

MATRESS MODEL OF LIPID-PROTEIN INTERACTIONS IN MEMBRANES

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ABSTRACT A thermodynamic model is proposed for describing phase diagrams of mixtures of lipid bilayers and amphiphilic proteins or polypeptides in water solution. The basic geometrical variables of the model are the thickness of the hydrophobic region of the lipid bilayer and the length of the hydrophobic region of the proteins. The model incorporates the elastic properties of the lipid bilayer and the proteins, as well as indirect and direct lipid-protein interactions expressed in terms of the geometrical variables. The concept of mismatch of the hydrophobic regions of the lipids and proteins is an important ingredient of the model. The general phase behavior is calculated using simple real solution theory. The phase behavior turns out to be quite rich and is used to discuss previous experiments on planar aggregations of proteins in phospholipid bilayers and to propose a systematic study of synthetic amphiphilic polypeptides in bilayers of different thicknesses. The model is used to interpret the influence of the lipid-protein interaction on calorimetric measurements and on local orientational order as determined by deuterium nuclear magnetic resonance.

INTRODUCTION

A substantial fraction of biological activity takes place in association with membranes. Many of the proteins that mediate membrane-associated biological activity span the entire thickness of the membrane, which usually takes the form of a bilayer composed of amphiphilic phospholipid molecules. The systematic investigation of the three-dimensional structures of such transmembrane or integral membrane proteins has progressed more slowly than that of proteins, which function in an aqueous environment, primarily because the high resolution determination of molecular structure using diffraction methods requires good three-dimensional crystals and such crystals have not yet been produced systematically for integral membrane proteins (Henderson, 1980). Nevertheless, some important insight into integral membrane protein structures has resulted from low resolution structural studies of bacteriorhodopsin, a protein that spontaneously forms two-dimensional crystals in its natural membrane (Stoeckenius, 1980; Henderson and Unwin, 1975).

It now seems likely that the three-dimensional structure of most integral membrane proteins consists of α -helices connected by segments having several amino acids, each α -helix having predominantly hydrophobic residues and spanning the hydrophobic region of the phospholipid bilayer (Henderson, 1981). Thus, the building blocks of

integral membrane proteins can be taken as α -helices whose organization into a specific three-dimensional structure is governed partly by the interaction energy associated with the matching of the hydrophilic and hydrophobic portions of the protein with those of its lipid environment, and partly by the tendency towards self-association of small hydrophilic regions of the predominantly hydrophobic portions of different α -helices within one protein. These same interactions may also mediate indirect protein-protein interactions and thus affect the lateral distribution of proteins to give rise to biologically important differentiated regions within the plane of a single membrane. It is known that the presence of proteins in lipid bilayers influences the main gel-fluid (liquid crystalline) phase transition that takes place in the lipid matrix, usually at temperatures below physiological temperatures (Quinn and Chapman, 1980), since this influence is sometimes accompanied by protein aggregation (see, e.g., Bienvenue et al., 1982).

Any attempt to understand physiological functions of membrane-associated proteins must come to grips, as precisely and quantitatively as possible, with the mechanism by which the physical state and bulk phase behavior of the lipid matrix may modulate the activity of membrane-bound proteins. It is important to approach this problem independently from the structural (physical) side and the functional (physiological) side. In this paper, we

shall be concerned with the former approach and propose a theoretical model, the "mattress" model, which will be useful for calculating the phase behavior of wet lipid bilayers with proteinlike, imbedded, transmembrane, amphiphilic macromolecules. This model will be shown to constitute a convenient framework within which various aspects of lipid-protein interactions can be rationalized simply and easily.

The development of a theory to treat a complex system such as a mixture of phospholipid and protein molecules cannot make progress without a parallel set of clear-cut, quantitative experiments to test ideas as they arise. In view of the small number of integral membrane proteins whose structure is known, such an experimental program is very difficult to envisage at the present time. Our theory has been motivated by the recent development of a model membrane suitable for the systematic investigation of interactions between lipids and the type of polypeptide molecules in integral membrane proteins (Davis et al., 1982). This model membrane consists of a phospholipid bilayer containing a given concentration of synthetic, amphiphilic, transmembrane polypeptide molecules with a predetermined (and variable) number of hydrophobic residues and a smaller, fixed number of hydrophilic residues at each end. By varying the number of hydrophobic residues and/or the acyl chain lengths of the phospholipid molecules, the mismatch between their hydrophobic regions can be varied. We shall show in this paper that the mattress model reveals an impressive range of possible experimental consequences of the single aspect (hydrophobic mismatch) of lipid-peptide interactions considered here. Variations in the mismatch can cause inhomogeneities in the lateral distribution of peptide molecules and modify the temperature range of co-existence of lipid phases.

We wish to emphasize that the mattress model is worth studying in detail even in the event that mismatch does not exist *in vivo*; i.e., the properties of bacteriorhodopsin in this regard are general for all integral membrane proteins. Just as careful study of the temperature dependence of the properties of biological systems is essential to the understanding of their behavior at a given operating temperature, study of the properties of lipid bilayers as a function of the mismatch of lipids and proteins is required to understand the static and dynamic properties of matched lipids and proteins. An interesting demonstration of the influence of mismatch of lipids and proteins on a dynamical property of proteins has recently been presented (Kusumi and Hyde, 1982; see especially Fig. 5).

A qualitative description of the mattress model and how it is related to previous theoretical work on lipid-protein interactions is given in the next section. This is followed by a quantitative formulation of the model and its treatment using thermodynamics. The general phase behavior of a lipid bilayer containing proteins is described. The problems of determining the model parameters of the specific systems are then discussed in relation to applications of the

theory to a multilamellar lipid-polypeptide dispersion and the aggregation of proteins in lipid bilayers. In the following section, the mattress model is used to interpret previous experiments on the segregation of proteins in lipid bilayers, on the influence of the lipid-protein interaction on calorimetric measurements, and on the local orientational order as determined by deuterium nuclear magnetic resonance. The concluding section of the paper includes proposals for further experiments on lipid-protein interactions in the light of insight provided by the mattress model.

THE MATTRESS MODEL AND ITS RELATION TO PREVIOUS WORK ON THE LIPID-PROTEIN INTERACTION

We do not attempt to review in detail here the variety of experimental studies that have stimulated recent theoretical work on lipid-protein interactions (see, however, the reviews by Caillé et al., 1980; Seelig and Seelig, 1980; Quinn and Chapman, 1980). Clearly the strategy and physical intuition adopted by both experimentalists and theoreticians in approaching the problem of lipid-protein interactions has been strongly influenced by the success of mean field theories in relating local orientational order as a function of depth in phospholipid bilayer model membranes to the thermodynamic properties of the gel and liquid crystalline phases. Much experimental effort involving electron spin resonance, nuclear magnetic resonance, and fluorescence polarization has been directed toward determining the influence of proteins on the orientational order of acyl chains of phospholipid molecules and on the related gel-to-liquid crystalline phase transition. This preoccupation of the experimental spectroscopists has been translated theoretically, in some approaches, into assumptions regarding boundary conditions at the lipid-protein interface on the lipid orientational order.

The recent breakthrough on the structure of bacteriorhodopsin leads naturally to a different focus insofar as guessing the nature of the fundamental lipid-protein interaction is concerned, namely to the energy associated with the matching of the amphiphilic features of the protein and phospholipid molecules (Owicki et al., 1978; Owicki and McConnell, 1979). This is represented schematically in Fig. 1, which shows a cross section of lipid bilayers with protein-like molecules that do not match the equilibrium bilayer thickness and thus give rise to a spatial undulation of the bilayer thickness. On an even more microscopic level, the three-dimensional structure of integral membrane proteins is pictured as being stabilized by the matching of pairs of oppositely charged or polar residues of different α -helices within the hydrophobic depths of the membrane (Henderson, 1981; McLachlan and Henderson, 1980; Engelman and Zaccai, 1980).

At this relatively early stage of development of a theory, the differing intuitions regarding the nature of the basic lipid-protein interactions, which follow from spectroscopic

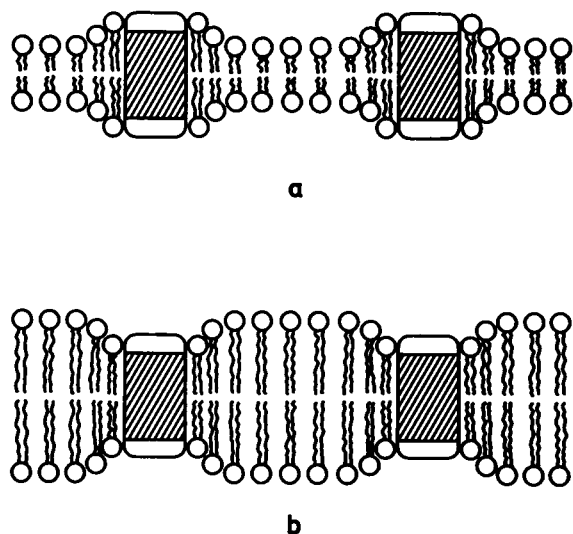


FIGURE 1 Cross section of two lipid bilayers each containing amphiphilic transmembrane impurities (proteins or polypeptides). The lipid molecules are indicated schematically by a circular polar head region and two flexible acyl chains. The impurities are shown as rod-shaped objects with hydrophilic ends and intermediate hydrophobic regions (cross-hatched). Two situations with a mismatch are illustrated: the impurity is longer (a) and shorter (b) than the lipid bilayer thickness.

and structural studies, have not led to any significant differences in the form of the theories. Most theories of lipid-protein interactions have been formulated in terms of some sort of "order parameter"—"membrane surface area per molecule," "hydrocarbon chain orientational order," "bilayer thickness," etc.—and have examined the influence of the lipid-protein interaction on the order parameters and on the phase equilibrium properties of the lipids. For geometrical reasons, these various types of order are not, in fact, independent of each other, and until the parameters of the theories are subjected to critical experimental tests, any theory expressed in terms of a single-order parameter can be used to represent any of the various types of interactions so far considered.

From the standpoint of statistical mechanics, the influence of proteins on the phase behavior and thermodynamic properties of the lipid matrix has been studied along two different routes. One is by means of phenomenological Landau theories (Owicki et al., 1978; Owicki and McConnell, 1979; Jähnig, 1981a, b; Schröder, 1977), which have proven useful in describing phase transitions in a great variety of other physical systems. In these theoretical developments, the dependence of various thermodynamic quantities, such as transition temperature and heat of melting on protein concentration, has been described qualitatively. Special attention was given to how the perturbation of the lipid order decays away from the surface of the individual proteins, as expressed in terms of a coherence length for spatial fluctuations. In all cases, the protein was treated formally as a rigid boundary condition for the lipid order parameter. It has been shown that quantities such as

heat capacity and lateral compressibility, which are closely associated with fluctuations, are enhanced near the phase transition and that the membrane may be driven toward a "critical" point when the protein concentration is increased (Jähnig, 1981a, b; Owicki et al., 1978). A common feature of the Landau theories is that they are formulated in terms of mainly unknown expansion parameters, which are difficult to relate to measurable physical properties. More important, these approaches have assumed homogeneous dispersions of proteins and have therefore excluded, a priori, the possibility of lateral phase separation, which is known to occur in most lipid-protein mixtures, especially in the gel phase (Quinn and Chapman, 1980; Bienvenue et al., 1982).

The other route taken by theories of lipid-protein interactions (Marčelja, 1976; Pink and Chapman, 1979; Tessier-Lavigne et al., 1982) has involved the use of detailed microscopic statistical mechanical models previously developed to describe the main gel-fluid transition in pure lipid bilayers by taking into account the nature of the molecular interaction forces, as well as the statistics of the hydrocarbon chain conformations (Marčelja, 1974; Caillé et al., 1980). In Marčelja's model, the protein is introduced as a cylindrical boundary condition on the lipid orientational order, and nonspecific lipid-protein interactions are assumed. The model does not allow for phase separation, and the derived results are very similar to those obtained from the Landau-type theories. In the approach used by Pink and co-workers (Pink and Chapman, 1979; Tessier-Lavigne et al., 1982), a much more detailed microscopic model is drawn upon, which includes specific lipid-protein interactions that depend on the configurational state of the individual hydrocarbon chains. The Pink model has proven useful in describing a variety of experimental observations for different lipid-protein mixtures. The basic drawback of such microscopic model calculations is the introduction of a large number of model parameters that can only be determined by elaborate and often somewhat questionable fitting to experimental data.

In this paper, we present a phenomenological thermodynamic model, the mattress model, devised to describe the phase behavior of lipid membranes with imbedded, protein-like amphiphilic molecules that span the membrane but do not necessarily match the pure lipid bilayer equilibrium thickness, as represented schematically in Fig. 1. The energy stored in the undulations of the membrane surface caused by the mismatch is related, within the model, to the elastic properties of the lipids and proteins. In contrast to the microscopic theories mentioned above, the mattress model makes no attempt to describe the properties of the pure lipid system itself but accepts as input data the known thermodynamic properties of the pure lipid bilayer, including the properties of the phase transition, and seeks only to model the perturbations produced by the proteins. Our method of modeling the elastic distortion has been stimulated directly by a closely related "plate and spring" model,

which has recently been advanced by Dahn et al (1982) to describe staging in intercalation batteries. In addition to the elastic distortion forces, the model incorporates indirect lipid-protein interactions induced by the mismatch as well as direct lipid-protein van der Waals-like interactions between the hydrophobic parts of the lipid bilayer and the proteins. The model is solved within the framework of a two-component real solution theory that includes the possibility of phase separation.

In a sense, the mattress model combined with solution theory falls between the two theoretical approaches outlined above. Like the microscopic theories, the mattress model includes specific physical interactions. However, the interactions are included in a highly phenomenological way. As in the case of the Landau-type theories, a free energy expansion is used, but the various terms in the expansion, using solution theory, have a more straightforward physical interpretation and lend themselves more easily to experimental measurements than those of the Landau-type theories. A deficiency of our model is that its "average" nature excludes the possibility of describing spatial fluctuations of the order parameter and the decay of perturbations near individual proteins. However, by not attempting to describe these microscopic details of lipid-protein interactions, we can treat, in a simple and transparent manner, bulk phase separation, which we believe to be of major importance from a biological standpoint.

QUANTITATIVE CHARACTERIZATION OF THE MATTRESS MODEL

The mattress model is a phenomenological thermodynamic model designed to describe phase equilibria for two-component mixtures of lipid bilayers with embedded,

protein-like impurities. The elastic properties of the lipid matrix and the protein-like molecules are incorporated as a basic feature of the model. The system can be visualized schematically as a "mattress" of an elastic medium (the lipids, L) characterized by a single spring constant, A_L , and a distribution of localized springs (the proteins, P) characterized by another spring constant, A_P .

We restrict ourselves in this paper to the low concentration regime for the impurities so that the possible phases, α , of the mixture can be identified with the phases of the pure lipid bilayer itself, i.e., $\alpha = g$ (gel) and $\alpha = f$ (fluid). The basic geometrical variables are taken to be the equilibrium average lengths, d_L^α and d_P^α , of the hydrophobic regions of the two components in the mixture. The geometrical picture modeling the corresponding physical situations of Fig. 1. Fig. 2 stresses the "average" nature of the mattress model, which does not treat in detail the local curvature of the bilayer surface around an impurity.

To the lowest approximation, the elastic energy stored in the simultaneous vertical deformation of the two components relative to their individual equilibrium lengths, d_L^α and d_P^α , is given by

$$H_E^{\text{elastic},\alpha} = n_L^\alpha A_L^\alpha (d_L^\alpha - d_L^{\alpha,0})^2 + n_P^\alpha A_P^\alpha (d_P^\alpha - d_P^0)^2, \quad (1)$$

where n_L^α and n_P^α are the number of molecules of the two species in the phase α .

Various forms of the L-P interaction can be included in the model. We consider here an explicit form for the hydrophobic energy associated with the exposure of the longer of L or P to either water or the hydrophilic parts of the neighboring molecules. This leads to an energy contribution that is a function of the mismatch $|d_P^\alpha - d_L^\alpha|$. A

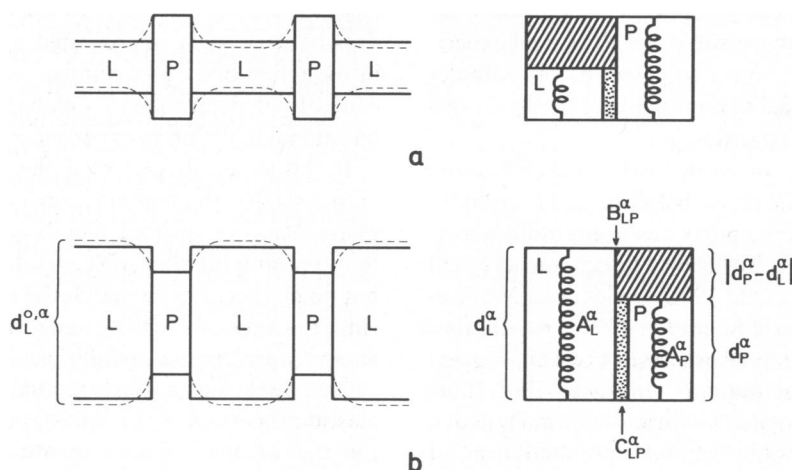


FIGURE 2 To the left are given a schematic representation of the two physical situations (a and b) of Fig. 1. Only the hydrophobic regions of the lipid bilayer (L) and the amphiphilic impurity (protein or polypeptide, P) are indicated. The dashed line indicates schematically a more realistic shape of the lipid surface, which is represented in the model by a uniform average bilayer thickness. The right-hand side of the figure gives a compact representation of the corresponding mattress model, defining the basic geometrical variables, d_L^α and d_P^α , and the mismatch $|d_P^\alpha - d_L^\alpha|$. The elastic constants of the two components are given by A_L^α and A_P^α , and the strengths of the hydrophobic and adhesive interactions are denoted by B_{LP}^α and C_{LP}^α , respectively. The cross-hatched areas correspond to hydrophilic regions and the dotted regions represent the adhesive.

simple choice for the form of the hydrophobic energy is sufficient for our present purposes. Expressed in terms of a positive interaction constant, B_{LP}^α , corresponding to a repulsive interaction and the explicit concentration dependence used in standard regular solution theory (Guggenheim, 1952; King, 1969), we get

$$H_E^{\text{hydrophobic},\alpha} = \frac{n_L^\alpha n_P^\alpha}{n_L^\alpha + n_P^\alpha} B_{LP}^\alpha |d_P^\alpha - d_L^\alpha|. \quad (2)$$

The direct L-P interaction is modeled by an attractive adhesive interaction:

$$H_E^{\text{adhesive},\alpha} = \frac{n_L^\alpha n_P^\alpha}{n_L^\alpha + n_P^\alpha} C_{LP}^\alpha \min(d_P^\alpha, d_L^\alpha) \quad (3)$$

with $C_{LP}^\alpha < 0$. This term accounts for the van der Waals attraction between the hydrophobic parts of the two types of molecules. Strictly speaking, the interaction parameters B_{LP}^α and C_{LP}^α in regular solution theory involve not only lipid-protein but also lipid-lipid and protein-protein contributions. However, in the limit of low protein concentration, the interaction parameters can be interpreted in terms of lipid-protein interactions alone.

The headgroup interactions between lipid molecules are taken into account through the standard free energy obtained from thermodynamic measurements of the properties of the pure lipid component. For the present, we do not model explicitly the interaction between the hydrophilic headgroups whenever an impurity molecule is involved.

The total excess enthalpy then takes the form

$$H_E^\alpha = H_E^{\text{elastic},\alpha} + H_E^{\text{hydrophobic},\alpha} + H_E^{\text{adhesive},\alpha}. \quad (4)$$

A schematic representation of the mattress model incorporating the interactions of Eq. 4 is given in Fig. 2. The model free energy is now written

$$G^\alpha = G_{\text{ideal}}^\alpha + H_E^\alpha. \quad (5)$$

The free energy of an ideal two-component mixture is

$$G_{\text{ideal}}^\alpha = n_L^\alpha \mu_L^{\circ,\alpha} + n_P^\alpha \mu_P^{\circ,\alpha} + RT \left[n_L^\alpha \ln \left(\frac{n_L^\alpha}{n_L^\alpha + n_P^\alpha} \right) + n_P^\alpha \ln \left(\frac{n_P^\alpha}{n_L^\alpha + n_P^\alpha} \right) \right], \quad (6)$$

where $\mu_L^{\circ,\alpha}$ and $\mu_P^{\circ,\alpha}$ are the standard chemical potentials in the α -phase. The standard states chosen are the pure lipid bilayer for L and the infinite dilution limit for P. By choosing the latter and not including direct P-P interactions in H_E^α , the model free energy contains no information on the properties of possible bulk phases of the P component. These properties will normally not be known and are of little relevance for the study of the low concentration regime. More important, by choosing the infinite dilution limit as reference state, we get automatically, as shown below, an expression for $\mu_P^{\circ,\alpha}$ in terms of the model parameters of Eq. 5. In other words, the assumption is made that

only the one-particle information on the impurity component is necessary to describe the dispersed phases of the mixture. In this respect, our treatment of the mixture differs from the conventional phase diagram calculations for binary mixtures, where symmetrically defined pure component standard states are chosen (Lee, 1977).

With the reference states chosen as indicated above, the excess enthalpy finally takes the form

$$H_E^\alpha = n_L^\alpha A_L^\alpha (d_L^\alpha - d_L^{\circ,\alpha})^2 + n_P^\alpha A_P^\alpha [(d_P^\alpha - d_P^{\circ,\alpha})^2 - (d_P^{\circ,\alpha} - d_P^\alpha)^2] + \frac{n_L^\alpha n_P^\alpha}{n_L^\alpha + n_P^\alpha} \{ B_{LP}^\alpha (|d_P^\alpha - d_L^\alpha| - |d_P^{\circ,\alpha} - d_L^{\circ,\alpha}|) + C_{LP}^\alpha [\min(d_P^\alpha, d_L^\alpha) - \min(d_P^{\circ,\alpha}, d_L^{\circ,\alpha})] \} \quad (7)$$

where $d_P^{\circ,\alpha} = \lim_{n_P \rightarrow 0} d_P^\alpha$ is the length of the P component when it is infinitely diluted in the lipids. In the general case of $A_P^\alpha < \infty$ and $B_{LP}^\alpha \neq 0$, we have $d_P^{\circ,\alpha} \neq d_P^\alpha$.

The standard chemical potential $\mu_P^{\circ,\alpha}$ consistent with Eq. 7 is

$$\mu_P^{\circ,\alpha} = B_{LP}^\alpha |d_P^{\circ,\alpha} - d_L^{\circ,\alpha}| + A_P^\alpha (d_P^{\circ,\alpha} - d_P^\alpha)^2 + C_{LP}^\alpha \min(d_P^{\circ,\alpha}, d_L^{\circ,\alpha}). \quad (8)$$

The standard chemical potential for the lipids, $\mu_L^{\circ,\alpha}$, contains the complete information on the pure lipid system. In particular, it accounts for the endothermal first-order gel-to-fluid phase transition taking place at $T = T_m$. Expanding about T_m , we have

$$\mu_L^{\circ,\alpha}(T) = \mu_L^{\circ,\alpha}(T_m) + \frac{h_L^\alpha}{T_m} (T - T_m) + \dots, \quad (9)$$

where h_L^α is the molar heat of melting. In the following section, the phase behavior is investigated over a limited temperature range about T_m , and we shall therefore make the usual assumption that h_L^α is independent of temperature and only retain the terms written explicitly in Eq. 9. The enthalpy of melting is defined as $\Delta H_L(T_m) = h_L^g - h_L^f$.

In thermodynamic equilibrium, the values of d_L^α and d_P^α are derived from the condition $\partial G^\alpha / \partial d_L^\alpha = \partial G^\alpha / \partial d_P^\alpha = 0$. We restrict ourselves to the case where the mismatch is always present and the hydrophobic energy term is unsaturated. The total excess enthalpy in Eq. 7 can then be expressed in terms of the model parameters and concentration variables exclusively:

$$H_E^\alpha = (n_L^\alpha + n_P^\alpha) x_P^\alpha \{ a^\alpha x_L^\alpha x_P^\alpha + b^\alpha [(x_L^\alpha)^2 - 1] \}, \quad (10)$$

where

$$a^\alpha = \frac{(B_+^\alpha)^2 - 2\eta^\alpha B_{LP}^\alpha B_+^\alpha + (1 + \eta^\alpha) C_{LP}^\alpha B_+^\alpha}{4A_L^\alpha} + \frac{2\eta^\alpha B_{LP}^\alpha B_-^\alpha + (1 - \eta^\alpha) C_{LP}^\alpha B_-^\alpha}{4A_P^\alpha}, \quad (10a)$$

$$b^\alpha = \frac{(B_-^\alpha)^2}{4A_P^\alpha}, \quad (10b)$$

and

$$\eta^\alpha = \text{sign}(d_P^\alpha - d_L^{\alpha,\alpha}) = \frac{d_P^\alpha - d_L^{\alpha,\alpha}}{|d_P^\alpha - d_L^{\alpha,\alpha}|} \quad (10c)$$

$$B_i^\alpha = \eta^\alpha \left[B_{LP}^\alpha - \frac{(1 \pm \eta^\alpha)}{2} C_{LP}^\alpha \right]. \quad (10d)$$

In Eq. 10, we have introduced the molar fractions x_i^α , which refer to the total mixture:

$$x_i^\alpha = \frac{n_i^\alpha}{n_L^g + n_P^g + n_L^f + n_P^f}. \quad (11)$$

GENERAL PHASE BEHAVIOR OF THE MATTRESS MODEL

We now consider the general situation where the equilibrium state of the system may be a homogeneous one-phase state or a two-phase state (phase separation). By neglecting possible free energy contributions from the interfaces in a two-phase state, the total free energy of the system is

$$G = G^g + G^f. \quad (12)$$

For a given composition $x_P (= x_P^g + x_P^f)$ and $x_L (= x_L^g + x_L^f = 1 - x_P)$, G can be written as a function of the model parameters and two independent concentration variables, e.g., x_P^g and x_L^g . The state of thermodynamic equilibrium, i.e., the phase and the composition, is then determined by minimizing $G(x_P^g, x_L^g)$ with respect to x_P^g and x_L^g , subject to the constraint $0 \leq x_P^g \leq x_P$ and $0 \leq x_L^g \leq x_L$. In the case of a two-phase coexistence region, this procedure is equivalent to the standard double-tangent construction (Lee, 1977).

The determination of the phase boundaries governed by $G(x_P^g, x_L^g)$ of the preceding section cannot be determined analytically in the general case, and numerical methods have to be employed. Before giving the general results of the numerical calculations, it is instructive to study the solution for the phase boundaries in the limit $x_P^g \ll 1$, where the well-known laws of dilute solutions apply. In this limit, the phase boundaries are given by

$$x_P^g \approx \frac{\Delta\mu_L^0}{RT_m(1 - K_d)} \quad (13)$$

and

$$x_P^f \approx \frac{\Delta\mu_L^0}{RT_m(K_d^{-1} - 1)}, \quad (14)$$

where, using Eq. 9,

$$\Delta\mu_L^0 = \mu_L^{0,g} - \mu_L^{0,f} = \frac{\Delta H_L}{T_m} (T - T_m). \quad (15)$$

The distribution constant, K_d , is defined as

$$K_d = \frac{x_P^f}{x_P^g} = \exp\left(\frac{\mu_P^{0,g} - \mu_P^{0,f}}{RT_m}\right). \quad (16)$$

In order to demonstrate the importance of the mismatch for the initial behavior of the phase boundaries, we consider briefly the following simple example:

$$C_{LP}^\alpha = 0, B_{LP}^g = B_{LP}^f = B_{LP}, A_P^\alpha \rightarrow \infty (d_P^{g,s} = d_P^{f,s} = d_P^0),$$

$$\text{and } d_L^{0,f} < d_P^0 < d_L^{0,g},$$

i.e.,

$$\Delta\mu_P^0 = \mu_P^{0,g} - \mu_P^{0,f} = -2B_{LP} \left[d_P^0 - \frac{1}{2}(d_L^{0,g} + d_L^{0,f}) \right]. \quad (17)$$

Eq. 17 says that for this special case, the difference in affinity of the impurity for the two lipid phases depends on the position of d_P^0 relative to the median $\bar{d}_L^0 = \frac{1}{2}(d_L^{0,g} + d_L^{0,f})$. For $d_P^0 > \bar{d}_L^0$, the P molecules are more soluble in the gel phase ($d_L^{0,g} > d_L^{0,f}$), and for $d_P^0 < \bar{d}_L^0$, the P molecules are more soluble in the fluid phase. In other words, for $d_P^0 > \bar{d}_L^0$, the phase boundaries will both be pushed above T_m , and for $d_P^0 < \bar{d}_L^0$, the phase boundaries will both be pushed below T_m . The two situations are illustrated by the initial behavior of the phase diagrams in Fig. 3 *a* and *b*. The situation will of course be more complicated when $C_{LP}^\alpha \neq 0$, but the example demonstrates the importance of the position of d_P^0 in the discontinuity gap of the lipid bilayer thicknesses at the pure lipid phase transition.

Reverting to the general case where non-ideal behavior will influence the phase boundaries, the question arises

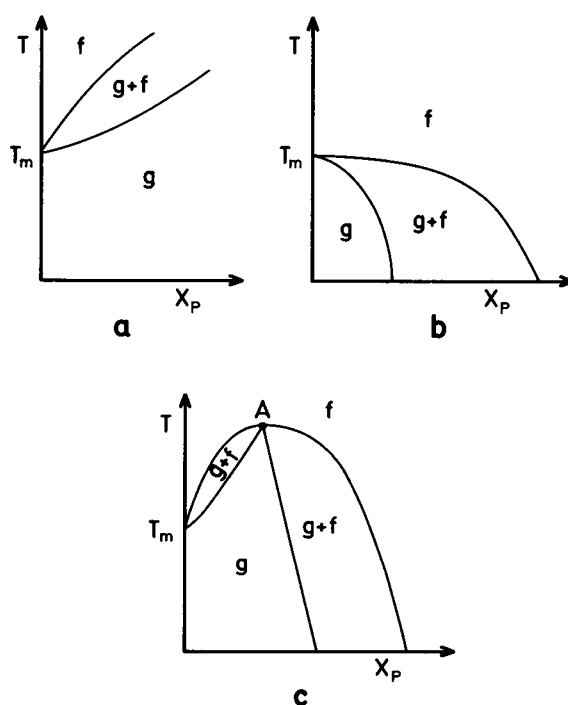


FIGURE 3 Phase diagrams (T, x_P) derived from the mattress model. (a) $\Delta\mu_P < 0$, corresponding to the protein being more soluble in the gel than the fluid phase at low protein concentrations; (b) $\Delta\mu_P > 0$; (c) $\Delta\mu_P < 0$ and azeotropic behavior (A = azeotropic point). T_m is the transition temperature of the pure lipid bilayer.

whether the interactions of the model may change the phase behavior in a qualitative way. To shed some light on this question, we study the excess enthalpy (Eq. 10) in the case of a one-phase region and for rigid impurities, $A_p^a \rightarrow \infty$ and $b^a \rightarrow 0$:

$$H_E^a = (n_L^a + n_P^a) a^a x_L^a (x_P^a)^2. \quad (18)$$

For $B_{LP}^g = B_{LP}^f = B_{LP}$, a^a takes the forms

$$a^g = -\frac{B_{LP}^2}{4A_L^g}, a^f = \frac{-(B_{LP} - C_{LP}^f)^2}{4A_L^f}. \quad (19)$$

Since for lipid bilayers, $A_L^g > A_L^f$, we find $0 \geq a^g > a^f$, which implies that the excess enthalpy favors the fluid phase. Therefore, if $\Delta\mu_P^a > 0$ and the phase boundaries initially are pushed below T_m , this effect is enhanced by the non-ideal contribution. Conversely, if $\Delta\mu_P^a < 0$ and the initial low x_P behavior of the phase boundaries favors the gel phase, the non-ideality counteracts this behavior when x_P is increased and may in some cases lead to the occurrence of an azeotropic point (an upper melting point), where $\partial T/\partial x_P = 0$ and the slope of the phase boundaries changes sign.

Some general examples of the phase behavior that are obtained for numerical calculations on the model are given in Fig. 3, *a-c*. Of particular interest is Fig. 3 *c*, which shows the situation with an azeotropic point (*A*) originating from the competition mentioned above. An important implication of azeotropic behavior is that the two-phase region may extend over temperatures on both sides of T_m .

DETERMINATION OF THE MODEL PARAMETERS

There are two classes of model parameters to be determined in order to compare the mattress model with experiment. The first class of model parameters is associated with the thermodynamic and geometrical properties of pure phospholipid bilayers. We restrict ourselves here to the best-characterized systems of this type, the lecithins, which have a phosphatidylcholine (PC) headgroup, and we limit ourselves to those having saturated acyl chains of length given by the number (n_c) of carbon atoms. The choice of some of the model parameters requires justification even for this well-characterized system. We illustrate this with the values given in Table I for dimyristoylphosphatidylcholine (DMPC; $n_c = 14$). The numbers in Table I and corresponding ones for other lecithins are obtained from information in review articles (Chapman, 1975; Seelig, 1981), dilatometry measurements (Nagle and Wilkinson, 1978), x-ray and neutron diffraction measurements (Janiak et al., 1976; Inoko and Mitsui, 1978; Zaccai et al., 1979; Lewis and Engelman, 1983a; Lis et al., 1982), and micromechanical thermodynamic measurements on individual lipid bilayer vesicles (Kwok and Evans, 1981; Evans and Kwok, 1982). These papers also form the basis of the following discussion on Table I.

TABLE I
MODEL PARAMETERS CHOSEN FOR DMPC ON
THE BASIS OF EXPERIMENTAL MEASUREMENTS
AND ARGUMENTS GIVEN IN THE TEXT

Geometrical parameters	Thermomechanical parameters
$d_L^{gs} = 23 \text{ \AA}$	$T_m = 296^\circ\text{K}$
* $d_L^{gf} = 37 \text{ \AA}$	$\Delta H_L = 6.7 \text{ kcal/mol}$
$q_L^f = 67 \text{ \AA}^2$	* $A_L^f = 0.0048 \text{ kcal/(mol \AA}^2)$
* $q_L^g = 52 \text{ \AA}^2$	* $A_L^g = 0.0086 \text{ kcal/(mol \AA}^2)$

Some of these quantities are known to depend on temperature and are approximated as constants for numerical calculations over a small range of temperature. Parameters whose values require further experimental investigation, as discussed in the text, are indicated with an asterisk.

The values of T_m , d_L^{gs} , and q_L^f (the cross-sectional area per molecule) in Table I require no discussion since they are obtained directly from diffraction and thermodynamic measurements. Note, however, that empirical studies have shown that q_L^f is approximately independent of n_c and $d_L^{gs} \approx 1.75 (n_c - 1) \text{ \AA}$ (Lewis and Engelman, 1983a).

The choice of values for d_L^{gs} , q_L^g , and ΔH_L is complicated by the fact that DMPC exhibits two phase transitions, the main "chain melting transition" from the P_β phase to the L_α phase at 296°K and the "pre-transition" from the L_β phase to the P_β phase at 286°K . The P_β phase is characterized by a rippled bilayer and the L_β phase by a tilt of the hydrocarbon chains relative to the bilayer normal by $\sim 30^\circ$. The pre-transition is not present in large single-bilayer vesicles (Evans and Kwok, 1982), where it is found that the enthalpy of the main transition is equal to the sum of the enthalpy changes of both transitions for multilamellar dispersions. Since small amounts of impurities also eliminate the P_β phase (Chapman et al., 1977), we have used the value of Evans and Kwok (1982) for ΔH_L in Table I.

It is also anticipated that the introduction of small quantities of transmembrane impurities into the bilayer should eliminate the tilt of the acyl chains in the low temperature phase. For this reason, we have estimated the values of d_L^{gs} and q_L^g given in Table I for the "unperturbed" phospholipid state by dividing the measured value of d_L^{gs} in the L_β state by $\cos\theta_{\text{tilt}}$ and multiplying that of q_L^g by $\cos\theta_{\text{tilt}}$, where we have used the approximation $\theta_{\text{tilt}} \approx 30^\circ$. Obviously, this ad hoc procedure for obtaining d_L^{gs} and q_L^g will require further examination.

The elastic constants are directly related to the elastic area compressibility modules at constant temperature, K_e , by (Evans and Skalak, 1979)

$$A_L^a = \frac{q_L^a K_e^a}{2(d_L^{gs} + 11 \text{ \AA})^2} \quad (20)$$

where the thickness of each polar headgroup region has been taken as 5.5 \AA (Lewis and Engelman, 1983a). The values listed in Table I are based on values of $K_e^f = 140 \text{ dyne/cm}$ and $K_e^g = 800 \text{ dyne/cm}$ given by Evans and Kwok (1982) for single-bilayer vesicles. In view of the widely

different values of K_c obtained by other workers (Lis et al., 1982; Jacobs et al., 1977) and the need for better theoretical justification for relating A_L^a directly to K_c^a as we have done in Eq. 20, these values should be treated with caution. It would be best to treat A_L^i and A_L^e as parameters capable of assuming a wider range of values than the other parameters of Table I.

The second class of model parameters involves the geometrical features of the transmembrane particles and their interaction with the phospholipid molecules. Generally speaking, the geometrical properties can be estimated to a reasonable accuracy from a molecular model when the three-dimensional structure is known. For bacteriorhodopsin, $d_p^o \approx 30 \text{ \AA}$ (Lewis and Engelman, 1983b). The cross-sectional area is estimated to be $q_p \approx 960 \text{ \AA}^2$ and the perimeter $\rho_p \approx 110 \text{ \AA}$ (Cherry, 1979). For a synthetic α -helical polypeptide, d_p^o is the number of hydrophobic residues multiplied by the rise per residue, 1.5 \AA , apart from end effects. We take the cross-sectional area to be $q_p \approx 110 \text{ \AA}^2$ for, e.g., leucines in an α -helix.

For present purposes, we shall assume that $A_p \gg A_L^a$. In this limit, A_p plays no role in the theory since $d_p \approx d_p^o \approx d_p^e$. In the event that vertical flexibility of proteins proves to be important, e.g., through a discrete set of conformational states, the appearance of A_p in the general formulation of the model may be useful in relating the physiological function of transmembrane proteins to the interactions between protein and phospholipid molecules.

Finally, we discuss the interaction parameters B_{LP}^a and C_{LP}^a . In the most naive interpretation of the mattress model (see Fig. 2), the effective repulsive coupling between hydrophobic and hydrophilic material (Tanford, 1973) can be expressed in terms of the free energy of transfer of hydrocarbon to water, which has been measured to be $\gamma \approx 50 \text{ cal}/(\text{mol} \cdot \text{\AA}^2)$ at 25°C (Reynolds et al., 1974), as $B_{LP} \approx \gamma \cdot \rho_p$. This simple relation neglects effects such as the different degree of hydrophobicity of hydrocarbon in contact with polar headgroup material rather than water. It also neglects the fact that the hydrophobicity of typical protein side chains has been estimated to be $\sim 50\%$ that of hydrocarbons (Tanford, 1973), so that if $d_L^{o,e} > d_p^o > d_L^{o,f}$, we would have $B_{LP}^e \approx 2B_{LP}^i$, or $\gamma^e \approx 2\gamma^i \approx 50 \text{ cal}/(\text{mol} \cdot \text{\AA}^2)$.

In the same way as in the above discussion, we can represent the adhesive energy in the form $C_{LP}^a \approx \nu^a \rho_p$. The value of ν^a is expected to depend on the lipid phase. For example, the arguments presented above may also give $|\nu^e| > |\nu^i|$. The van der Waals contribution to ν may be roughly estimated from dilatometry measurements to be $\sim 20 \text{ cal}/(\text{mol} \cdot \text{\AA}^2)$ (Nagle and Wilkinson, 1978). However, ν^a should also depend on the distribution of protein polypeptide residues and is unpredictable because of a lack of quantitative information on the solubility of proteins in lipids (Sanderman, 1978; Tanford, 1973).

It is obvious from the above discussion that the values of B_{LP}^a and C_{LP}^a are not well established experimentally even if

we restrict ourselves to the most naive interpretation of the mattress model, in which the thickness of the bilayer is taken to be uniform and equal to its average value right up to the protein surface. A more refined analysis of the interaction constants would relate the B_{LP} and C_{LP} interaction coefficients to the fundamental lipid-protein interactions discussed above, taking into account the boundary conditions at the lipid-protein interface. For example, the values of γ and A_L presented in this section give a very large value (>100) for the ratio of the hydrophobic to the elastic energy terms of Eq. 2 and 1, respectively. Presumably, this represents a substantial overestimate of the ratio since the level of the lipid near the protein interface would readjust itself at the cost of a relatively small amount of elastic energy to minimize the total free energy. For the moment, we propose to explore the theoretical physical properties of lipid-protein mixtures, treating the interaction coefficients of the mattress model as fitting parameters.

INTERPRETATION OF EXPERIMENTS USING THE MATTRESS MODEL

Although many experimental studies have been carried out with the purpose of investigating the interactions between phospholipid and protein molecules, the experimental data available at the present time are inadequate to provide a definitive quantitative test of the mattress model. Nevertheless, it is useful, in planning better experiments, to discuss the relationships between quantitative predictions of the mattress model and the aspects of lipid-protein interactions on which a substantial amount of experimental work has been done. In this section, we compare the predictions of the mattress model with three types of experimental studies. First, we review the influence of proteins on local orientational order in phospholipid bilayers as determined by deuterium nuclear magnetic resonance (^2H NMR) measurements. Then, we show how the influence of small concentrations of proteins on the thermodynamic properties of the gel-fluid phase transition can be characterized quantitatively by the mattress model. Finally, we discuss the factors that lead to protein segregation in fluid and gel bilayers.

Influence of Proteins on Local Orientational Order

A number of spectroscopic techniques provide information on "local orientational order" (Seelig and Seelig, 1980; Marsh and Watts, 1982; Devaux, 1983). In the simplest cases, the energy levels of a spectroscopically active part of a molecule are governed by an interaction that depends on orientation. Those molecular motions that are rapid on the "spectroscopic time scale," i.e., motions characterized by frequencies greater than the range of frequency shifts or

splittings produced by the interaction, give rise to time-averaged spectral shifts or splittings caused by the interaction modulated by the motions. Thus, the quadrupolar interactions give rise to a splitting in ^2H NMR spectroscopy that is proportional to a "local orientational order parameter," S_{CD} , defined by

$$S_{\text{CD}}^{(i)} = \left\langle \frac{1}{2} (3\cos^2\theta_i - 1) \right\rangle, \quad (21)$$

where θ_i , for a deuteron bonded to the i th carbon atom on an acyl chain of a phospholipid molecule, is the angle between the CD bond direction and the normal to the bilayer, which is the axis of symmetry for the rapid motions of the acyl chains. The characteristic variation of S_{CD} with position on the acyl chains in the fluid phase of pure phospholipid bilayer (model) membranes and its discontinuous increase in the more ordered gel phase are generally credited with having stimulated the development of a number of useful theoretical models for describing quantitatively the gel-fluid phase transitions in lipid bilayer systems (Seelig, 1977; Seelig and Seelig, 1980).

A large number of ^2H NMR studies have been carried out on the influence of proteins on local orientational order. As reviewed in several articles (Seelig and Seelig, 1980; Jacobs and Oldfield, 1981; Devaux, 1983), a variety of natural and reconstituted membranes have been studied. The general, and somewhat unanticipated, result was that the values of S_{CD} obtained in the fluid phase of pure lipid bilayer systems were not changed very much by the presence of proteins even when the amount of protein in the membrane was 50% (by weight) or more. At temperatures characteristic of the gel phase, by contrast, the proteins always cause a decrease in S_{CD} . Since proteins are usually regarded as having fairly rigid structures, this result has provoked considerable discussion. The absence of a large change in S_{CD} of the fluid phase caused by the lipid-protein interaction has been ascribed by some workers (Seelig and Seelig, 1980; Jacobs and Oldfield, 1981) to the disordering effects of the protruding amino acid side chains, i.e., roughness of the protein surface. Another conjecture is that the protein surface is fluidlike, providing a fluid mechanical match with the fluid lipid bilayer (Bloom, 1979).

A simpler and more general interpretation of the influence of the lipid-protein interaction on S_{CD} is provided by the mattress model. This model assumes that the dominant lipid-protein interaction involves the mismatch of the lengths of the hydrophobic regions of the phospholipid and protein molecules. If the equilibrium lengths $d_{\text{L}}^{\text{o.f}}$ and d_{P}^{o} of the hydrophobic regions of the lipid and protein molecules were closely matched, as is believed to be the case in the fluid bilayer phase for most biological membranes, the lipid-protein interaction would not modify $d_{\text{L}}^{\text{o.f}}$. It is anticipated that there will be a correlation between $d_{\text{L}}^{\text{o.f}}$

and the average order parameter $S_1 = \langle S_{\text{CD}} \rangle$ for the entire acyl chains of the lipids. Indeed, for a lattice model of acyl chain conformations, there is a precise relationship between $d_{\text{L}}^{\text{o.f}}$ and S_1 (Schindler and Seelig, 1975; Seelig and Seelig, 1980, p. 38; Zaccai et al, 1979, p. 705). We suggest that the reason that experiments thus far indicate that proteins do not perturb fluid lipid values of S_{CD} much is that most experiments have been carried out on phospholipid and protein mixtures that were either those found in biological membranes or those considered to be of importance in understanding biological membranes. This ensures that the value of $d_{\text{L}}^{\text{o.f}}$ is reasonably well matched to the protein d_{P}^{o} so that little perturbation to S_1 occurs, providing that the acyl chains of the phospholipid molecules are sufficiently mobile to average the quadrupolar interactions relative to the bilayer normal on the ^2H NMR time scale.

There are other indications that this interpretation is probably correct. One is that measurements of S_1 on lipids with perdeuterated acyl chains have shown that when the protein concentration is increased at temperatures corresponding to the lipid gel phase, S_1 steadily decreases until it reaches the value of S_1 that is characteristic of the lipid fluid phase (Paddy et al., 1981; Bienvenue et al., 1982). Although the interpretation of these experiments is complicated by the segregation of the proteins with their associated lipids in the gel phase region (Bienvenue et al., 1982), they do demonstrate that the lipid-protein interaction tends to change $d_{\text{L}}^{\text{o.f}}$ so as to match d_{P}^{o} . A second indication is that experiments carried out on an amphiphilic polypeptide, spanning phospholipid and soap bilayers in the form of an α -helix and having $d_{\text{P}}^{\text{o}} > d_{\text{L}}^{\text{o.f}}$, produced markedly increased values of S_1 (Davis et al., 1982, 1983).

There is an interesting, and possibly controversial, corollary to the above remarks. Suppose that for all the lipid-protein mixtures studied thus far in the lipid fluid phase, there are many lipids bound to the protein surfaces for times that are long on the ^2H NMR time scale. Such a possibility has been rejected because the average values of S_{CD} observed in the presence of proteins are very similar to those in the absence of proteins, as discussed above. If, however, $S_{\text{CD}}^{\text{bound}} \approx S_{\text{CD}}^{\text{fluid}}$ because of the constraint $d_{\text{L}}^{\text{bound}} \approx d_{\text{P}}^{\text{o}} \approx d_{\text{L}}^{\text{o.f}}$, the strong case against the existence of intermediate or long-lived boundary lipids on the basis of the analysis of ^2H NMR spectra evaporates. We should emphasize that the "rough protein surface" (Seelig and Seelig, 1980; Jacobs and Oldfield, 1981) and "squishy protein" (Bloom, 1979) explanation of the small influence of proteins on S_{CD} values for the lipid chains would lead to the same possibility. However, the mattress model does provide a more plausible explanation for why the S_{CD} values remain unchanged as large amounts of proteins are added to fluid bilayers. The very large amount of electron spin resonance, (ESR) work on lipid-protein interactions

and the role of boundary lipids has been reviewed recently by Marsh and Watts (1982) and Devaux (1983).

Thermodynamic Properties of the Gel-Fluid Phase Transition

Calorimetric measurements on lipid bilayers with low concentrations of proteins such as bacteriorhodopsin (Alonso et al., 1982; Heyn et al., 1981), (Ca²⁺ - Mg²⁺)ATPase (Gomez-Fernandez et al., 1980), glycophorin (Van Zoelen et al., 1978), lipophilin (Boggs and Moscarello, 1978; Curatolo et al., 1977), and the polypeptide gramicidin A (Chapman et al., 1977) have demonstrated that the enthalpy change $\Delta H(x_p)$ associated with the broadened transition decreases linearly with x_p . If the proteins were dispersed homogeneously in the gel phase just below the phase transition for low protein concentration, the observed broadened transition could be associated with the two-phase region. Since all of the proteins mentioned above satisfy $d_p^\circ < \bar{d}_L^\circ$, a phase diagram of the type of Fig. 3 *b* would apply, in accordance with the experiments that indicate a slight lowering of the "mid-point" transition temperature. To the lowest order in x_p , the enthalpic part of Eq. 6, used in conjunction with Eq. 8 and the interaction parameters described in the previous section, gives the result

$$\begin{aligned}\Delta H(x_p) &= x_L \Delta H_L(T_m) + x_p(\mu_p^{\alpha f} - \mu_p^{\alpha g}) \\ &= \Delta H_L(T_m) + x_p[\rho\Gamma - \Delta H_L(T_m)],\end{aligned}\quad (22)$$

where

$$\Gamma = \gamma^f(d_p^\circ - d_L^{\alpha f}) - \gamma^g(d_L^{\alpha g} - d_p^\circ) + \nu^f d_L^{\alpha f} - \nu^g d_p^\circ. \quad (23)$$

The two proteins bacteriorhodopsin and (Ca²⁺ - Mg²⁺)ATPase are sufficiently well characterized to allow estimates of $\rho \approx 110$ Å and $\rho \approx 220$ Å, respectively. Using the experimental values of $\Delta H_L(T_m)$ for pure DMPC and dipalmitoylphosphatidylcholine DPPC and the experimental values of the slope of $\Delta H(x_p)$ vs. x_p for the two proteins, we find that, in units of kcal/(mol·Å)

$$\Gamma_{\text{DPPC}} = -1.66, \Gamma_{\text{DMPC}} = -1.10 \quad (\text{bacteriorhodopsin})$$

$$\Gamma_{\text{DPPC}} = -1.50, \Gamma_{\text{DMPC}} = -1.24 \quad [(Ca^{2+} - Mg^{2+})ATPase]$$

Since d_p° is estimated to be ~ 30 Å for both of these proteins, Eq. 23 predicts that Γ_{DPPC} and Γ_{DMPC} should be approximately the same for the two proteins studied. Indeed, the difference in Γ for the two proteins is only $\sim 10\%$ in each case.

Since the values of the interaction parameters, γ^α and ν^α , in Eq. 23 are of the order of 50 cal/(mol·Å²) and the geometrical parameters are of the order of 30 Å, the simple version of the mattress model that we have developed thus far is capable of accounting for the order of magnitude of the experimental values of Γ . However, we believe that it is premature at this time to draw any conclusions about the

validity of the mattress model on the basis of calorimetric data. More experiments are required to determine the interaction parameters thus far introduced into the mattress model, as well as those neglected, e.g., the Coulomb interactions associated with the charged amino acid residues in those parts of the protein buried within the hydrophobic region (Henderson, 1981; Engelman and Zaccai, 1980). At the moment, the assumption that the proteins are homogeneously distributed in the gel phase seems difficult to reconcile with strong experimental indications (see, e.g., Quinn and Chapman, 1980; Alonso et al., 1982; Bienvenue et al., 1982) that protein segregation occurs. Indeed, the extremely large value of $-\rho\Gamma/RT_m \approx 250$ gives an enormous partition coefficient, K_d , according to Eqs. 16 and 22, which would imply that segregation of the proteins and/or the formation of protein complexes with a number of lipid molecules occurs.

Protein Segregation in Lipid Bilayers

The mattress model may be applied to the study of segregation of proteins within a single phase (gel or fluid) of the lipids. There is an effective, lipid-mediated, attractive interaction potential between pairs of proteins in the lipid bilayer because the distortion energy of the bilayer caused by a pair of identical proteins depends on their separation and is less than the sum of distortion energies that are due to two individual proteins. This gives the possibility of a phase transition within a single lipid phase from a homogeneous distribution of proteins to one in which a fraction of the proteins are segregated into a relatively small region. In order to make theoretical predictions about such phenomena, it is necessary to have information on the free energy $G^{\text{seg},\alpha}$ of the segregated phases. Although insufficient information is available at present to estimate $G^{\text{seg},\alpha}$ for any system, we can make some general, qualitative predictions on protein segregation on the basis of the behavior of G^α of Eqs. 5–7 and the values of the mattress model parameters, since segregation will occur only if $G^{\text{seg},\alpha} < G^\alpha$. Circumstances that tend to favor segregation are then found to be: (a) large values of the elastic constant, A_L^α , i.e., rigid bilayers; (b) large values of the mismatch, $|d_p^\circ - d_L^{\alpha f}|$. These predictions imply that: (a) Segregation is more likely to occur in the gel phase than the fluid phase. For lipid bilayers that differ only with respect to the degree of saturation of the hydrocarbon chains, a smaller degree of saturation facilitates segregation since more unsaturation is found experimentally to soften the bilayer (Lis et al., 1982). (b) Bilayers of extreme thickness facilitate segregation.

Since most experimental studies of biological and reconstituted membranes carried out thus far have involved lipids and proteins for which $d_p^\circ \approx d_L^{\alpha f}$ and since $A_L^f > A_L^g$, conditions (a) and (b) are consistent with a large number of physical properties including calorimetry (Quinn and Chapman, 1980; Alonso et al., 1982), freeze fracture

(Devaux, 1981), ESR (Marsh and Watts, 1982; Devaux, 1983), and ^2H NMR (Bienvenue et al., 1982), which are easily understood on the basis of segregation of proteins occurring in the gel phase of the lipids but not in the fluid phase. All of the available observations are consistent with the segregated protein phase having a sufficient number of lipids to coat the protein surface, the molar lipid-to-protein ratio having been measured to be 30 in the case of rhodopsin-DMPC mixtures (Bienvenue et al., 1982). As described earlier, the lipid chains are sufficiently mobile in the segregated protein phase to give rise to ^2H NMR orientational order parameters that are characteristic of the thickness of hydrophobic region of the proteins. It has also been found recently (MacKay et al., 1983) that the proteins themselves have a measurably smaller amount of internal motion in the segregated phase when the lipid-to-protein ratio is less than the equilibrium number of lipids required to solvate the proteins (i.e., 30, in the case of rhodopsin-DMPC mixtures). This implies that in estimating $G^{\text{seg},\alpha}$, it will be necessary to include contributions associated with internal degrees of freedom of the proteins.

A particularly interesting freeze-fracture study of segregation of bacteriorhodopsin in fluid lipid bilayers of lecithins of varying thickness has recently been reported by Lewis and Engelman (1983b), who found that segregation only occurs for extreme bilayer thicknesses. An interesting asymmetry found in these studies is that segregation only occurred for $d_p^o - d_L^o \geq 10 \text{ \AA}$ and $d_L^o - d_p^o \geq 4 \text{ \AA}$. This asymmetry is indeed predicted by the mattress model (to lowest order in x_p) simply because $B_{LP}^f > B_{LP}^i$.

Concluding Remarks and Suggestions for Further Experiments

The mattress model in its present formulation only accounts for lipid phases with dispersed proteins. There is ample experimental evidence that many proteins have very low solubility in gel lipid phases. This suggests that for such systems one should expect phase diagrams with eutectic behavior of the type illustrated in Fig. 4 (Chapman et al., 1979). In the absence of a free energy term to account for the bulk segregated phases of the proteins with their associated lipids, the mattress model is only able to describe phase behavior along paths of the type (a) of Fig. 4. It is along paths like this that the calorimetric data were analyzed in the previous section. For proteins with very low solubility, the point *D* in Fig. 4 may occur very close to the $x_p = 0$ axes, leaving only a very small concentration range of a dispersed gel phase. For higher values of x_p , two phase changes may occur, e.g., along paths of the type *b* of Fig. 4. Recent calorimetric measurements on bacteriorhodopsin in DMPC and DPPC (Alonso et al., 1982) revealed a low temperature shoulder on the main heat capacity peak. These observations may be interpreted as crossing a two-phase region along path *b*. Temperature scans along paths

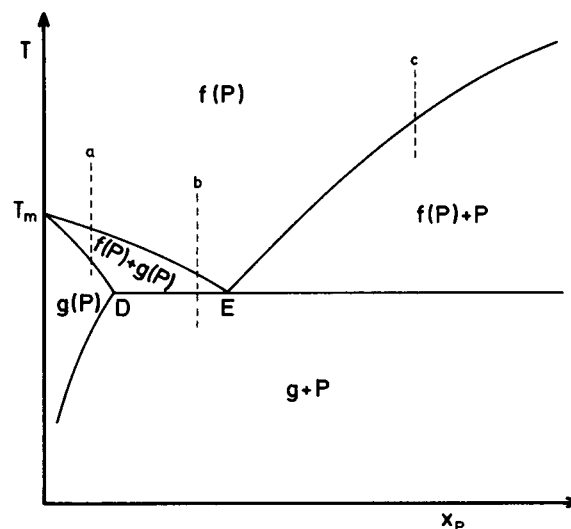


FIGURE 4 General phase diagram of a lipid-protein mixture exhibiting eutectic behavior. The lipid phases with dispersed proteins are labeled $f(P)$ and $g(P)$. g and P indicate the pure lipid gel phase and the segregated protein phase, separately. E denotes the eutectic point. Along path *a*, only dispersed phases are observed. For decreasing temperatures along path *b*, two phase changes occur, the latter of which corresponds to complete protein segregation. Along path *c*, partial protein segregation takes place in the fluid lipid phase.

of the type *c* in Fig. 4 would lead to segregation in the fluid phase. To determine the position of the phase boundary along path *c* as a function of the mattress model parameters in such cases, we would require more experimental information on $G^{\text{seg},f}$ than is presently available, as discussed in the preceding section. In fact, Lewis and Engelman (1983b) searched unsuccessfully for this boundary in their freeze-fracture experiments. It would be interesting if experiments such as theirs were repeated at higher protein concentrations.

A major problem in applying the mattress model to biological membranes and to lipid bilayers containing real proteins, as we attempted to do in the preceding section with limited success, is that some of the most important interactions in such systems may not yet have been included in the model. For example, the charged residues buried in the bilayer, whose intra-protein interactions are of vital importance in determining protein three-dimensional structure (Henderson, 1981; Engelman and Zaccai, 1980), probably give rise to direct inter-protein interactions that play an important role in processes such as protein segregation, which we considered in the previous section. However, it is important, in the development of a useful intuition about lipid-protein interactions, to explore and isolate the range of phenomena that can result from the most basic interactions. In this regard, we believe that a most useful type of system will be model membranes of the type being explored by Davis et al. (1982), which involve synthetic, amphiphilic polypeptides incorporated into phospholipid bilayers. For such polypeptides involving a

hydrophobic region, which assumes an α -helical conformation spanning the bilayer, it will be possible to vary d_p^0 by varying the number of hydrophobic residues, while maintaining the hydrophilic part of the polypeptide constant. A systematic study of the physical properties of such a membrane as a function of d_p^0 will enable us to isolate the influence of the interactions introduced thus far in the mattress model. Studies carried out on DPPC containing the polypeptide lysine₂-glycine-leucine₂₄-lysine₂-alanine amide with a lipid-to-protein ratio of 43:1 indicate that the polypeptide is probably homogeneously dispersed in the bilayer in the gel and fluid phases (Davis et al., 1982, 1983). It will be interesting to see how the physical properties of such model membranes depend on the number of leucines and on the nature of the hydrophilic ends of the polypeptides. Indeed, the mattress model with its present assumptions is most likely to be applicable to these types of model membranes, since the polypeptides are smoother than proteins and have no specific binding sites.

In addition to the physical properties, it is obviously of paramount interest to investigate the influence of the geometrical parameters of the model on physiological processes. It is appropriate, therefore, to conclude this paper by drawing attention to some recent attempts to isolate experimentally the influence of bilayer thickness on some physiological functions of integral membrane proteins (see, e.g., Montecucco et al., 1982). Of course, a theory for the influence of d_L^{of} on physiological processes is beyond the scope of the present model, since such an influence is likely to involve not only the effect of d_L^{of} on d_p^0 , but also its effect on the dynamical structure of integral membranes. Still, it does provide a useful perspective on the long-range goals of this type of research.

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