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ELASTIC RESPONSE OF BILAYERS WITH INTRINSIC PROTEINS

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Intrinsic membrane proteins affect the ordering of neighbouring lipid chains. We have used a model of protein-lipid interactions in bilayers proposed by Owicki et al. (Owicki, J.C., Springgate, M.W. and McConnell, H.M. (1978) Proc. Natl. Acad. Sci. U.S.A. 75, 1616–1619) to show that near the lipid phase transition this effect may significantly increase the magnitude of a membrane's lateral compressibility (or correspondingly, decrease the magnitude of the membrane's elastic moduli).

Introduction

A physical property of bilayer membranes which is of considerable interest is the elastic response to an external or an internal stress. It is believed that higher lateral compressibility of the membrane is responsible for the increase in passive permeability near the lipid phase transition [1–5]. The value of lateral compressibility may also affect the activity of intrinsic membrane proteins [6]. The form and the magnitude of curvature elastic energy is expected to control some of the features of the cellular shape (e.g. in erythrocytes [7]) or function (e.g. pinocytosis or reversible stacking of thylakoid membranes).

It is now known from many studies that intrinsic membrane proteins affect the physical state of neighbouring lipid chains. The protein molecule acts as an external field, imposing a certain alteration in motion and order on nearby lipid chains. These chains therefore do not go through a well defined melting transition, but rather change their order gradually with temperature.

The lateral compressibility of bilayer membranes (denoted by κ) is one of the physical properties strongly affected by the ordering of lipid chains. From a general consideration of the statistical mechanics of phase transitions (and in particular the statistical mechanics of small [8] or inhomogeneous [9] systems) one would expect that κ is also affected by the presence of intrinsic proteins. The discontinuity in area per molecule (which produces the singularity in κ) at the phase transition of an homogeneous bilayer may be reduced, or disappear entirely. In that case an increase in κ would be observed over a wider temperature range. Because of the intrinsic interest in lateral compressibility of membranes mentioned earlier, and because the appropriate experimental measurements are very difficult, we have undertaken the present theoretical study of the intrinsic protein effect on κ.

Compressibility of single lipid bilayers with intrinsic proteins

In principle, any of the existing models [10–13] of bilayer membranes with intrinsic proteins could be used to obtain qualitative information on the change of bilayer lateral compressibility with the

introduction of intrinsic proteins. However, some of the models are rather complex, which makes the application difficult. For example, with the first and quantitatively most accurate model [10], a large increase in computational accuracy would be required to obtain information on k. We have therefore selected the model of Owicki et al. [12], based on the Landau-de Gennes expansion of the free energy [14], which has the advantage of relative simplicity while still retaining the essential physics of the problem. Jähnig [26] has recently published a calculation of the compressibility of lipid-protein membranes using a more complicated model, and arriving at a similar conclusion.

Owicki et al. define an order parameter u by

$$u=(a_F-a)/(a_F-a_S) \tag{1}$$

where a is the interfacial area per molecule and the subscripts F and S denote values in the fluid and solid phases at the transition temperature T_0. (Thus defined the order changes from zero to one at the phase transition and is less than zero at higher temperatures and greater than one at lower temperatures. This differs from the usual definition of the order parameter corresponding to that measured by magnetic resonance experiments, but the two ought to be strongly correlated).

In Landau-de Gennes' theory the (time averaged) free energy per molecule is expanded in terms of the order thus:

$$g(u)=-\Pi(a_F-a_S)u+\frac{1}{2}A(T-T^*)u^2-\frac{1}{3}Bu^3+\frac{1}{4}Cu^4 \tag{2}$$

where Π is the lateral pressure and the parameters T^*, A, B and C may be determined from comparison with the data. An elastic energy which is quadratic in $\nabla u(r)$ is added to take into account spatial variation in u.

The order of lipid molecules as a function of radial displacement from a protein is that function $u(r)$ which minimizes the free energy of the system subject to suitable boundary conditions. Owicki et al. [12] solve this boundary value problem by either a numerical integration of the associated Euler-Lagrange equation or by selecting a trial function $u(r)$ and adjusting its parameters to obtain the lowest possible free energy which is consistent with such a function. They find that, for a system in which an infinite, homogeneous bilayer is perturbed by one intrinsic protein molecule, an excellent approximation to the exact radial variation of u is

$$u(r)=u_B+(u_1-u_B)\exp[-(r-r_1)/\lambda] \tag{3}$$

where λ is a characteristic length to be determined, r_1 is the radius of the protein (assumed cylindrical) and u_1 and u_B are, respectively, the values of u at $r=r_1$ and $r\to\infty$ (i.e. the order parameter of the bulk lipids). r, r_1 and λ are expressed in reduced units.

Strictly, this model may only accommodate one protein molecule. To model a bilayer incorporating many intrinsic proteins we set the protein molecules on a hexagonal lattice. Next we invoke the Wigner-Seitz approximation [15] and replace each hexagonal cell by a circle with the same area. The trial function $u(r)$ must satisfy the boundary conditions:

$$u(r)=u_1 \quad at \quad r=r_1,$$

and

$$\frac{\partial u(r)}{\partial r}=0 \quad at \quad r=r_2$$

where r_2 is the radius of the Wigner-Seitz cell. Thus a convenient trial function is:

$$u(r)=u_2+(u_1-u_2)\exp[-(r-r_1)/\lambda]+(u_1-u_2)\exp[-(2r_2-r_1-r)/\lambda] \tag{4}$$

where u_2 is the value of the order parameter at $r=r_2$ ($u_2=u_B$ for large r_2).

The lateral compressibility is defined as

$$\kappa=-\frac{1}{A}\cdot\frac{\partial A}{\partial \Pi}\bigg|_T \tag{5}$$

where $A=\pi(r_2^2-r_1^2)$ is the area of the Wigner-Seitz cell. To calculate κ we put r_1 constant but $r_2=r_2(T,\Pi,N)$ where N is the ratio of the number of lipid molecules to the number of protein molecules (i.e. the number on both sides of bilayer in each Wigner-Seitz cell). To determine r_2 and hence

calculate $\left.\frac{\partial A}{\partial \Pi}\right|_T$, a fixed value of N was chosen where:

$$\frac{N}{2} = \int_{r_1}^{r_2} \frac{2\pi r \cdot dr}{a(r)} \tag{6}$$

and $a(r) = a[u(r)]$ is the interfacial area per lipid at given T and Π as defined by Eqn. 1.

What is an appropriate value for the boundary value u_1? Elsewhere [6] we have argued that the hydrophobic surface of a protein will in general be less regular than the surface of a crystalline array of solid lipids and so the adjacent lipids will be less ordered than are solid lipids, but that the motion of fluid lipids near a protein will be restricted by the presence of its hydrophobic surface which we presumed more rigid than liquid alkane chains. Kang et al. [23] explain their nuclear magnetic resonance results by proposing that the boundary layer of lipids have a larger amplitude of chain motion than do fluid lipids, but that the frequency of this motion is rather lower. The combination of a geometrical disordering and a restriction of motion imposed by proteins on fluid lipids [16] will affect the area they occupy in the membrane (and thus the order parameter u) but there are no data on this effect.

In these calculations we have imposed the boundary condition $u_1 = 0.75$. For large lipid:protein ratios, this value produces a mean value of u equal to 0.50 in the boundary layer ($r_1 < r \lesssim r_1 +$ 0.7 nm) when the bulk lipids are fluid. This choice is arbitrary, but its effect should qualitatively resemble that of the interactions described above, and by varying this value we have determined that the results are not critically dependent on this choice. The parameters of Eqn. 2 are determined from the properties of dipalmitoylphosphatidylcholine (DPPC) and the zero of Π is chosen so that the transition temperature of the (bulk) lipid is 279 K at $\Pi = 0$, i.e. to correspond to a bilayer under negligible tension or compression. (Variations in Π, and the relation between lateral pressures in membranes and bilayers are discussed by Gruen and Wolfe [25]. Under physiological conditions, bulk variations in Π are only of the order of 10 mM $\cdot$ m^{-1}, which variations have only a small effect on κ [6,21].)

The change in $\kappa(T)$ occasioned by the introduction of intrinsic proteins may be seen in Fig. 1. In this figure are presented $\kappa(T)$ at $\Pi = 0$ for a pure lipid bilayer and for a bilayer lipid membrane with intrinsic proteins and protein lipid ratio of approx. 600 (i.e. $N = 600$). The protein has a radius (r_1) of 2 nm (thus the ratio of the area of a protein in the plane of the membrane to that of a lipid is about 25). A very similar anomaly is obtained for $\kappa(\Pi)$ curves at various temperatures. However, at temperatures above the critical temperature or at pressures above the critical pressure the anomaly is smaller and has no discontinuity.

Very interesting results are obtained when the relative change in κ due to intrinsic proteins is examined as a function of the lipid/protein ratio N. Fig. 2 shows such results at $\Pi = 0$ and a range of temperatures above and below the pure lipid phase transition temperature. The effect is greatest for $N \approx 110$.

The reason for this behaviour can be understood with reference to Fig. 3, which shows the radial profile of the order parameter across the Wigner-Seitz cell. At large values of N, u is close to its bulk value throughout most of the cell, and κ is also close to its bulk value. For N very small, the remaining lipids are practically immobilized (at an appropriate u value) by proteins and cannot

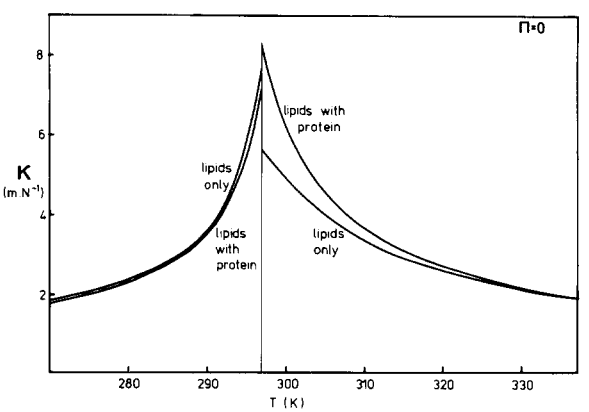

Fig. 1. The calculated lateral compressibility (as a function of temperature) of a bilayer of pure lipid compared with that of a lipid bilayer with intrinsic proteins (lipid-protein ratio 600 : 1). Parameter values corresponding to dipalmitoylphosphatidylcholine were used in the computation. The effect on κ is greater above than below T_2 because of the choice of boundary value ($a_1 = 0.75$) discussed earlier. This means that the proteins have a greater effect on the order of fluid lipids than they do on solid lipids.

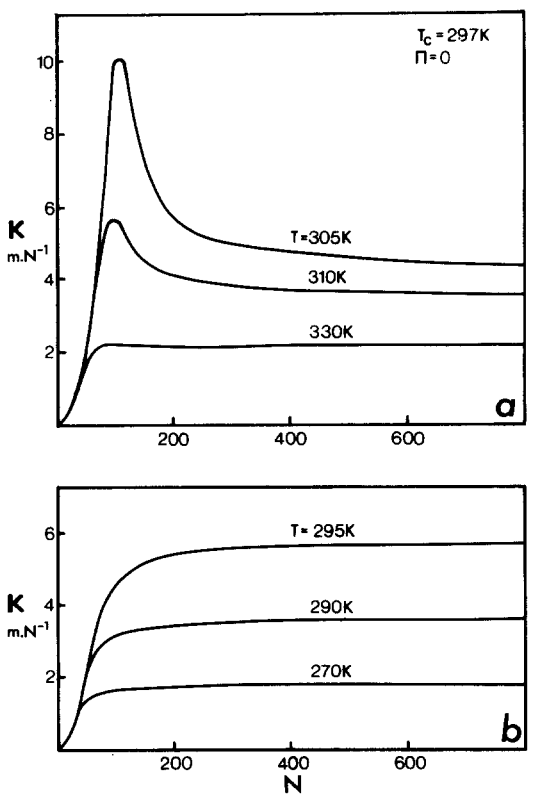

Fig. 2. The lateral compressibility as function of lipid-protein ratio (N) at different temperatures for a bilayer with intrinsic proteins. (a) Temperatures above the transition temperature at zero pressure (T_0) and (b) temperatures below T_0. (For temperatures very close to T_c computational difficulties render the calculations inaccurate.)

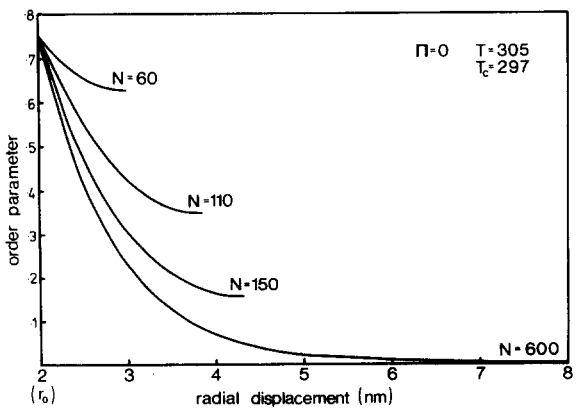

Fig. 3. Theoretical radial profiles at constant T and II of the order parameter of lipids about proteins for different sized Wigner-Seitz cells.

strongly respond to an elastic stress. At intermediate value of N however, the equilibrium value of the order of many of the lipid molecules is in the unstable region between the solid order and the fluid order. Thus their order is easily changed by small elastic stresses and this leads to the large values of κ.

It should be noted that all the results were obtained with the assumption of maximal separation between protein molecules. For small lipid:protein ratios the system may show partial or total aggregation of proteins, particularly if there is no electrostatic repulsion between individual protein molecules. In that case, the anomaly in κ would be greatly reduced.

Discussion

The results presented here show that under favourable circumstances (i.e. near the lipid phase transition, and with an appropriate lipid:protein ratio - both of which circumstances may obtain in biological membranes) lateral compressibility of the interior of lipid bilayers is significantly enhanced by incorporation of intrinsic protein molecules. The corresponding elastic modulus [17,18] describing the resistance of the membrane to lateral stress depends on contributions from both the lipid chain region and the lipid polar head region *. This elastic modulus will be significantly decreased under those conditions which produce a high lateral compressibility of the bilayer interior.

The bending of a membrane requires expansion and compression of the lipid chain region in the respective halves of the bilayer. Bending elastic moduli [17,18] will therefore reflect the presence of intrinsic proteins in a way qualitatively similar to the alteration of the stretching elastic modulus.

When considering the effect of intrinsic proteins on the compressibility of membranes formed from more than one species of lipid, it is necessary to consider not only the broadening of the temperature range of high κ induced by lateral phase separations among the lipids, but also the possible

* Under some circumstances the elastic response may consist of a more complex rearrangement of lipid packing, e.g. a collective tilt caused by changes in pH of the aqueous phase [10].

effect of proteins on the lipid composition in the vicinity of a protein. Contrary predictions [6,12,21] have been made for the composition. Some authors [12] expect that for rather rigid proteins in mixtures of lipids with differing hydrocarbon chains, the lipid species with the higher melting point would be preferentially adsorbed onto the protein. This effect may be quantified by computing the change in the Gibbs free energy of a lipid molecule as it is shifted from the bulk to the neighbourhood of a protein, using the model of Ref. 10. For several different values of the order parameter of the lipid-protein interface, it was found [6] that, although the changes in internal energy and entropy are large, their contributions to the free energy cancel almost exactly, and the change in free energy per hydrocarbon is less than one tenth of the thermal energy (κT). Thus, provided that specific geometric or packing effects do not influence lipid segregation [20], the protein does not disturb the homogeneity of the bilayer in which it is inserted. (Where lipids are covalently or electrostatically bound to a protein, then this protein-lipid complex replaces 'protein' in the foregoing argument, and homogeneity begins at the second neighbours.) The fluorescence quenching experiments of Caffrey and Feigenson [22] indicate that, for a large range of hydrocarbon chains, Ca^{2+}-ATPase does not perturb the homogeneity of bilayers comprising mixtures of phosphatidylcholine with different chains.

From the above argument it follows that outside the coexistence region, binary lipid mixtures with intrinsic proteins will behave similarly to single lipid phase, and the results of the previous section could be applied. Within the coexistence region, the very high lateral compressibility of the lipids [5] overwhelms any anomaly due to intrinsic proteins.

The possible physiological significance of the anomalies in the physical properties of membranes in or near the phase coexistence region is a time-honoured theme for speculation. An interesting idea formulated by Linden et al. [2] associates high lateral compressibility in the phase separation range of temperatures with enhanced transport across the membrane. Further, any reaction which involves geometric deformation of a membrane or membrane-bound enzyme will have a reaction energy which depends on the membrane compressibility [6,20]. The nature of our calculations allows us only to calculate the 'bulk' area elastic modulus, i.e. the quantity that is measured in a macroscopic determination of that modulus, such as that of Kwok and Evans [29]. The dynamic compressibility of small region of the membrane (a few tens of molecules, say) is the quantity which would affect biochemical reactions at that site [6] and this quantity could vary from place to place. We expect, however, that this dynamic compressibility would have a similar dependence on local membrane composition. The anomolous biochemical and physiological properties ascribed to lipid-protein membranes in the lipid phase coexistence region would therefore be expected to appear at temperatures somewhat above the range of the phase coexistence.

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