly asymmetric phase diagram. At cholesterol concentrations above approximately 20 mol% the solid state is suppressed even at temperatures well below T_m. The reason for this is that the specific phosphatidylcholine–cholesterol interactions straighten the acyl chains of the PC in the liquid phase and, once this has been accomplished, the short-range phosphatidylcholine–cholesterol interactions are similar in the solid and the liquid. In that case, cholesterol clearly prefers the liquid phase.

The liquid-liquid phase separation with a critical point at relatively low cholesterol contents arises from the fact that cholesterol prefers a particular conformational state of the PC molecule. It is perhaps tempting to ascribe the asymmetry of the miscibility gap to an asymmetry in the cholesterol–phosphatidylcholine interactions as being caused by for example a 1 : 4 complex. The calculations of this paper show that it is unnecessary to invoke complications beyond binary additive interactions in order to account for the asymmetry, provided one introduces distinct conformational states. It is interesting to note that the model calculations apart from their explanatory role also have some predictive power in the sense that it is difficult to find a parameter set that produces only two of the characteristic features of the phase diagram without giving the third. This gives strong support for the idea that these features are basically generated by one common molecular mechanism.

Although we have throughout the paper used DPPC (dipalmitoylphosphatidylcholine) as the specific example of lecithin bilayers for which we have compared theoretical predictions with experimental measurements, Figs. 1–4, it should be noted that a similar phase behaviour is expected for other PC bilayers. In the case of DMPC (dimyristoylphosphatidylcholine), where some experimental information is available about part of the phase diagram [10,11,26,32], the data indeed suggest a phase diagram with a topology of the type of Fig. 1. Moreover, the two low-temperature phase lines bounding the so-lo coexistence region are not sensitive to the acyl chain length.

The phosphatidylcholine-cholesterol system has been extensively studied by a number of different experimental techniques and we briefly discuss the relation between the present model calculations and some of these studies. A key feature of the model is that cholesterol tends to promote conformationally ordered acyl chains. It has been conclusively demonstrated by NMR studies that this is indeed the case [14–17]. At high cholesterol contents and low temperatures, the system behaves like a liquid, albeit viscous, as shown by several experimental criteria [32,33] and it is fully consistent with the present models. It is sometimes stated, e.g. on the basis of fluorescence anisotropy measurements [3], that cholesterol makes phosphatidylcholine membranes less fluid. This is an imprecise statement since it neglects the fact that the PC bilayer is a highly anisotropic system in which fluidity has components of microviscosity or lateral mobility as well as of acyl chain conformational order [33,51]. In the liquid phase, cholesterol leads to an increase in the acyl-chain conformational order [14] but does not induce a concomitant decrease of molecular mobility [20,33].

Cholesterol is an important regulator of the physical properties of biological membranes and membrane function [1–3]. The range of cholesterol concentrations considered in the present paper is within physiological ranges for many membranes, e.g. the plasma membranes of erythrocytes. It is therefore interesting to point out that the phase behaviour of the phosphatidylcholine-cholesterol system has important bearings on membrane biology and that the unusual features of the phase diagram emphasise that cholesterol is a unique molecular species whose function in membranes is unlikely to be mimicked by other biochemical substances [2,52].

A. Appendix

A general description of the microscopic model is given in Section 3. This appendix is devoted to a description of the (i) energy function (the Hamiltonian) for this model and (ii) the details of the calculation of the phase diagram in Fig. 4. The Hamiltonian has already been described in detail in Refs. 39 and 43 for pure phosphatidylcholine systems in the absence of cholesterol.

The microscopic model is a lattice model defined on a triangular lattice. Each site 'i' of the lattice represents either an acyl chain or a cholesterol molecule. In the case of an acyl chain the site 'i' has two associated conformational states, the ordered (o) state and the disordered stage (d). For each o-state there are Q Potts states which give an approximate description of the crystalline nature of the so phase. The Hamiltonian can now be expressed in terms of the following occupation variables: $\mathscr{L}_{o,p}(i)$, $\mathscr{L}_d$ and $\mathscr{L}_{C,p}(i)$. $\mathscr{L}_{o,p}(i)=1$ if the chain on site 'i' is in the conformationally ordered state and the p-th Potts state ($p=1,\ldots,Q$) and $\mathscr{L}_{o,p}(i)=0$ otherwise. Similarly, $\mathscr{L}_d(i)$ and $\mathscr{L}_{C,p}(i)$ are the occupation variables for a conformationally disordered chain and a cholesterol molecule in the p-th Potts state ($p=1,\ldots,Q$). The sum of all the occupation variables at a lattice site must be unity, i.e.

$$\sum_{p=1}^{Q}[\mathscr{L}_{o,p}(i)+\mathscr{L}_{C,p}(i)]+\mathscr{L}_d(i)=1 \tag{4}$$

The total Hamiltonian for the phosphatidylcholine-cholesterol system is

$$\mathscr{H}=\mathscr{H}_0+\mathscr{H}_{int} \tag{5}$$

$\mathscr{H}_0$ is the Hamiltonian for the intra-molecular contribution to the energy from the acyl chains and the cholesterol molecules

$$\mathscr{H}_0=\sum_i\Big\{\sum_p[(\epsilon_o+\Pi A_o)\mathscr{L}_{o,p}(i)+\Pi A_C\mathscr{L}_{C,p}(i)] + (\epsilon_d+\Pi A_d)\mathscr{L}_d(i)\Big\} \tag{6}$$

ϵ_o and ϵ_d are conformational energies and A_o and A_d are the areas corresponding to the two conformational states. The cholesterol cross-sectional area is A_C and Π is the internal lateral pressure.

$\mathscr{H}_{int}$ represents the inter-molecular interactions and it has several contributions

$$\mathscr{H}_{int}=\mathscr{H}_{oo}+\mathscr{H}_{od}+\mathscr{H}_{dd}+\mathscr{H}_{oo}^{C}+\mathscr{H}_{oC}+\mathscr{H}_{dC}+\mathscr{H}_{CC} \tag{7}$$

The interaction energy $\mathscr{H}_{oo}$ between nearest neighbour acyl chains in the o-state is

$$\mathscr{H}_{oo}=-J_0/2\sum_{\langle i,j\rangle}\sum_{\langle p,p'\rangle}(1-J_P(1-\delta_{pp'}))\mathscr{L}_{o,p}(i)\mathscr{L}_{o,p'}(j) \tag{8}$$

J_0 is the van der Waals interaction between neighbouring chains. J_P is the strength of the Potts interaction with respect to J_0. The next terms in Eqn. 7 are

$$\mathscr{H}_{od}=-J_0/2\sum_{\langle i,j\rangle}\sum_p I_d(\mathscr{L}_{o,p}(i)\mathscr{L}_d(j)+\mathscr{L}_d(i)\mathscr{L}_{o,p}(j)) \tag{9}$$

and

$$\mathscr{H}_{dd}=-J_0/2\sum_{\langle ij\rangle}I_d^2\mathscr{L}_d(i)\mathscr{L}_d(j) \tag{10}$$

The terms in Eqns. 8–10 are the pure phosphatidylcholine contributions [43]. I_d and I_d^2 give the o-d and the d-d van der Waals interactions in units of J_0. To describe the effect of cholesterol on the PC system we introduce the occupation variable

$$\mathscr{L}_C(i)=\sum_p\mathscr{L}_{C,p}(i) \tag{11}$$

and the quantity

$$\mathscr{L}_c(i,j)=\prod_{k_i;k_i\neq j}^{z}(1-\mathscr{L}_C(k_i))\prod_{k_j;k_j\neq i}^{z}(1-\mathscr{L}_C(k_j)) \tag{12}$$

where k_i and k_j represent nearest neighbours of i and j. $\mathscr{L}_C(i,j)$ is thus zero if a cholesterol molecule is present in the neighbourhood of i or j. The presence of a cholesterol molecule will weaken the Potts interaction between the surrounding acyl

chains

$$\mathcal{H}_{oo}^{C} = -J_0/2 \sum_{\langle i,j\rangle} \sum_{\langle p,p'\rangle} J_P j_C'(1-\delta_{p,p'}) \times(1-\mathcal{L}_C(i,j))\mathcal{L}_{o,p}(i)\mathcal{L}_{o,p'}(j) \quad (13)$$

The remaining Potts interactions will be accounted for by the ordered phosphatidylcholine chain–cholesterol interaction term

$$\mathcal{H}_{oC} = -J_0/2 \sum_{\langle i,j\rangle} \sum_{\langle p,p'\rangle} (I_{oC} - J_P(1-j_C')(1-\delta_{p,p'})) \times(\mathcal{L}_{o,p}(i)\mathcal{L}_{C,p'}(j) + \mathcal{L}_{C,p'}(i)\mathcal{L}_{o,p}(j)) \quad (14)$$

I_{dC} and I_{oC} denote the van der Waals interaction between the acyl chains and the cholesterol in units of J_0. j_C' parametrizes the strength of cholesterol as a 'crystal-breaker'. The last terms in Eqn. 7 are

$$\mathcal{H}_{dC} = -J_0/2 \sum_{\langle i,j\rangle} \sum_{p} I_{dC}(\mathcal{L}_d(i)\mathcal{L}_{C,p}(j) + \mathcal{L}_{C,p}(i)\mathcal{L}_d(j)) \quad (15)$$

and

$$\mathcal{H}_{CC} = -J_0/2 \sum_{\langle i,j\rangle} \sum_{\langle p,p'\rangle} I_{CC}\mathcal{L}_{C,p}(i)\mathcal{L}_{C,p'}(j) \quad (16)$$

The phase behaviour of the model is governed by the free energy

$$F = \mathrm{Tr}(\rho\mathcal{H}) + k_B T \mathrm{Tr}(\rho \ln(\rho)) \quad (17)$$

where ρ is the equilibrium distribution function. In mean field theory ρ is approximated by a product probability

$$\rho = \prod_{i=1}^{N} \rho_i,\ \rho_i = \rho_1 \quad (18)$$

where ρ_1 is the single-site distribution function. The occupation variables $\mathcal{L}_{o,p}(i)$, $\mathcal{L}_d(i)$ and $\mathcal{L}_C(i)$ can then be replaced by their mean-field values $\langle\mathcal{L}_{o,p}\rangle$, $\langle\mathcal{L}_d\rangle$ and $\langle\mathcal{L}_{C,p}\rangle$ in $\mathrm{Tr}(\rho\mathcal{H})$. In our mean-field analysis of F we fix the value of $\langle\mathcal{L}_C\rangle$ and afterwards investigate the stability of $F(\langle\mathcal{L}_C\rangle)$. ρ_1 is obtained by minimizing F with respect to ρ_1 under the constraints $\mathrm{Tr}(\rho_1) = 1$ and $\mathrm{Tr}(\rho_1\mathcal{L}_C) = \langle\mathcal{L}_C\rangle$

$$\frac{\delta(F + \lambda\,\mathrm{Tr}(\rho_1) + \mu\,\mathrm{Tr}(\rho_1\mathcal{L}_C))}{\delta\rho_1} = 0 \quad (19)$$

The Lagrange multipliers λ and μ must be chosen so that the constraints are fulfilled. This equations now permit a self-consistent determination of $\langle\mathcal{L}_d\rangle$, $\langle\mathcal{L}_{o,p}\rangle$ and $\langle\mathcal{L}_{C,p}\rangle$; $p = 1,\ldots,Q$. $\langle\mathcal{L}_C\rangle$ is not the molar fraction of cholesterol in PC because each PC molecule occupies two sites in the triangular lattice. Therefore

$$\langle\mathcal{L}_C\rangle = \frac{x_C}{2-x_C} \quad (20)$$

The phase diagram is calculated by minimizing the function

$$F(x_C, x_1, x_2) = \frac{(x_2 - x_C)F(x_1) + (x_C - x_1)F(x_2)}{x_2 - x_1};$$
$$x_1 \le x_C,\ x_2 \ge x_C \quad (21)$$

with respect to x_1 and x_2 for fixed temperature. The values obtained for x_1 and x_2 determine the phase boundaries of a coexistence region if they are different from x_C.

The values of the model parameters associated with pure phosphatidylcholine bilayers are taken from previous theoretical studies of DPPC [39,43]. The main phase transition temperature (41 °C) is fixed by the values of J_0 and J_P. We choose $J_0 = 0.847 \cdot 10^{-3}$ erg and $J_P = 0.91$. An area of $A_C = 32$ Å^2 is ascribed to cholesterol [53]. The internal lateral pressure is chosen as $\Pi = 30$ dyn/cm [39]. The remaining parameters are I_{oC}, I_{dC} and I_{CC} and j_C'. The phase diagram shown in Fig. 4 was obtained for $I_{oC} = 0.84$, $I_{dC} = 0.28$, $I_{CC} = 0.12$, and $j_C' = 0.35$. The topology of the phase diagram is only weakly influenced by the value of I_{CC} in the low-cholesterol concentration region, so the chosen value of $I_{CC} = 0.12$ does not necessarily reflect the interaction between cholesterol molecules in a physical system.

Acknowledgements

This work was supported by the Danish Natural Science Research Council under grant J. No. 5.21.99.72., by NSERC of Canada, and by Le

FCAC du Quebec. Fruitful discussions with Erich Sackmann, David Needham, Alex Georgallas, and Jim Davies are gratefully acknowledged. One of us (M.J.Z.) wishes to thank Vera and Carl Johan Michaelsens Legat for funding a visiting professorship at The Technical University of Denmark and wishes to thank Dennis Chapman for a very stimulating discussion on the effects of cholesterol in lipid bilayers.

References

1 Demel, R.A. and De Kruyff, B. (1976) Biochim. Biophys. Acta 457, 109–132
2 Yeagle, P.L. (1985) Biochim. Biophys. Acta 822, 267–287
3 Presti, F.T. (1985) in Membrane Fluidity in Biology (Aloia, R.C. and Boggs, J.M., eds.), pp. 97–146, Academic Press, New York
4 Chapman, D., Williams, R.M. and Ladbrooke, B.D. (1967) Chem. Phys. Lipids 1, 445–475
5 Ladbrooke, B.D., Williams, R.M. and Chapman, D. (1968) Biochim. Biophys. Acta 150, 333–340
6 Mabrey, S., Mateo, P.L. and Sturtevant, J.M. (1978) Biochemistry 17, 2464–2468
7 Estep, T.N., Mountcastle, D.B., Biltonen, R.L. and Thompson, T.E. (1978) Biochemistry 17, 1984–1989
8 Imaizumi, S. and Hatta, I. (1984) J. Phys. Soc. Japan 53, 4476–4487
9 Melchior, D.L., Scavitto, F.J. and Steim, J.M. (1980) Biochemistry 19, 4828–4834
10 Recktenwald, D.J. and McConnell, H.M. (1981) Biochemistry 20, 4505–4510; Shimshick, E.J. and McConnel, H.M. (1973) Biochem. Biophys. Res. Commun. 53, 446–451
11 Kusumi, A., Subczynski, W.K., Pasenkiewicz-Gierula, M., Hyde, J.S. and Merkle, H. (1986) Biochim. Biophys. Acta 854, 307–317
12 Presti, F.T. and Chan, S.I. (1982) Biochemistry 21, 3821–3830
13 Presti, F.T., Pace, R.J. and Chan, S.I. (1982) Biochemistry 21, 3831–3835
14 Stockton, G.W. and Smith, I.C.P. (1976) Chem. Phys. Lipids 17, 251–263; Jacobs, R. and Oldfield, E.G. (1979) Biochemistry 18, 3280–3285
15 Dufourc, E.J., Parish, E.J., Chitrakorn, S. and Smith, I.C.P. (1984) Biochemistry 23, 6062–6071
16 Vist, M.R. (1984) Partial Phase Behaviour of Perdeuterated Dipalmitoyl Phosphatidyl Choline-Cholesterol Model Membranes. MSc. Thesis, University of Guelph, Ontario, Canada
17 Davis, J.H. (1986) in Proc. Int. School Phys. 'Enrico Fermi' on Medical Applications of NMR, Varenna, in the press
18 Alecio, M.R., Golan, D.E., Veatch, W.R. and Rando, R.R. (1982) Proc. Natl. Acad. Sci. USA 79, 5171–5174
19 Smutzer, G. and Yeagle, P.L. (1985) Biochim. Biophys. Acta 814, 274–280
20 Van Ginkel, G., Korstanje, L.J., Van Langen, H. and Levine, Y.K. (1986) Faraday Discuss. Chem. Soc. 81, 49–61
21 Lippert, J.L. and Peticolas, W.L. (1971) Proc. Natl. Acad. Sci. USA 68, 1572–1576
22 Pink, D.A., Green, T.J. and Chapman, D. (1981) Biochemistry 20, 6692–6698
23 Levin, I.W., Keihn, E. and Harris, W.C. (1985) Biochim. Biophys. Acta 820, 40–47
24 O'Leary, T.J. and Levin, I.W. (1986) Biochim. Biophys. Acta 854, 321–324
25 Rooney, M., Tamura-Lis, W., Lis, L.J., Yachnin, S., Kucuk, O. and Kauffman, J.W. (1986) Chem. Phys. Lipids 41, 81–92
26 Knoll, W., Schmidt, G., Ibel, K. and Sackmann, E. (1985) Biochemistry 24, 5240–5246
27 McIntosh, T.J. (1978) Biochim. Biophys. Acta 513, 43–58
28 Hui, S.W. and He, N.-B. (1983) Biochemistry 22, 1159–1164
29 Copeland, B.R. and McConnell, H.M. (1980) Biochim. Biophys. Acta 599, 95–109
30 Hicks, A., Dinda, M. and Singer, M. (1987) Biochim. Biophys. Acta 903, 177–185
31 Lentz, B.R., Barrow, D.A. and Hoechli, M. (1980) Biochemistry 19, 1943–1954
32 Evans, E. and Needham, D. (1986) Faraday Discuss. Chem. Soc. 81, 267–280
33 Lindblom, G., Johansson, L.B.A. and Arvidson, G. (1981) Biochemistry 20, 2204–2207
34 Rubenstein, J.L.R., Smith, B.A. and McConnell, H.M. (1979) Proc. Natl. Acad. Sci. USA 76, 15–18
35 Kawato, S., Kinosita, K. and Ikegami, A. (1978) Biochemistry 17, 5026–5031
36 Ulmius, J., Wennerström, H., Lindblom, G. and Arvidson, G. (1975) Biochim. Biophys. Acta 389, 197–202
37 Lee, A.G. (1977) Biochim. Biophys. Acta 472, 285–344
38 Doniach, S. (1978) J. Chem. Phys. 68, 4912–4916
39 Caille, A., Pink, D.A., de Verteuil, F. and Zuckermann, M.J. (1980) Can. J. Phys. 58, 581–611
40 Karlström, G. (1985) J. Phys. Chem. 89, 4962–4964
41 Mouritsen, O.G. and Zuckermann, M.J. (1987) Phys. Rev. Lett. 58, 389–392
42 Mouritsen, O.G. and Zuckermann, M.J. (1987) Chem. Phys. Lett. 135, 294–298
43 Zuckermann, M.J. and Mouritsen, O.G. (1987) Eur. Biophys. J. 15, 77–86
44 Mouritsen, O.G. and Zuckermann, M.J. (1985) Eur. Biophys. J. 12, 75–86
45 Georgallas, A. and Zuckermann, M.J. (1986) Eur. Biophys. J. 14, 53–61
46 Slater, G. and Caille, A. (1981) Phys. Lett. 86A, 256–258
47 Snyder, B. and Freire, E. (1980) Proc. Natl. Acad. Sci. USA 77, 4055–4059
48 Marčelja, S. (1976) Biochim. Biophys. Acta 455, 1–7
49 Jähnig, F. (1981) Biophys. J. 36, 329–345, 347–357
50 O'Leary, T. (1983) Biochim. Biophys. Acta 731, 47–53
51 Johansson, L.B.A. and Lindblom, G. (1980) Q. Rev. Biophys. 13, 63–118
52 Bloch, K.E. (1983) CRC Crit. Rev. Biochem. 14, 47–92
53 Engelman, D.M. and Rothman, J.E. (1972) J. Biol. Chem. 247, 3694–3697