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A SIMPLE THEORETICAL MODEL FOR THE EFFECTS OF CHOLESTEROL AND POLYPEPTIDES ON LIPID MEMBRANES

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A simple theoretical model for the effects of impurities on biomembranes is proposed. The model accounts for the cholesterol-induced decrease of membrane phase transition temperature, membrane condensation above the gel to liquid crystalline phase transition, and increase in lateral compressibility. The model also predicts that addition of molecules such as cholesterol and polypeptides to membranes results in unmasking of a continuous phase transition. This results in a second broad peak in the calorimetric curves for melting of lipid-cholesterol mixtures, and the appearance of a second melting transition in membranes modified by the incorporation of polypeptides. The theory assumes that the membrane may be adequately described by a kink model, and that impurities are randomly distributed in the membrane. The difference in size and shape of impurity molecules, compared to membrane lipids, results in a spatial disordering in the membrane which in turn causes increased chain disorder and membrane condensation, as well as a decrease in the cooperativity of melting. The second transition results from a second expansion of the condensed, partially disordered membrane, which takes place over a several degree temperature range. This transition, although unmasked by boundary effects of non-lipid molecules, does not correspond to melting of a boundary annulus or phase separation.

Introduction

The influence of cholesterol on the thermodynamic properties of biological membranes has been extensively investigated experimentally. These investigations have shown that cholesterol has at least three major effects on phospholipid membranes which may be biologically significant: decrease of membrane phase transition temperature [1], condensation of the membrane [2] and increased lateral compressibility [3]. These changes are accompanied by an apparent change in the phase transition from first order to continuous [4]. Calorimetric investigations have shown that for a wide range of cholesterol concentrations both a sharply peaked first-order component and a much broader second component are present. These peaks have been attributed by many experimentalists to lateral phase separation followed by melting first of free lipid and then of a lipid-cholesterol complex.

Polypeptides have membrane effects which are in many ways similar to those of cholesterol. Polypeptides may either increase [5] or decrease [6] the phase transition temperature, as well as induce a second transition at a temperature above that of the main transition. The main transition has generally been attributed to melting of bulk lipid, while the higher temperature transition has often been attributed to the melting of immobilized boundary lipid [7].

Only recently have potential mechanisms for these effects been subjected to theoretical scrutiny [8–17]. Three principle approaches (Landau theories [8–11], molecular field theories [12,13] and lattice theories [14–19]) have been employed in an attempt to understand the mechanisms of cholesterol and protein effects. All of the theories suffer from one or more limitations. The Landau theories, for example, require a free parameter which is in principle temperature-dependent to describe the effects of impurities on the phase transition, and do not account for the membrane-condensing effects of cholesterol. The lattice models of Pink et al. [14–17], which assume that rigid chains interact less strongly with cholesterol than with other rigid chains, do not describe the physics which underly this assumption, and achieve their excellent agreement with experiment in part as a result of two free (although temperature-independent) parameters. The one-chain molecular field models [12,13] share with the Landau theories the requirement of a temperature-dependent boundary order parameter, and also ignore the details of chain packing which are thought to be very important in describing the phase transition.

The recent work of Cornell et al. [18] on random arrays of cholesterol and lipid suggests another model of membrane-impurity interactions. These authors assumed that cholesterol is randomly distributed in arrays of membrane lipid and on that basis calculated the number of lipid-lipid, lipid-cholesterol and cholesterol-cholesterol contacts which would be expected for various cholesterol concentrations. From this information, and the assumption that the enthalpy of transition decreases linearly as the number of lipid-cholesterol contacts increases, they estimated the amount of cholesterol required to destroy the phase transition in dipalmitoylphosphatidylcholine (DPPC). This analysis was performed without reference to specific properties of any model or details of the phase transition. The results do not provide a detailed understanding of cholesterol-induced changes in membrane phase transition temperature or area. In addition, the model cannot account for differences in the effects of cholesterol on the transition in DPPC and dimyristoylphosphatidylcholine (DMPC). If a justification for the assumption that the number of lipid-cholesterol contacts determines the enthalpy of transition could be found, however, the results of this study would be very illuminating.

In the present work we use the random mixing assumption in conjunction with a molecular model for the phase transition in order to describe the major effects of cholesterol on biomembranes. The basic model which we consider is the 'kink model' proposed by Jackson [19]. On the basis of the random mixing assumption we may calculate the effects of impurities on the configurational free energy of the membrane, and thus on the phase transition. We show in the context of this model that it is the number of lipid-lipid contacts, which is almost inversely related to the number of lipid-cholesterol contacts, which determines the enthalpy of transition, thus providing some theoretical justification for the approach used by Cornell et al. [18].

The Jackson kink model

Jackson [19] has modeled the membrane as a slab in which phospholipid molecules can exist in all-*trans* states or can form either of two types of β-coupled *gauche* kinks. No other form of *gauche* rotation is allowed. The 'forcing kink', which may initiate a sequence of 'falling kinks', consists of a chain kink which forces the remainder of the chain to be pushed into close proximity to its neighbors, a state which is highly unfavorable energetically. The energy E_f required to form a forcing kink in the membrane is given by

$$E_f = (nw/(A - A_0)) + 2E_g \quad (1)$$

where n is the level of the membrane at which the kink occurs (starting at the tail), w is an adjustable parameter (0.7308 for a 14-carbon chain with a phase transition at 297 K), E_g is the energy of a *gauche* rotation (0.5 kcal/mol), A is the membrane area per chain and A_0 is the area per chain of a completely condensed membrane. The first term in this expression represents the interchain contribution to the energy of kink-sequence formation which results from pushing one chain into very close proximity to its neighbors (excluded volume effect), while the second is an intrachain contribution resulting from the energy difference between

trans and *gauche* conformers. The energy of a falling kink, which may be formed in the vacancy formed by either a forcing kink or another falling kink, is $2E_g$. The disorder/kink P is given by $p/2$, where p is the number of lipid chains surrounding the chain in question. Using the method of Lagrange multipliers, Jackson calculates the configurational free energy of the membrane as

$$U = -kTN \ln[1 + f(\epsilon - \epsilon^{m+1})/(1-\epsilon)] \quad (2)$$

where f is the probability of filling a vacancy, given by

$$f = P \exp(-2E_g/kT)/[1 + P \exp(-2E_g/kT)], \quad (3)$$

N is the number of chains in the membrane and

$$\epsilon = \exp(-w/[kT(A - A_0)])[1 + P \exp(-2E_g/kT)] \quad (4)$$

The membrane is held together by a semi-empirically determined potential of the form

$$U(A) = (m+2)[(-3760/A^{2.5}) + (12389000/A^{5.5})] + 160/A + 0.33(A - A_0) \quad (5)$$

This membrane model is easily understood and its thermodynamic properties are readily computed. Its advantages include a detailed accounting for excluded volume interactions, the dominant feature of the lipid bilayer phase transition. The kink model is unrealistic in ignoring totally other forms of *gauche* rotation; kinks, while probably the most numerous type of *gauche* rotation, particularly near the headgroup, cannot sensibly account for the total enthalpy of transition [20]. The phase-transition temperature is shifted artificially upward by a severe undercounting of states, and the nature of the membrane expansion does not give qualitatively correct Π-A curves. Nevertheless, the model seems to provide a reasonable accurate description of membrane changes at $\Pi = 0$, contains the essential physical features of the phase transition, and is easily adapted to consider the effects of cholesterol on spatial and chain order in the membrane.

The cholesterol model

We assume that the thermodynamics of the membrane is adequately described by the kink model above. We further assume that cholesterol is randomly distributed among membrane lipids as if both were circular discs of equal area. With these assumptions the number of lipid-lipid, lipid-cholesterol and cholesterol-cholesterol contacts can be estimated [24]. These assumptions should result in only minor quantitative errors which come from ignoring the fact that neither cholesterol or phospholipids are cylindrically symmetric. We assume that the disorder per kink P is given by

$$P = 0.5(1 + 5C_{11}/6) \quad (6)$$

where C_{11} is the average number of lipid-lipid contacts per lipid. the form of this equation attempts to account for the fact that in pure phospholipid membrane each chain has six nearest neighbors, one of which is always adjacent to another lipid chain. The average number of lipid and cholesterol molecules adjacent to each lipid is shown in Table I, together with the disorder/kink at various cholesterol concentrations. We see that cholesterol's first effect is to prevent the propagation of kinks and reduce the entropy of kinking.

Impurities may also modify bilayer properties by introducing free volume into the membrane because of irregular shapes and incomplete penetration into the bilayer. Irregular shapes promote the formation of vacancies at all levels, while

TABLE I

EFFECTS OF IMPURITIES ON LIPID COORDINATION NUMBER AND PARAMETERS FOR THE JACKSON MODEL

Impurity (lipid)	Mole fraction impurity	C_{11}	C_{1c}	P	w
Cholesterol (DMPC)	0.0	6.0	0.0	3.0	0.7308
	0.1	4.9	1.1	2.6	0.6170
	0.2	4.1	1.9	2.2	0.5290
Polypeptide (DPPC)	0.1	4.2	0.2	2.3	0.5600

incomplete penetration promotes the formation of vacancies only below the level of penetration. The effect of these vacancies is to allow the formation of 'sequence-initiating' falling kinks (which is not allowed in the unmodified Jackson model). We may alternatively view the 'chain initiating falling kink' as another form of forcing kink which requires a smaller than usual energy to form. While it is possible in principle to account for the energetic effects on a level by level basis, the result is more complicated than the nature of the basic model justifies. To simplify, we assume that the proportionality constant w is related to the number of lipid-cholesterol contacts at all levels such that

$$w = w_0 - ((w_0 - w_c)C_{lc}/C_{lcc}) \tag{7}$$

where w_0 is the proportionality constant for a pure lipid, w_c is the proportionality constant which results in apparent disappearance of the first-order phase transition at the correct cholesterol concentration (0.5290 for DMPC), C_{lc} is the average number of lipid-cholesterol contacts per lipid and C_{lcc} is the average number of lipid-cholesterol contacts per lipid at the cholesterol concentration at which the first-order phase transition disappears in analogy with the entropy of the falling kink. The parameter w_c is always substantially smaller than w_0, reflecting the fact that in the presence of cholesterol forcing kinks may be formed more easily than in its absence, since free volume is created by the cholesterol molecules. Thus we have simply described the effects of cholesterol on kink models of the bilayer. In principle, cholesterol might also modify the attractive forces of the membrane, but to included such an effect would require the introduction of another free parameter. We prefer to assume that the attractive potential has the same dependence on lipid area as in the absence of cholesterol, even though this leads to poorer quantitative agreement with experiment.

In Fig. 1 we show a plot of $G(A,T)$ vs. A for the Jackson model at various temperatures in the absence of cholesterol. The moderate 'hump' in the G-A curves results in a first order phase transition at $T = 297$ K, as the location of the free energy minimum changes abruptly from 40.6 Å^2/lipid to 45.0 Å^2/lipid. In addition there is a very small continuous melting transition which expands the membrane from 45.0 to 45.2 Å^2/lipid as the temperature is raised two degrees. In the presence of cholesterol, the first order phase transition is shifted to increasingly lower temperatures as the fraction of cholesterol is increased, with the 'hump' in the G-A curve, and hence the first-order transition, nearly disappearing at 20% cholesterol in DMPC (Fig. 2). The expansion which occurs in

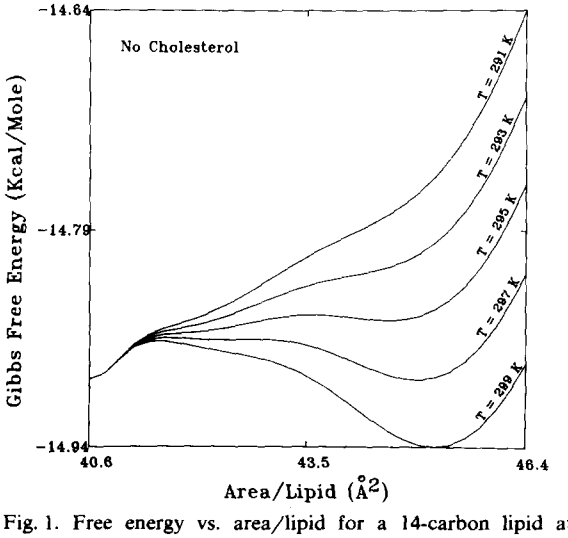

Fig. 1. Free energy vs. area/lipid for a 14-carbon lipid at various temperatures in the absence of cholesterol.

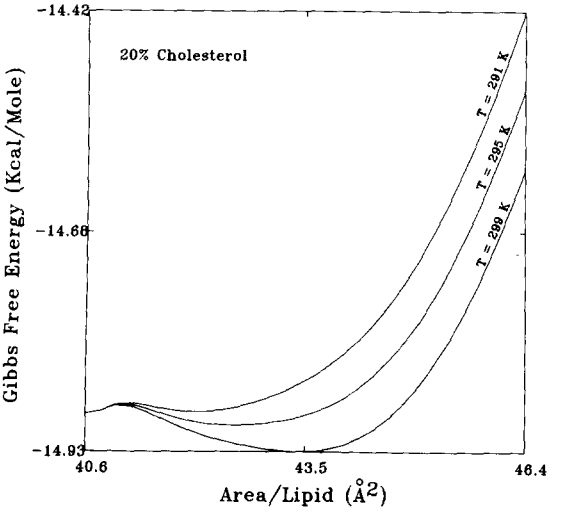

Fig. 2. Free energy vs. area/lipid for a 14-carbon lipid at various temperatures in the presence of 20% cholesterol. The first order component of the phase transition is almost gone, and a distinct continuous component is present.

the first order transition is greatly reduced in the presence of cholesterol, and the area/lipid is further reduced as cholesterol concentrations increase. The continuous melting becomes much more pronounced in relation to the first-order component in the presence of cholesterol, allowing a second distinct transition to appear in plots of heat capacity vs. temperature.

At high cholesterol concentrations one sees that the change in membrane free energy is very small as the membrane area is varied about its equilibrium value, especially when compared to the membrane without cholesterol; i.e. cholesterol increases the compressibility of the membrane. This may be associated with significant functional change, as discussed by Jahnig [10,11] and by O'Leary [21].

Fig. 3 shows the effects of cholesterol on membrane heat capacity curves. The height of the first order component is reduced with increasing concentrations, and becomes inapparent at approx. 20% cholesterol (although examination of $G(A,T)$ curves shows that an exceedingly weak first order component is still present). The peak is shifted to lower temperatures, in qualitative (though not quantitative) agreement with experiment. A second peak corresponding to the continuous melting transition appears at 10% cholesterol, although obvious broadening of the first-order component is seen by 5%. With increasing cholesterol, this second peak is also broadened and reduced in amplitude. Increasing cholesterol concentrations result in a decrease in the enthalpy of melting which is curved when plotted against cholesterol concentration, as predicted by Cornell et al. [18].

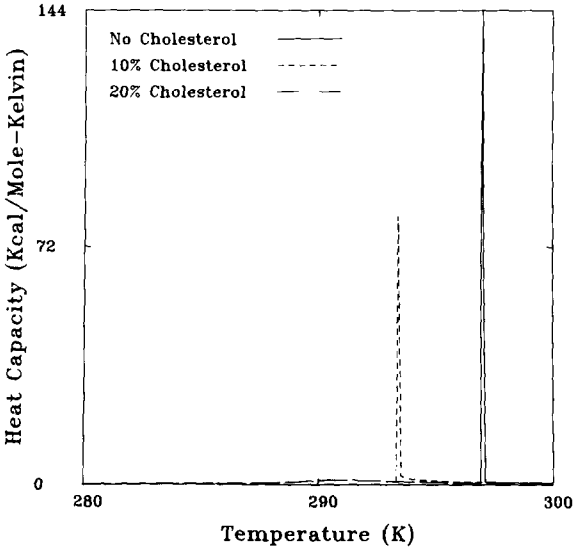

Fig. 3. Heat capacity vs. temperature for a 14-carbon lipid with various cholesterol concentrations. At 10% cholesterol a very small and broad continuous component is present in addition to the first-order component; at 20% cholesterol the first-order component is no longer seen.

While all of these results are qualitatively correct, there are important quantitative differences between the theoretical and the experimental results. In particular, the cholesterol-induced shift in the main phase transition temperature is too great. This is not surprising given the simplicity of the model, and it is possible that this defect could have been remedied by allowing the attractive portion of the free energy to depend on cholesterol concentration. There is no straightforward way to do this without resorting to curve fitting, however; this would do little to increase our understanding of the physics of lipid melting. Of greater importance is the observation of two components in the melting curve. This demonstrates that neither lateral phase separation nor immobilization of adjacent lipids is required for this type of behaviour to occur.

The polypeptide model

The basic model is the same as for cholesterol, but polypeptides are assumed to be twice the diameter of lipids. The numbers of lipid-lipid, lipid-polypeptide, and polypeptide-polypeptide contacts are taken from Dodd's work on experimental arrays [22]; thus the effects of packing irregularities are taken into consideration. We ignore possible effects of polypeptides on the form of the cohesive potential.

In Fig. 4 we see a dramatic possible effect of such proteins on the heat capacity curve for DPPC. A second transition appears which is distinct and sharp at relatively low polypeptide concentrations. Both transitions may be eliminated at high concentrations, as with cholesterol, and the second transition is not seen for all polypeptide concentrations and proportionality constants w. The appearance of a distinct second transition is consistent with experimental results obtained by Ra-

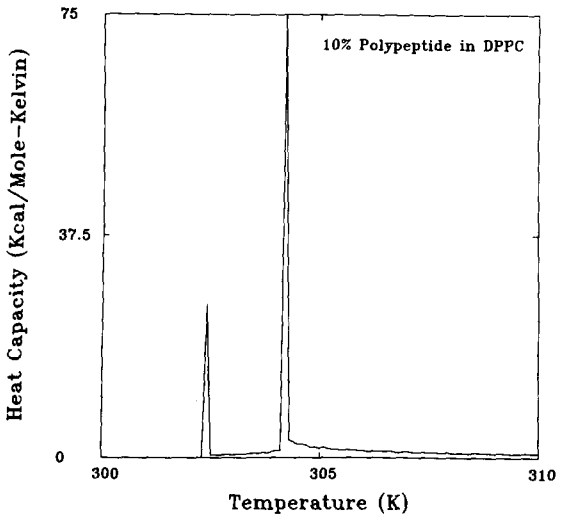

Fig. 4. Heat capacity vs. temperature for a 16-carbon lipid with 10% polypeptide impurity. The polypeptide has an area twice that of a lipid. Both a first-order and a continuous component are seen; the continuous component is apparent at lower concentrations than are required for cholesterol, and is sharper.

man spectroscopy, in which various polypeptides, such as mellitin, have been shown to induce such a transition at low concentrations. As in the case of cholesterol, this has been attributed by some experimentalists to melting of an immobilized boundary layer around the polypeptide; since this model does not formally distinguish such a boundary layer, we conclude that it is not necessary to invoke this concept to explain the experimental results. The model appears to favor increasing temperature separations between the two peaks as phospholipid chains become longer, in accord with experiment, although the presence of a free parameter which may depend on both the impurity and the phospholipid makes it impossible to test this quantitatively.

There is an important difference between the melting seen in the model for polypeptide impurities and that for cholesterol; because the cholesterol molecule has approximately the same surface projection as a lipid molecule, the melting transition in this case is analogous to the melting of a two-dimensional crystal with substitution impurities; in contrast, the differences in size between polypeptides and lipid makes melting of these mixtures more nearly analogous to the melting of a glass; the result is a more dramatic effect since even small concentrations of impurities can introduce rather significant distortions in the crystal order.

Discussion

An ideal model for the effects of cholesterol and polypeptide in lipid membranes would be exactly solvable in three dimensions and would incorporate the dominant steric interactions responsible for the phase transition. Such a model has been obtained for pure phospholipid monolayer in two dimensions only, and seems unobtainable for impure mixtures in three dimensions. For this reason we have restricted ourselves to a mean-field model based on the kink model of Jackson. In spite of potentially unrealistic features in the model, a qualitatively correct description of the static effects of cholesterol and polypeptides is achieved. Cholesterol-induced membrane stabilization, phase transition temperature shifts, reduction in melting enthalpy, and eventual disappearance of the first order phase transition are all accounted for, have correct signs and approximately correct magnitudes, in spite of the simple assumptions of the theory and inclusion of only a single arbitrary parameter beyond those incorporated in the Jackson kink model. It is possible in this model to separate the effects of free volume introduction from those of kink-sequence interruption in the model. Not surprisingly, the effect of free volume introduction in the absence of kink-sequence interruption is to depress the phase transition temperature and expand the membrane. Boundary effects which terminate kink sequences raise the phase transition temperature and condense the membrane by reducing the entropy of the phase transition, which depends directly on the number of lipid-lipid contacts. Only by considering both forms of interaction is a correct qualitative description of cholesterol effects obtained, The boundary effects predicted by this model are similar to those suggested by other workers, but are less restrictive since they are predetermined by the selection of a single temperature-independent parameter; in essence, the boundary order parameter is allowed to be temperature-dependent.

While predictions that the calorimetrically ob-

served first order transition will disappear at sufficiently high impurity concentrations have been made by others, the appearance of a second transition is seen only in this and in the lattice theories. This suggests that the simple expedient of defining a temperature independent boundary order parameter is inadequate to describe impurity effects. Unlike the Landau theories in which symmetry changes cause a real change in the transition from first order to continuous, a very small first order component is always present in the transition of the present theory; it becomes too small to observe, however, in the presence of cholesterol.

This model suggests a general approach to interpreting experimental results on phospholipid melting. In the absence of impurities, the main transition consists of a first order melting transition which is superimposed on a continous expansion. As impurities are added to the membrane, the temperatures of the two transitions become different, and the enthalpy of the second transition becomes larger in comparison to that of the first. As impurity concentrations are further increased, both peaks become smaller and eventually disappear. Neither lateral phase separation nor immobilization of boundary lipid is required to explain this phenomenon, nor are they excluded by its occurrence. More exacting experiments are required to reliably select among these possibilities.

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