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Dependence of lipid membrane phase transition temperature on the mismatch of protein and lipid hydrophobic thickness

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Abstract. A two-component solution theory is studied which incorporates hydrophobic matching as a major contribution to the lipid-protein interactions in biological membranes. A special geometrical constraint has been discovered which has important implications for the quantitative interpretation of physical effects to lipid-protein interactions. The theory has an advantage over conventional Landau-type phenomenological descriptions in that it accounts for phase separation. A certain class of experimental systems, photosynthetic reaction centre and antenna proteins reconstituted into synthetic lipid membranes of different hydrophobic thicknesses, are considered with a view to determining the parameters of the theory. The theoretical predictions are found to be in good agreement with experimental measurements of shifts in the phase transition temperature.

Key words: Lipid membrane, lipid-protein interactions, phase diagram, hydrophobic thickness, solution theory, membrane elasticity

Introduction

The quantitative interpretation of experimental studies of the physical effects due to lipid-protein interactions in lipid membranes is made difficult by the lack of information on the three-dimensional structure of integral membrane proteins. So far, only a few structures have been worked out, notably that of bacteriorhodopsin from *Halobacterium halobium* (Henderson and Unwin 1975) and the photosynthetic reaction centre of *Rhodopseudomonas viridis* (Deisenhofer et al. 1985), of which only the latter is a high-resolution structure (3 Å). The limited information on protein structure has precluded detailed theoretical modelling of lipid-protein interactions. On the other hand, even if the structure was known, its anticipated complexity would make any attempt to model membranes with integral proteins in any detailed fashion a formidable task which is outside the range of current computational capabilities.

That theoretical modelling of lipid-protein interactions in membranes, despite these difficulties, is nevertheless feasible is related to three fundamental experimental observations: *(i)* Integral membrane proteins are amphiphilic molecules with a hydrophobic core and two hydrophilic ends anchored in the polar regions of the lipid bilayer membrane. *(ii)* All integral membrane proteins, for which some structural information is available, consist predominantly of separately identifiable subunits, α-helices or β-sheets, which each traverse the bilayer (Henderson 1981; Eisenberg 1984). *(iii)* The physical perturbation of fluid lipid membranes due to integral proteins and α-helical polypeptides exhibits some universal trends with only little protein specificity (Bloom and Smith 1985). These observations suggest that the physical effects due to lipid-protein interactions may be caused by the very physical constraints which the lipid bilayer membrane puts on its imbedded proteins. This points to modelling based on some simple physical principles.

In this paper we investigate the thermodynamic consequences of the above suggestion by studying a simple model of integral membrane proteins in lipid bilayers. The model incorporates matching between protein and lipid hydrophobic thicknesses as an important element of lipid-protein interactions. The model, which is obtained as the diluted limit of the so-called mattress model advanced by Mouritsen and Bloom (1984), is treated within a two-component solu-

Abbreviations: Dn_cPC: diacyl-glycero-PC with n_c acyl chain carbon atoms; *D12*PC: L-α-dilauroyl PC; *D13*PC: L-α-ditridecanoyl PC; *D14*PC: L-α-dimyristoyl PC; *D15*PC: L-α-dipentadecanoyl PC; *D16*PC: L-α-dipalmitoyl PC; LHCP: light harvesting chlorophyll protein; NMR: nuclear magnetic resonance; PC: phosphatidylcholine; RC: reaction centre

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tion theory which allows for phase separation. The idea underlying a study of the thermodynamic phase behaviour of lipid-protein mixtures is that the modifications by proteins of the sharp first-order chain-melting phase transition in lipid bilayers (for a recent review, see Mouritsen 1987) are related in a very intimate way to the interactions between lipids and proteins. That the hydrophobic thickness of the protein must therefore be a key parameter for these interactions is underscored by the experimental observation that the lipid bilayer hydrophobic thickness may change by as much as 30% during the phase transition (Evans and Kwok 1982; Inoko and Mitsui 1978).

Most theoretical modelling of lipid-protein interactions (for a recent review see Abney and Owicki 1985), has been based on the assumption that the protein can be treated as some kind of rigid object of little specificity which presents itself as a boundary condition with which the lipid bilayer has to comply (Marčelja 1976; Schröder 1977; Owicki et al. 1978; Scott and Cherng 1978; Owicki and McConnel 1979; Jähnig 1981; Scott and Coe 1983). All these studies, being guided by the experimental preoccupation with lipid order-parameter profiles (Seelig and Seelig 1980; Devaux 1983), have considered the effects of a single protein or a quenched homogeneous dispersion of proteins in the lipid bilayer. These restrictions lead to a simple phase diagram without phase separation. Similarly, the recent studies of lipid-polypeptide interactions (Scott 1986; Edholm and Johansson 1987) using computer-simulation methods on models with highly realistic potentials were carried out for single proteins thus leading to very detailed information about the statics and dynamics of the order-parameter profile but no information about cooperative behaviour and the thermodynamic manifestations of lipid-protein interactions. The approach taken by Pink and collaborators (for a review, see Pink 1984) is built on microscopic interaction models allowing for phase separation and it gives the most detailed description so far of the phase behaviour of lipid-protein mixtures. The models used include a number of specific lipid-protein interaction parameters which have to be determined for each system separately.

The strategy adopted in the present paper is to determine, in the simplest possible setting, the parameters in a phenomenological model of protein solubility in lipid membranes by using experimental data of transition temperature shifts for specific integral membrane proteins. These parameters are assumed to have some general validity and may be used to make predictions for other systems which can be tested against appropriate experiments. In the spirit of the mattress model (Mouritsen and Bloom 1984), the model takes as its input the known thermodynamic properties, and hence the phase transition, of pure lipid bilayers of different hydrophobic thicknesses. The main purpose of the model is to identify the perturbations caused by the mismatch between lipid and protein hydrophobic thicknesses.

Our work has been stimulated by recent systematic experimental studies of the phase behaviour of photosynthetic RC proteins and antenna proteins (LHCP) reconstituted into lipid bilayers of saturated 1,2-diacyl-*sn*-glycero-3-PCs with different acyl chain lengths (Riegler and Möhwald 1986; Peschke et al. 1987). We find that the results of these experiments can be rationalized within the framework of the proposed model. Moreover they yield a set of model-parameter values which lead to predictions for other lipid-protein systems. As a by-product, our study reveals some general properties of linear solution theories applied to lipid-protein mixtures.

The organization of the paper is as follows: Firstly, we introduce the model and discuss its various parameters and how some of these may be obtained from experiments. Secondly, we apply the model to the data for RC and LHCP proteins in lecithin bilayers (Riegler and Möhwald 1986) and arrive at a set of parameters which can then be applied to predict the phase behaviour of other proteins in lecithin membranes (Peschke et al. 1987). Our results are compared to those obtained from Landau-type theories based on the elastic properties of membranes (Jähnig 1981). It is concluded that it is possible to describe the experimental data within a simple solution theory and that it is unnecessary to include effects of membrane elasticity at low protein concentrations.

Thermodynamic model

The mattress model of Mouritsen and Bloom (1984) pictures the membrane as planar elastic lipid sheet in which the protein molecules are imbedded normal to the membrane plane. Considering different hydrophobic thicknesses of the two components, d_L and d_P, situations of hydrophobic mismatch, $|d_L - d_P| > 0$, can arise. It should be noted that the model does not account in any detailed way for the wetting of the lipid-protein interface which in the real system will induce a curvature of the membrane surface near the interface. We shall here assume that the protein concentration is so low that macroscopic effects of the wetting can be neglected and that the "average" mismatch can therefore be related to the protein solubility. Along the same lines, we shall neglect the energy of the elastic distortion of the lipid sheet as well as possible distortions of the much less compressible proteins (Mouritsen and Bloom 1984; Jähnig 1981). This implies that d_L and d_P remain at their unperturbed values, independent of the protein concentration. The

effects of including membrane elasticity will be considered separately.

Under these assumptions of high dilution, the equilibrium free energy of the two-component system can be written

$$G = x_L \mu_L^0 + RT[x_L \ln x_L + x_P \ln x_P] + x_L x_P [B_{LP} |d_L - d_P| + C_{LP} \min\{d_L, d_P\}], \quad (1)$$

where x_L and x_P are the composition variables, $x_L + x_P = 1$, μ_L^0 is the standard chemical potential of the lipids, and B_{LP} and C_{LP} are interaction parameters. B_{LP} is related to the hydrophobic effect of the mismatch, and C_{LP} is the direct lipid-protein hydrophobic van der Waals-like interaction which is associated with the interfacial contact of the two species.

The standard chemical potential of the lipids, μ_L^0, refers to the pure lipid system and its difference in the two lipid phases, the low-temperature gel phase (g) and the high-temperature fluid phase (f), can therefore be related to the transition enthalpy ΔH_L

$$\Delta\mu_L^0 = \mu_L^{0,g} - \mu_L^{0,f} \cong \frac{\Delta H_L}{T_m}(T - T_m), \quad (2)$$

where T_m is the phase transition temperature of the pure system. By choosing the standard state of the proteins as the infinite-dilution limit (Mouritsen and Bloom 1984), it is possible to express the standard chemical potential of the proteins in terms of the model parameters as

$$\mu_P^0 = B_{LP} |d_P - d| + C_{LP} \min\{d_P, d_L\}. \quad (3)$$

By now imposing the phase structure of the pure lipid system, with phases $\alpha = g$ or f, on Eqs. (1)–(3), the equations for the phase boundaries in the dilute region, $x_P \ll 1$, of the phase diagram (T, x_P) can be determined

$$T^g(x_P) \cong T_m + x_P(1 - K_d)\frac{RT_m^2}{\Delta H_L}, \quad (4)$$

$$T^f(x_P) \cong T_m + x_P(K_d^{-1} - 1)\frac{RT_m^2}{\Delta H_L}. \quad (5)$$

The distribution constant K_d is approximately given by

$$K_d \cong \exp\left[\frac{\Delta\mu_P^0}{RT_m}\right], \quad (6)$$

where $\Delta\mu_P^0 = \mu_P^{0,g} - \mu_P^{0,f}$ is a measure of the solubility difference of the proteins in the two different lipid phases. $\Delta\mu_P^0$ is the primary quantity of our solution theory.

We shall now assume that the hydrophobic part of the transmembrane proteins has the form of a column with a smooth surface and a cross-section which is uniform along the long axis of the protein. This assumption is commonly used in protein modelling (Jähnig 1981; Sadler and Worcester 1982; Sadler et al. 1984) and appears to be justified in those cases where the structure is known. It follows from this, that B_{LP} and C_{LP} and thus $\Delta\mu_P^0$ are proportional to the perimeter ϱ_P of the protein cross-section

$$\Delta\mu_P^0 = -\varrho_P \Gamma, \quad B_{LP} = \varrho_P \gamma, \quad C_{LP} = \varrho_P \nu. \quad (7)$$

γ and ν are the basic reduced energy parameters of the model. The parameter ν, being related to the direct interaction between the hydrophobic parts of the lipids and the proteins, will depend explicitly on the lipid phase, i.e. $\nu = \nu^\alpha$. The parameter γ measures the interaction between the hydrophobic part of the longer species and the hydrophobic material to which this species is exposed owing to the mismatch. Hence, γ only depends on the lipid phase in an indirect way and its value will depend on the relation between d_L^g, d_L^f, and d_P, where $d_L^g > d_L^f$ is invariably the case. If we assign the value of γ to be 2ε in the case of lipid acyl chains in contact with hydrophilic material and note with Tanford (1973) that the hydrophobicity of typical protein side chains is about half of that of hydrocarbons, we arrive at the following three cases

(a) $d_P > d_L^g > d_L^f$: $\gamma^g = \gamma^f = \varepsilon$, (8)

(b) $d_L^g > d_P > d_L^f$: $\gamma^g = 2\gamma^f = 2\varepsilon$, (9)

(c) $d_L^g > d_L^f > d_P$: $\gamma^g = \gamma^f = 2\varepsilon$. (10)

The parameter Γ of Eq. (7) is a function of γ and ν as well as of the geometrical parameters d_L and d_P. In the three cases (a)–(b) referred to in Eqs. (8)–(10) Γ takes the form

(a) $d_P > d_L^g > d_L^f$:

$$\Gamma = (d_L^f - d_L^g)\varepsilon + d_L^f \nu^f - d_L^g \nu^g \quad (11)$$

(b) $d_L^g > d_P > d_L^f$:

$$\Gamma = (3d_P - 2d_L^g - d_L^f)\varepsilon + d_L^f \nu^f - d_P \nu^g \quad (12)$$

(c) $d_L^g > d_L^f > d_P$:

$$\Gamma = (2d_L^f - 2d_L^g)\varepsilon + d_P \nu^f - d_P \nu^g \quad (13)$$

Depending on the sign of Γ, two types of low-x_P phase behaviour arise as shown in Fig. 1. With the definition of the midpoint transition temperature

$$\bar{T}(x_P) = \tfrac{1}{2}[T^g(x_P) + T^f(x_P)], \quad (14)$$

we introduce the so-called shift in midpoint transition temperature, $\Delta T(x_P)$, which can be given an analytical expression

$$\Delta T(x_P) = \bar{T}(x_P) - T_m \cong \frac{RT_m^2}{\Delta H_L} x_P \sinh\left(\frac{\varrho_P \Gamma}{RT_m}\right). \quad (15)$$

As it will turn out, $\Delta T(x_P)$ is a convenient quantity to compare with experimental measurements.

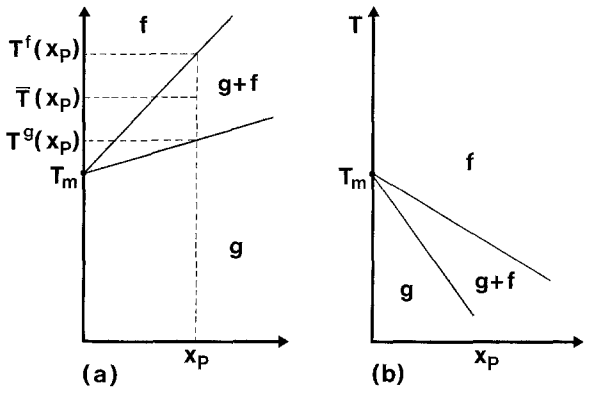

Fig. 1 a and b. Schematic phase diagrams in temperature T vs. protein concentration x_P of lipid-protein bilayers in the dilute regime, $x_P \ll 1$, Eqs. (4) and (5). g and f denote gel and fluid lipid phases, and f + g is the two-phase region. (**a**) corresponds to a situation of $\Delta\mu_P^0 < 0$ and (**b**) to $\Delta\mu_P^0 > 0$, cf. Eqs. (4)–(6). $\bar{T}(x_P)$ is the midpoint phase transition temperature, Eq. (14)

Geometric and thermodynamic model parameters

In this section we discuss the geometrical parameters d_L^g, d_L^f, d_P, and ϱ_P, the thermodynamic parameters ΔH_L and T_m, and how these parameters may be estimated from experiments. We shall focus on lecithin bilayers of diacyl-glycero-PCs, Dn_cPC, with two saturated chains of each n_c carbon atom, $n_c \geqq 12$. This class of lipid bilayers has the best-known physical and thermodynamic properties.

The hydrophobic thicknesses d_L^g and d_L^f are most accurately obtained from continuous x-ray scattering (Lewis and Engelmann 1983 b) which measures the distance between the phosphate groups across the bilayer. The hydrophobic thickness may then be obtained by subtracting the thickness of the polar headgroup region known from neutron-diffraction studies of specifically deuterated samples (Büldt et al. 1979; Zaccai et al. 1979). This approach avoids uncertainties which may be introduced via the corrections for interlamellar water thickness and specific lipid volumes. Such corrections have to be made in order to derive the lipid hydrophobic thickness from lamellar repeat distances (Janiak et al. 1976, 1979; Cornell and Separovic 1983) and from measurements of the cross-sectional area per molecule (Lis et al. 1982; Evans and Kwok 1982; Cornell and Separovic 1983). Unfortunately, the method of Lewis and Engelman (1983 b) has so far only been applied to fluid bilayers. Another direct estimate of d_L^α may be derived from the average acyl chain orientational order parameter obtained from the first moment of the distribution of quadrupolar splittings in a deuterium-NMR spectrum (Schindler and Seelig 1975; Mouritsen and Bloom 1984; Covello et al. 1983). It appears that all these measures of d_L^α are in reasonably good agreement with each other (e.g. see Bloom and Mouritsen 1988).

It turns out from the bulk of the experimental data referred to above that d_L^f is, to a very good approximation, a linear function of the acyl chain length (Lewis and Engelman 1983 b)

$$d_L^f \cong 1.75(n_c - 1)\ [\text{Å}], \tag{16}$$

and that the bilayer hydrophobic thickness is about 30% larger in the gel phase (Janiak et al. 1976; Inoko and Mitsui 1978). Since the jump in thickness at the transition is large compared with the variations in d_L^g and d_L^f with temperature on both sides of the transition, we can safely neglect the latter temperature effect and assume d_L^g and d_L^f separately to be independent of temperature. Since pure Dn_cPC bilayers have a tilted rippled gel phase ($P_{\beta'}$) below T_m and since it is known that minor amounts of impurities remove the tilt as well as the ripples (Chapman et al. 1977; Silvius 1982), the experimentally determined values of the gel-phase hydrophobic thickness have to be corrected by a factor of cos θ, θ being the tilt angle, in order to arrive at a value of d_L^g which applies to lipid bilayers with integral proteins. The tilt angle has been measured to be around 30° immediately below T_m, approximately independent of the chain length (Janiak et al. 1976). Hence, we have the relation

$$d_L^g \cong 1.3\, d_L^f/\cos 30° \cong 1.50\, d_L^f. \tag{17}$$

Equations (15) and (16) suggest that the mean lipid hydrophobic thickness

$$\bar{d} = \tfrac{1}{2}(d_L^g + d_L^f) \cong 2.19\,(n_c - 1)\ [\text{Å}] \tag{18}$$

is a useful quantity to characterize the individual Dn_cPC lipid bilayers. Equations (17) and (18) together with Eq. (15) now show that at fixed x_P, the shift in transition temperature $\Delta T(\bar{d})$ is a hyperbolic function of $\bar{d}$.

Much less information is available on hydrophobic thicknesses of integral membrane proteins. In fact in many cases d_P is obtained indirectly from measurements of the lipid membrane thickness assuming hydrophobic matching (e.g. see Sadler et al. 1984; Johannsson et al. 1981). As we shall point out later, such indirect estimates may be in error if matching is deduced from a criterion of zero transition temperature shift. For proteins where the three-dimensional structure is known, d_P and ϱ_P may be determined rather accurately provided that the shape is reasonably regular and the region between the hydrophobic and hydrophilic regions is not too fuzzy. If the primary structure is known, d_P may in some cases be gauged from hydrophobicity plots (Kyte and Doolittle 1982; Eisenberg 1984; Klein et al. 1985) and the value of ϱ_P may be estimated from measurements of rotational diffusion of proteins (Cherry 1979).

The thermodynamic properties of the lipid bilayer phase transition are known from a great variety of

experimental techniques (Silvius 1982; Seelig 1981; Nagle and Wilkinson 1978). The striking result obtained from an analysis of the data for the Dn_cPC bilayers is that ΔH_L as well as T_m to a very good approximation are simple functions of n_c and thus, by Eq. (18), in $\bar{d}$:

$$\Delta H_L \text{ [kcal/mole]} \cong 0.59\,\bar{d} \text{ [Å]} - 9.52, \tag{19}$$

$$T_m\text{[K]} = \frac{-1262}{n_c - 3} + 412 = \frac{-1262}{(\bar{d}\text{ [Å]}/2.19 - 2)} + 412. \tag{20}$$

The linear relations, Eqs. (17) and (18) lead to a remarkable statement about the feasibility of obtaining the protein solubility parameters, v^α and ε, from experimental measurements of transition temperature shifts in lipid membranes with different values of $\bar{d}$ incorporated with proteins of different hydrophobic thicknesses: If the series of systems studied is confined solely to one of the cases (a)–(c) in Eqs. (11)–(13), measurements of $\Delta T(\bar{d})$ do not give access to the solubility parameters, no matter how many combinations of $\bar{d}$ and d_P are being considered. This statement holds exactly in the limit of $x_P \ll 1$ if the linearity conditions are rigorously fulfilled. If this is not the case, the solubility parameters may be determined from measurements of ΔT but with an accuracy which reflects the deviation from linearity. Similarly and paradoxically, imprecise data give access to ε and v^α. This remarkable geometrical constraint, which is explained in detail in the Appendix, suggests that protein solubility parameters are best determined by choosing lipids and proteins with values of $\bar{d}$ and d_P which fall in more than one of the brackets (a)–(b) in Eqs. (11)–(13).

Solubility parameters from experiment

At present, only limited experimental data are available from quantitative systematic studies of the physical effects due to mismatch of protein and lipid hydrophobic thicknesses. Most physical studies of lipid-protein interactions are concerned with measurements of $\Delta H(x_P)$ for a single type of protein in one or two different types of lipid bilayers with only small variations in $\bar{d}$ (Alonso et al. 1982; Gomez-Fernandez et al. 1980; Boggs and Moscarello 1978; Heyn et al. 1981; Morrow et al. 1986; van Zoelen et al. 1978; Chapman et al. 1977). Other studies concentrate on structural, dynamic, and functional effects due to lipid-protein interactions (for lists of references, see e.g. Bloom and Smith 1985; Mouritsen 1987). None of these studies have produced data which allow the solubility parameters of the present model to be determined. The systematic search for segregation of bacteriorhodopsin in fluid bilayers (Lewis and Engelman 1983a) demonstrates that this protein remains dispersed over a wide range of fluid lipid bilayer thicknesses, but no quantitative information is obtained on the phase behaviour as such. Conversely, the extensive studies by Davis et al. (Morrow et al. 1985; Huchilt et al. 1985) on amphiphilic polypeptide-phospholipid model membranes with large variations in hydrophobic mismatch give very precise information on the phase diagram and thus $\Delta T(x_P)$. However, these model membranes have an extra degree of freedom which cannot easily be accounted for by our model. This degree of freedom is the tilting of the long axis of the thin polypeptide rod. Tilting might be expected to occur when $d_P > d_L^\alpha$. It was recently demonstrated by a molecular dynamics calculation on the hydrophobic segment of the transmenbrane protein glycophorin in a membrane environment (O. Edholm and F. Jähnig unpublished) that tilting only required an energy of the order of the thermal fluctuations. Thus the solubility parameters have to be derived from model membranes incorporated with a bulky protein (with several transmembrane polypeptide segments) whose long axis will stay appoximately normal to the membrane plane. Only in this case will the effective values of ϱ_P and d_P be constant, phase-independent quantities.

The recent systematic experimental studies of the phase behaviour of bacterial photosynthetic RC proteins and antenna proteins reconstituted into Dn_cPC bilayer vesicles with $n_c = 12, 13, 14, 15$, and 16 (Riegler and Möhwald 1986; Peschke et al. 1987) provide a set of data for $\Delta T(x_P)$ and $\Delta T(\bar{d})$ which makes possible an attempt towards determination of the solubility parameters, ε, v^g, v^f. By detecting transmission changes due to light scattering, Möhwald and collaborators monitored the phase transition as a function of $\bar{d}$ and x_P. The experimental data are reproduced in Fig. 2 and 3. It is obvious that hydrophobic mismatch has a dramatic influence on the phase behaviour. Firstly, we note from Fig. 3 that the $\Delta T(x_P)$ data are consistent with the assumption of high dilution since it is found that, for both proteins, $\Delta T_{exp} \propto x_P$, cf. Eq. (15). Secondly, it is observed that the case (b): $d_L^g > d_P > d_L^f$, Eq. (12), applies since $d_P \cong 28$ Å for the RC protein and $d_P \cong 31$ Å for LHCP (Riegler and Möhwald 1986; Williams et al. 1983, 1984). Hence, according to the analysis in the Appendix, any determination of the solubility parameters will rely on deviations from perfect linearity or imprecise data.

For determining the solubility parameters using the experimental data of Figs. 2 and 3 we have adopted the following strategy: Since $\Delta T(\bar{d})$ has the most marked variation, we use the data of Fig. 2 as the main basis of a fit to Eq. (15). However, the data in Fig. 3 are considered to be the quantitatively most reliable (Peschke et al. 1987) with only insignificant protein-degradation during reconstitution and consequently with the most accurate values of x_P. Therefore, we have included these data in the fit and therefore given

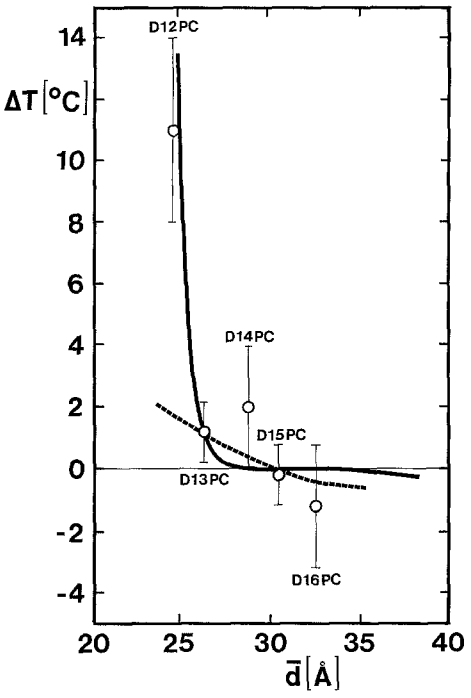

Fig. 2. Shift in midpoint transition temperature, $\Delta T(\bar{d})$, vs. mean lipid hydrophobic thickness $\bar{d}$ for a series of Dn_cPC lipid bilayers incorporated with RC protein in a molar concentration of $x_P = 10^{-4}$. Experimental data (Riegler and Möhwald 1986; Peschke et al. 1987) are indicated by *open circles* and appropriate errorbars. The *solid line* is the theoretical fit of Eq. (15) to the data using the solubility parameters, Eq. (21). The *dashed line* is the prediction from the Landau-de Gennes theory of elastic distortions, Eq. (25). Note that, in the work by Riegler and Möhwald (1986), no correction for tilt angle, Eq. (17), was made in the gel lipid bilayer hydrophobic thickness. Thus the horizontal axis of their Fig. 3 has been rescaled in the presentation of the present figure. A similar comment applies when comparing the dashed curve with Riegler and Möhwald's scale for $\bar{d}$

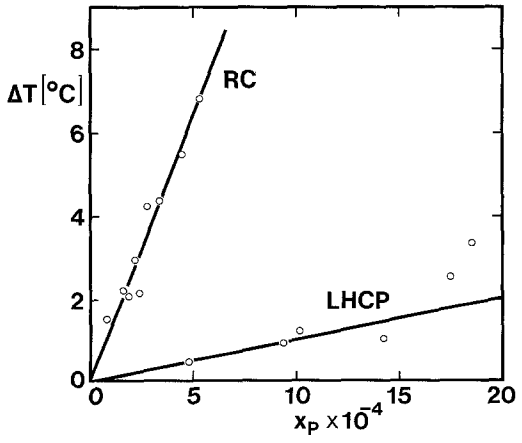

Fig. 3. Midpoint transition temperature shift, $\Delta T(x_P)$, vs. molar concentration, x_P, of RC proteins and LHCP protein, respectively, reconstituted into *D13*PC lipid bilayers. The experimental data of Peschke et al. (1987) are indicated by *open circles*, and the linear theoretical predictions from Eq. (15) are shown by *solid curves*

extra weight to the point for *D13*PC in Fig. 2. Finally, we have excluded from the fit the data for *D12*PC since the phase transition properties in this system may be obscured by the proximity of the water freezing point to $T_m(n_c = 12) \cong 0\,°C$. For the protein perimeters we have assumed $\varrho_P \cong 189$ Å for the RC protein (Williams et al. 1983, 1984) and $\varrho_P \cong 75$ Å for LHCP (Zuber 1985; Peschke et al. 1987). The best fit is obtained with the solubility parameters

$$\varepsilon \cong 1.6 \text{ cal/mol Å}^2,$$
$$v^f \cong 1.4 \text{ cal/mol Å}^2, \quad v^g \cong 0.3 \text{ cal/mol Å}^2. \tag{21}$$

The fit describes the entire set of data (even for RC proteins in *D12*PC) of Figs. 2 and 3 in a satisfactory way considering the experimental uncertainties involved in locating the midpoint transition temperature and in determining the precise protein content in the reconstituted membranes. The latter determination is based on photo absorption spectroscopy (Peschke et al. 1987) on the active proteins only and the actual total protein content for the data in Fig. 2 may be as much as two times larger than measured by photo absorption.

Predictions for other proteins

Assuming that the parameters, Eq. (21), are generally applicable to describe the solubility of amphiphilic transmembrane proteins in lipid bilayers, we can predict $\Delta T(\bar{d}, x_P)$ for other proteins. In Fig. 4, $\Delta T(\bar{d})$ is shown in the case of $x_P = 10^{-4}$ for a few "model proteins" characterized by different values of d_P and ϱ_P. The hyperbolic curve $\Delta T(\bar{d})$, Eq. (15), shown in Fig. 4 passes through $\Delta T = 0$ for a value of $\bar{d}$ which in the general case is different from d_P. Only in the case $v^g = v^f$ or $v^\alpha \ll \varepsilon$ does one obtain $\bar{d} = d_P$. This is an important observation since it implies that it is not strictly correct for a protein with unknown hydrophobic thickness to assign the value of d_P to be the mean hydrophobic thickness of that lipid bilayer which does not suffer from a transition temperature shift in the presence of the protein. However, the values of the solubility parameters in Eq. (21), and Figs. 2 and 4, indicate that $d_P \cong \bar{d}$ is a reasonable 10% approximation. When d_P is increased (for fixed ϱ_P), but still within $d_L^g > d_P > d_L^f$, the $\Delta T(\bar{d})$ curve is moved towards larger values of $\bar{d}$, although not in a perfect shape-conserving translation, cf. Eq. (A.1). Similarly, decreases in ϱ^P (for fixed d_P) act effectively to flatten the hyperbolic shape.

Considering the situation where d_P may be outside the range of the lipid bilayer gel and fluid hydrophobic thicknesses, Eqs. (11) and (13), a complicated scenario arises: For fixed d_P, there are singular points at $\bar{d} = d_P/1.2$ and $\bar{d} \cong d_P/0.8$ (given the solubility parameters of Eq. (21)). For $\bar{d} < d_P/1.2$, $\Delta T(\bar{d})$ is an increasing

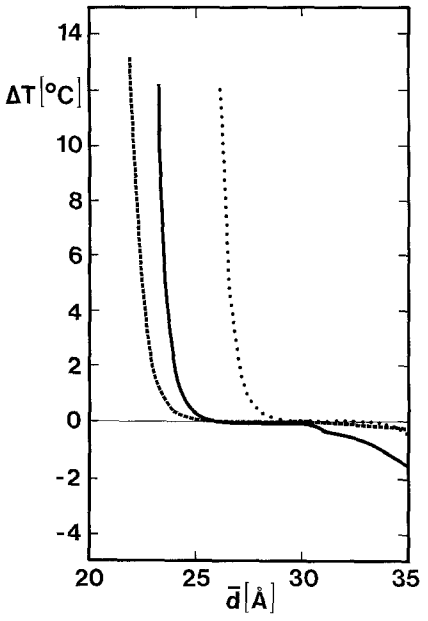

Fig. 4. Theoretical predictions from Eq. (15) of shifts in midpoint transition temperature $\Delta T(\bar{d})$ as a function of mean lipid hydrophobic thickness $\bar{d}$. Results are given for model proteins characterized by $(d_P, \varrho_P) = (25\,\text{Å}, 189\,\text{Å})$ (*dashed line*), (25 Å, 250 Å) (*solid line*), and (28 Å, 250 Å) (*dotted line*)

function and for $\bar{d} > d_P/0.8$ the decreasing behaviour of $\Delta T(\bar{d})$ seen in Fig. 2 is less strong. The latter behaviour is also illustrated in Fig. 4.

Finally, it should be pointed out that the strongly asymmetric shape of the curves in Fig. 4 will be less pronounced if the value of ε is increased. The uncertainty of ε is 10%–20%, and for e.g. $\varepsilon \cong 2\,\text{cal/mol Å}^2$, there is a much stronger decrease of ΔT on the negative axis before the singular point is reached, thereby more clearly exposing the hyperbolic shape of $\Delta T(\bar{d})$.

Comparison with elasticity theories

The theory of protein solubility in lipid bilayers presented in this paper considers linear effects of hydrophobic matching as well as direct hydrophobic-hydrophobic lipid-protein interactions as main determinants of the phase behaviour at low protein concentrations. Effects of elastic distortions of the lipid bilayer sheet have been neglected. Two types of theoretical approaches exist for lipid-protein interactions in membranes which explicitly incorporate elastic interactions: Landau-de Gennes theory (Owicki et al. 1978; Owicki and McConnell 1979; Jähnig 1981) and the mattress model (Mouritsen and Bloom 1984). Since there are some conceptual differences between these two approaches, we shall discuss their implications for our work separately.

These mattress moldel, from which the present solution theory is derived in the $x_P \ll 1$ limit adds to the free energy expression in Eq. (1) an elastic energy term of the form

$$G_{\text{el}} = A_L (d_L - d_L^0)^2, \tag{22}$$

where d_L^0 is the unperturbed lipid bilayer hydrophobic thickness. A_L is an elastic constant which can be related to the elastic area compressibility modulus at constant temperature (Mouritsen and Bloom 1984; Evans and Skalak 1979). For lecithins, micromechanical measurements by Evans and Kwok (1982) suggest $A_L^{\text{f}} \cong 4.8\,\text{cal/mol Å}^2$ and $A_L^{\text{g}} \cong 8.6\,\text{cal/mol Å}^2$. In equilibrium, Eq. (22) leads to

$$d = d_L^0 + \left(\frac{\varrho_P \gamma}{2 A_L}\right) x_P\,. \tag{23}$$

The equation implies that the elastic energy contribution only enters to second order in x_P. For $x_P \leqq 10^{-3}$ and with the solubility parameters of Eq. (21) the elastic interactions can safely be ignored.

Turning now to Landau-de Gennes theory, the free energy of the membrane is written (Jähnig 1981)

$$G = G^0 + \frac{2\pi b}{(d_L^{0,\text{f}} - d_L^{0,\text{g}})^2}\left(\frac{\varrho_P}{\pi \xi} + 1\right)(d_P - d_L)^2, \tag{24}$$

where G^0 is the free energy of the unperturbed membrane. b is a phenomenological constant related to an elastic energy of spatial density fluctuations in the membrane and ξ is a characteristic coherence length of the lipid membrane. The concentration dependence is now implicitly given through the equilibrium bilayer thickness d_L. It is to be noted that, in contrast to the mattress model, the Landau-de Gennes theory attributes the full excess free energy to an elastic distortion and that this excess free energy is proportional to the square of the mismatch rather than the square of the distortions of the lipid bilayer thickness, cf. Eq. (22). Furthermore, phase separation is not allowed within this description. From Eq. (24) the shift in transition temperature is found to be (Peschke et al. 1987)

$$\Delta T = 16\,\xi^2\left(\frac{\varrho_P}{\pi \xi} + 1\right)\left(\frac{\bar{d} - d_P}{d_L^{0,\text{f}} - d_L^{0,\text{g}}}\right) x_P\,. \tag{25}$$

Hence, within Landau-de Gennes theory, $\Delta T = 0$ for $d_P = \bar{d}$ which is a consequence of the neglect of the direct hydrophobic-hydrophobic lipid-protein interactions which are included explicitly in our solution theory. It should be noted that the ΔT of Eqs. (15) and (25) are in principle different quantities since a sharp phase transition remains at $x_P \neq 0$ in the Landau-de Gennes theory whereas a two-phase coexistence region is allowed for in our solution theory.

By assuming $\xi \cong 15$ Å (Jähnig 1981), Peschke et al. (1987) obtain good agreement with the experimental

data for $\Delta T(x_P)$ in the case of RC proteins in *D13*PC. However, for LHCP in *D13*PC bilayers, the predictions of $\Delta T(x_P)$ from Eq. (25) are an order of magnitude too large. Peschke et al. have to invoke aggregation of LHCP proteins into aggregates of approximately 50 molecules to modify the effective values of x_P and ϱ_P in order to bring the theory in accordance with experiments. There are some indications from freeze-fracture electron micrographs (Peschke et al. 1987) and independent optical experiments (Grondelle et al. 1983) that the small LHCP proteins are subject to some aggregation. It is, however, difficult to determine directly how large these aggregates are and what the size distribution is. For the RC protein Peschke et al. (1987) found that the proteins remain dispersed in the bilayers except for the gel phase of *D16*PC. If such aggregates are compact, uniform in size, and each comprises n molecules, x_P and ϱ_P of both theories should be renormalized by n and $1/\sqrt{n}$, respectively. It is far from clear that the protein aggregates are compact but rather incorporate a substantial part of connected interstitial lipid domains (Pink et al. 1981). Furthermore, the aggregates may have different size distributions in the two lipid phases. Therefore, we hesitate to make the issue of aggregation a central one for comparing the two theories.

Rather, we find it instructive to return to the data for $\Delta T(\bar{d})$ for the RC protein in Fig. 2 and compare the predictions from respectively the solution theory, Eq. (15), and the Landau-de Gennes theory, Eq. (25). It is obvious from such a comparison that, although the two theories equally well account for the experimental data at medium $\bar{d}$, they produce significantly different results at more extreme bilayer thicknesses. In particular, the elastic theory fails to reproduce the dramatic increase in ΔT for *D12*PC. This gives some indication that the solution theory is a more reliable theory, although it should be born in mind that the experimental data for *D12*PC may be influenced by the proximity of the water freezing point to $T_m(n_c = 12)$, Eq. (20) (Riegler and Möhwald 1986). We believe that comparison between theoretical and experimental data for $\Delta T(\bar{d})$ rather $\Delta T(x_P)$ presents a more critical test of the theories. More experimental data are required, however, to decide which of the two theoretical approaches discussed here is the more reliable one.

Conclusion

The physical forces underlying lipid-protein interactions in membranes are still a subject of considerable controversy. In this paper we have presented a two-component solution theory derived from the mattress model of Mouritsen and Bloom (1984) which identifies matching of protein and lipid hydrophobic thicknesses as an important parameter for the phase behaviour of membranes with integral proteins. The theory differs from Landau-type phenomenological elasticity theories in that it accounts for the direct interaction between the hydrophobic parts of the two species and it furthermore allows for phase separation. The solubility parameters of the theory have been determined by fitting to experimental data for phase transition temperature shifts obtained from systematic studies of RC and LHCP proteins reconstituted into lipid bilayers of varying hydrophobic thickness (Riegler and Möhwald 1986; Peschke et al. 1987). The experimental data are well described by the theory. Furthermore, we note that the spectroscopic and thermodynamic data for a great variety of proteins and polypeptides reconstituted into lipid membranes (Silvius 1982) are consistent with a decreasing transition temperature shift $\Delta T(\bar{d})$ when the bilayer thickness is increased.

The overall success of our theory in accounting for the existing experimental data suggests that it is unnecessary to invoke elastic interactions to explain, in the low-concentration regime, the thermodynamic consequences of mismatch of protein and lipid bilayer hydrophobic thicknesses. We believe that the solubility parameters of the theory have some general applicability which is supported by the recent finding of similar solubility parameter values for the mattress model of two-component lipid-lipid mixtures (J. Hjort Ipsen and O. G. Mouritsen unpublished). Consequently, there are reasons to believe that our solution theory may be useful for guiding and suggesting further experiments geared towards a fuller understanding of the physical forces governing lipid-protein interactions.

A final remark is in order on the possible significance of hydrophobic matching for the functioning of biological membranes. It is now anticipated that integral proteins are approximately matched to the fluid bilayer thickness of their natural membranes (Mouritsen and Bloom 1984; Bloom and Smith 1985; Bloom and Mouritsen 1988) in order to function optionally (for a recent list of references to pertinent experimental work, see Mouritsen 1987). Local or global changes in the conditions for matching, e.g. as induced by changes in lateral composition or by external chemical gradients or electrical fields, may have dramatic effects on protein activity and could indeed form the basis for trigger processes (Sackmann 1984). Finally, it has recently been suggested that the concept of hydrophobic matching may have played an important role in the evolution of biological membranes (Bloom and Mouritsen 1988).

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Appendix

Using Eqs. (17) and (18), the parameter Γ of Eqs. (11) to (13) may be written as a function of mean lipid hydrophobic thickness $\bar{d}$, e.g. in the case (b), Eq. (12),

$$\Gamma(\bar{d}) = (3\,d_P - 3.2\,\bar{d})\,\varepsilon + 0.8\,\bar{d}\,v^{\mathrm{f}} - d_P\,v^{\mathrm{g}}. \tag{A.1}$$

Using Eq. (15) we also have

$$\Gamma(\bar{d}) = \frac{RT_m(\bar{d})}{\varrho_P}\,\mathrm{arcsinh}\left(\frac{\Delta T(\bar{d})\,\Delta H_L(\bar{d})}{RT_M^2(\bar{d})\,x_P}\right) \tag{A.2}$$

Considering $T_m(\bar{d})$ and $\Delta H_L(\bar{d})$ to be known, a series of values of $\Gamma(\bar{d})$ can be obtained by measuring $\Delta T(\bar{d})$ for a variety of combinations of $\bar{d}$, x_P, and choices of protein (i.e. d_P and ϱ_P). For any three different measurements, labelled $i = 1, 2, 3$, the solubility parameters ε, v^{g}, and v^{f} should be obtained by solving the set of linear equations

$$\bar{\bar{A}}\,\boldsymbol{\eta} = \boldsymbol{F}, \tag{A.3}$$

where

$$\bar{\bar{A}} = \begin{pmatrix} 3\,d_P^{(1)} - 3.2\,\bar{d}^{(1)} & 0.8\,\bar{d}^{(1)} & -d_P^{(1)} \\ 3\,d_P^{(2)} - 3.2\,\bar{d}^{(2)} & 0.8\,\bar{d}^{(2)} & -d_P^{(2)} \\ 3\,d_P^{(3)} - 3.2\,\bar{d}^{(3)} & 0.8\,\bar{d}^{(3)} & -d_P^{(3)} \end{pmatrix} \tag{A.4}$$

is a (3×3) matrix, and

$$\boldsymbol{\eta} = \begin{pmatrix} \varepsilon \\ v^{\mathrm{g}} \\ v^{\mathrm{f}} \end{pmatrix}, \quad \boldsymbol{F} = \begin{pmatrix} \Gamma(\bar{d}^{(1)}) \\ \Gamma(\bar{d}^{(2)}) \\ \Gamma(\bar{d}^{(3)}) \end{pmatrix} \tag{A.5}$$

We now make the observation that $\det \bar{\bar{A}} = 0$. This implies that Eq. (A.3) either has no solution or an infinite set of solutions. Analysis of the rank of $\bar{\bar{A}}$ and the rank of the augmented matrix $(\bar{\bar{A}}, \boldsymbol{F})$ shows that it is the case of no solution which applies here. Basically, this finding is a consequence of the linearity relation, Eq. (17), which underlies Eq. (12). The same statement holds separately for the cases (a) and (c), Eqs. (11) and (13). Thus, if the linearity relations hold and measurements are made only within one of the brackets, (a)–(c), Eqs. (11)–(13), it is not possible to derive the solubility parameters from experimental measurements of transition temperature shifts. This is a remarkable observation which suggests that, in order to obtain solubility parameters from such experiments in the $x_P \ll 1$ limit, lipids and proteins should be chosen so as to fall within more than one of the brackets for the hydrophobic thickness relations. In the case of slight departure from the linearity relations for d_L^{α}, the same measures should be taken in order to improve the accuracy of the derived solubility parameters.

For higher protein concentrations, the statements in this Appendix will not be valid since the phase boundaries will, by Eq. (22) and (23), cease to be linear in x_P.

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