Simultaneous Modeling of Phase and Calorimetric Behavior in an Amphiphilic Peptide/Phospholipid Model Membrane†

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ABSTRACT: Differential scanning calorimetry (DSC) experiments have been performed on the amphiphilic peptide/1,2-bis(perdeuteriopalmitoyl)-*sn*-glycero-3-phosphocholine system for which partial phase diagrams have been measured by deuterium nuclear magnetic resonance. The solute concentration dependence of the transition enthalpy in such systems is often interpreted in terms of an annulus of lipid withdrawn, by the solvent, from participation in the transition while the bulk lipid melts with its fully enthalpy. This idea is equivalent to postulating ideal mixing between the lipid and the peptide/lipid complex, and there is little justification for such an assumption. Adaptation of regular solution theory to this system demonstrates that the peptide concentration dependence of the transition enthalpies can be incorporated into a thermodynamic model which reproduces the observed phase behavior fairly well without postulating that a complexing annulus of lipid around the peptide be withdrawn from participating in the chain-melting transition. The model parameters determined by simultaneous fitting of the phase behavior and transition enthalpies are used to simulate the DSC scan shapes. The asymmetry of the calorimetric scans for $X_2 \leq 0.02$ is reproduced by the model, but a broad component observed for higher concentration is not. In light of the results presented here, previous analyses of the calorimetric behavior of two-component systems in terms of symmetric transitions which do not account for the possible extent of a region of two-phase equilibrium must be questioned.

A number of authors have considered the phase behavior of two-component systems. Lee (1977, 1978) has described two-component lipid mixtures in terms of nonideal solution theory using an excess free energy term proportional to the product of the mole fractions of the components. Cheng (1980) has employed a similar model. There has, however, been less work done on treating the protein/lipid system as a two-component solution with a phase diagram displaying regions of two-phase coexistence. Eigenberg et al. (1982) have adapted Lee's approach in modeling the liquidus for mixtures of chlorophyll *a* and 1,2-distearoyl-*sn*-glycero-3-phosphocholine (DSPC).[1] Rowe (1983) has used a similar picture to model the liquidus in ethanol/lipid mixtures. Mouritsen & Bloom (1984) have presented a phenomenological model for the phase behavior of bilayers spanned by amphiphilic peptide molecules. Their model takes a form analogous to that of regular solution theory with the parameters of the excess free energy of mixing dependent on the mismatch between length of the bilayer-spanning portion of the peptide and bilayer thickness.

The presence of a region of two-phase coexistence would be expected to have a marked effect on the results of differential scanning calorimetry (DSC) experiments on two-component systems. A number of workers have recognized this relationship between the slope of the DSC trace and the nature of the two-phase region. One approach has been to use the onset and completion temperatures of the transition, as determined by DSC, to map out the phase boundaries. This type of analysis has been used on a number of lipid/lipid mixtures (Mabrey & Sturtevant, 1976; Keough & Davis, 1979; Silvius & Gagné, 1984a,b; Barenholz et al., 1983). Eigenberg et al. (1982) have also applied this method to chlorophyll *a*/lipid mixtures. The inverse of this approach, simulating the DSC trace by using known phase behavior, has been done by Mabrey & Sturtevant (1976) for a number of lipid/lipid mixtures. Simulations of DSC experiments have also been carried out for 1,2-dipalmitoyl-*sn*-glycero-3-phosphocholine (DPPC)/egg yolk phosphatidylcholine mixtures (Tinker & Low, 1982) and bacteriorhodopsin/1,2-dimyristoyl-*sn*-glycero-3-phosphocholine (DMPC) mixtures (Heyn et al., 1981).

Another aspect of DSC experiments is the dependence of the total transition enthalpy on solute concentration. For lipid/lipid mixtures displaying nearly ideal mixing, the enthalpy is simply a weighted average of the pure lipid enthalpies as has been demonstrated by Chen & Sturtevant (1981) and Silvius & Gagné (1984a,b). Apparently, however, only Mouritsen & Bloom (1984) have suggested that the integrated enthalpy might be derivable from the phase behavior for protein/lipid mixtures.

There has, however, been a great deal of DSC work done on mixtures of lipids with other molecules where the possibility of there being regions of two-phase equilibrium was not included in the analysis. For example, the broadening of the gel to liquid-crystalline transition with increasing concentration of a solute is sometimes discussed in terms of the van't Hoff equation and decreasing size of the cooperative unit. Such interpretations have been applied to the cases of bacteriorhodopsin/lipid mixtures (Heyn et al., 1981; Alonso et al.,

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[1] Abbreviations: DSPC, 1,2-distearoyl-*sn*-glycero-3-phosphocholine; DSC, differential scanning calorimetry; DPPC, 1,2-dipalmitoyl-*sn*-glycero-3-phosphocholine; DMPC, 1,2-dimyristoyl-*sn*-glycero-3-phosphocholine; DPPC-d_{62}, 1,2-bis(perdeuteriopalmitoyl)-*sn*-glycero-3-phosphocholine; ^{2}H NMR, deuterium nuclear magnetic resonance; peptide 16, Lys_2-Gly-Leu_{16}-Lys_2-Ala-amide; peptide 24, Lys_2-Gly-Leu_{24}-Lys_2-Ala-amide.

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1982) and dolichol/lipid and dolichyl phosphate/lipid mixtures (Vigo et al., 1984). In a general two-component system, however, it is possible for the transition to occur across a temperature range over which two phases of different composition may be in equilibrium. As we shall discuss below, the effects which influence the temperature extent of a region of two-phase equilibrium may be too complex to be summarized in a single parameter such as the cooperative unit size. For example, there is no reason to expect a calorimetric scan across a two-phase region to be symmetric. The van't Hoff equation, however, implies a particular shape for the transition, and when confronted with a sufficiently asymmetric DSC trace, some workers have chosen to resolve it into components which conform to the van't Hoff equation (Spink et al., 1982; Davis & Keough, 1983). Such an approach, taken without reference to the possibility of a region of two-phase equilibrium, seems to be an unjustified complication. A further difficulty, as has been recognized by Chowdry et al. (1984), arises in the inconsistency with the phase rule of interpreting such components to be transitions between distinct phases.

The concept of "boundary lipid" has been a common element of many analyses of DSC experiments on two-component membrane systems. In this picture, the decrease in transition enthalpy with increasing solute concentration is taken to be the result of an annulus of lipid, around the solute molecule, which is withdrawn from participation in the gel to liquid-crystalline transition. This type of analysis has been applied to mixtures of lipid with a number of solute molecules including cytochrome oxidase (Semin et al., 1984), bacteriorhodopsin (Alonso et al., 1982), glycophorin (Ruppel et al., 1982), ATPase (Gomez-Fernandez et al., 1980), and gramicidin A (Chapman et al., 1977). The concentration at which the enthalpy extrapolates to zero is taken to be that concentration for which all of the lipid has been withdrawn from the transition. That the annulus cannot be a static structure is well-known from deuterium nuclear magnetic resonance (^{2}H NMR) work on lipid/protein systems (Davis, 1983). The boundary lipid picture thus implies the existence of a dynamic lipid/solute complex. The idea that the lipids outside of the complex undergo the gel to liquid-crystalline transition with the full pure lipid enthalpy implies that they and the complexing lipids are thermodynamically independent. This type of analysis may not provide a complete picture of two-phase coexistence inherent in the phase behavior of such systems.

Recently, synthetic amphiphilic peptides have become available for use as protein analogues in model membrane systems (Davis et al., 1983). Huschilt et al. (1985) have explored the phase behavior of such systems with ^{2}H NMR difference spectroscopy. With their phase diagram, determined without the aid of calorimetric measurements, we have been able to explore the extent to which DSC experiments on this system can be shown to be consistent with the thermodynamics governing its phase behavior. We have chosen to model the phase behavior in terms of an adaptation of the nonideal solution theories discussed by Lee (1977, 1978) and Mouritsen & Bloom (1984). Unfortunately, a lack of independent knowledge about the peptide standard state prevents the unambiguous assignment of the model parameters on the basis of the observed phase behavior alone. The parameters which are found provide the best simultaneous approximation to both the phase and enthalpy data. Using the thermodynamic parameters found in conjunction with a fairly realistic model for the calorimeter response, we have simulated DSC scans for the range of peptide concentrations studied. We have also considered the question of whether the DSC results reflect equilibrium properties of the peptide/lipid system by comparing them to results obtained by further analysis of the ^{2}H NMR spectral moments obtained by Huschilt et al. (1985).

On this basis, we comment on a number of situations in which care should be exercised in analyzing DSC results on lipid/protein mixtures. These points have mainly to do with the shape of the DSC trace and the interpretation of the decrease in transition enthalpy with increasing solute concentration.

Materials and Methods

The synthesis of the peptide Lys_2-Gly-Leu_n-Lys_2-Ala-amide has been described previously (Davis et al., 1983). Peptide 16 has a hydrophobic core of 16 leucines with hydrophilic amino acids at either end. In peptide 24, the hydrophobic core consists of 24 leucines.

The mixtures of peptide and 1,2-bis(perdeuteriopalmitoyl)-*sn*-glycero-3-phosphocholine (DPPC-d_{62}) were prepared as described by Huschilt et al. (1985) for the NMR samples. The peptide mole fractions used were 0.005, 0.010, 0.0149, 0.0197, 0.0243, and 0.0323 for both peptides. Typical DSC samples were prepared by adding approximately 200 μL of 0.05 M phosphate buffer at pH 7.0 to approximately 5 mg of peptide/lipid mixture in the DSC cell. The sample was then vortexed and centrifuged before being incubated at 0 °C for up to 24 h. DSC runs were performed on a Microcal MC1 high-sensitivity differential scanning calorimeter at a scan rate of about 10 °C/h. In all cases, the first scan was discarded. All of the peptide 16 samples and many of the peptide 24 samples were scanned with the calorimeter interfaced to a slave microcomputer for data acquisition and storage. Data analysis was performed on an Intel series II microcomputer development system. The base line on either side of the transition was determined by least-squares fitting. A zero-order base line through the transition was determined by linear interpolation between the fitted base line on either side. This base line was then used to estimate the integral of the trace as a function of temperature. This integral was in turn used to construct a better base line by using the fraction of the transition completed to determine the fraction of each of the fitted base lines to combine at each point. The calculated base line was subtracted from the raw scan and the result used for further analysis and determination of the integrated enthalpies.

A number of the lipid/peptide 24 mixtures were run without computer data acquisition. For these scans, the base line was interpolated visually, and the peaks were integrated by planimeter.

The ^{2}H NMR results discussed here were obtained by further analysis of the spectra obtained by Huschilt et al. (1985).

Theoretical Procedures

Models for Two-Component Phase Behavior. The thermodynamics of binary mixtures have been discussed by a number of authors including Hildebrand & Scott (1950), Guggenheim (1952), and Swalin (1972). The free energy for a mixture of two components may be written

$$G^\alpha = n_1^\alpha \mu_1^\circ(\alpha,T) + n_2^\alpha \mu_2^\circ(\alpha,T) - T\Delta S_m^\alpha + \Delta H_m^\alpha \quad (1)$$

where ΔS_m^α and ΔH_m^α are the entropy and enthalpy, respectively, of mixing, n_i^α represents the number of moles of component i in phase α, and $\mu_i^\circ(\alpha,T)$ represents the chemical potential for the standard state of component i corresponding to phase α at temperature T. The mixing is said to be ideal if $\Delta H_m^\alpha = 0$ and

$$\Delta S_m^\alpha/R = -n_1^\alpha \ln X_1^\alpha - n_2^\alpha \ln X_2^\alpha = \Delta S_m^{\alpha,\text{ideal}}/R \quad (2)$$

where $X_i^\alpha = n_i^\alpha/(n_1^\alpha + n_2^\alpha)$ is the mole fraction of component i in phase α. There are various ways in which the mixture may exhibit nonideality. If $\Delta H_m^\alpha = 0$ but $\Delta S_m^\alpha \neq \Delta S_m^{\alpha,ideal}$, the mixing is said to be athermal. Such nonideality might, for example, arise if the solute and solvent molecules were of very different sizes. This case has been discussed by Flory (1942), who finds that the entropy of mixing can be approximated by

$$\Delta S_m^\alpha/R = -n_1^\alpha/\beta \ln \phi_1^\alpha - n_2^\alpha \ln \phi_2^\alpha \quad (3)$$

where component 1 has been designated as the solvent and β is the ratio of molecular volumes. In eq 3

$$\phi_i^\alpha = \frac{n_i^\alpha q_i}{n_1^\alpha q_1 + n_2^\alpha q_2} \quad (4)$$

is the effective volume fraction of component i in phase α and q_i is its effective molar volume. In Flory's treatment, which is for a polymer solute, β is the number of solvent molecules which may be replaced by a freely orienting segment of the polymer.

If $\Delta S_m^\alpha = \Delta S_m^{\alpha,ideal}$ but $\Delta H_m^\alpha \neq 0$, the mixing is said to be regular. This would come about, for example, if the solvent/solute interaction was not the average of the solvent/solvent and solute/solute interactions. If the solvent and solute molecules are of comparable size, a quasi-lattice treatment (Guggenheim, 1952) yields

$$\Delta H_m^\alpha = X_2^\alpha(1 - X_2^\alpha)\rho^\alpha \quad (5)$$

where ρ^α depends on the difference between solute/solvent and the average solute/solute and solvent/solvent interactions. Lee (1978) has used such a term to describe the nonideality of mixing in lipid/lipid mixtures but has identified it as excess free energy rather than as excess enthalpy of mixing.

If the solvent/solute interaction differs sufficiently from the average of the solvent/solvent and solute/solute interactions, the mixture will tend toward a nonrandom distribution of solute molecules and thus have a nonideal entropy as well as enthalpy of mixing. The resulting mixture is referred to by Swalin (1972) as nonregular and may be treated quasi-chemically as described by Guggenheim (1952).

We have chosen to model mixing in the peptide/lipid system with a free energy analogous to that used in regular solution theory. We thus restrict the model to six parameters which are formally identified with those of regular solution theory. We will, however, choose the peptide standard state to be its dilute mixture with the lipid. As we discuss below, such a choice requires a somewhat different interpretation of some model parameters. In attempting to account for the difference in size of the two molecules, we have introduced two additional parameters which are not allowed to vary in the fitting process. For the small peptide concentrations discussed here, the qualitative results are relatively insensitive to these size corrections.

The change in lipid standard chemical potential across the phase transition can be well approximated with parameters accessible from calorimetry on the pure lipid sample. Denoting the lipid by the letter l, it is common to take

$$\mu_1^\circ(l,T) - \mu_1^\circ(g,T) \simeq \Delta H_1 T_{c_1} - T\Delta H_1(T_{c_1})/T_{c_1} \quad (6)$$

where T_{c_1} is the melting temperature of the pure lipid and $\Delta H_1(T_{c_1})$ is its enthalpy of melting.

We choose the dilute solution of peptide in lipid phase α as the peptide standard state in phase α. This raises a question as to the distinction between the peptide chemical potential and the term derived from the excess enthalpy of mixing. Within regular solution theory, the chemical potential of the solute includes the terms $\mu_2^\circ(\alpha,T) + \rho^\alpha(1 - X_2^\alpha)^2$. Together, these terms describe the effect of solute/solvent interactions, through the influence of the peptide on the bilayer, on the change in free energy of the lipid/peptide system per mole of solute dissolved. Both terms survive at infinite dilution. Along with the more dominant entropy of mixing, the effect, per mole of peptide, of the peptide on the bilayer free energy includes $\mu_2^\circ(\alpha,T) + \rho^\alpha$. For nondilute solute concentrations, the effect of solute/solute interaction must be included, and this leads to the concentration dependence of the term $\rho^\alpha(1 - X_2^\alpha)^2$. A positive ρ^α implies that solute/solute contact lowers the free energy and indicates an inclination to cluster. If solute/solute contact raises the free energy, ρ^α is negative, and the mixing deviates from randomness by lowering the amount of solute/solute contact. Across the transition, the change in peptide chemical potential includes $[\mu_2^\circ(l,T) + \rho^l(1 - X_2^l)^2] - [\mu_2^\circ(g,T) + \rho^g(1 - X_2^g)^2]$. Of this amount, $[\mu_2^\circ(l,T) - \mu_2^\circ(g,T)] + (\rho^l - \rho^g)$ gives the effect of isolated solute on the change in μ_2 at the transition while the remainder represents, to a first approximation, the effect of solute/solute interactions. The distinction between $\mu_2^\circ(\alpha,T)$ and ρ^α is meaningful only for solute concentrations high enough that solute/solute interaction influences the phase behavior. We will approximate the change in peptide standard chemical potential with an expression analogous to that for the lipid component:

$$\mu_2^\circ(l,T) - \mu_2^\circ(g,T) \simeq \Delta H_2 - T\Delta S_2 \quad (7)$$

where ΔH_2 and ΔS_2 must be determined.

In the usual presentation of regular solution theory, the molecules interact only by direct contact. In the peptide/lipid system, it is unlikely that the interactions are restricted to nearest neighbors. One can imagine a number of effects which might contribute to the interactions. Mouritsen & Bloom (1984), in constructing a mattress model for protein/lipid interactions, have considered three effects: an elastic energy associated with changes in average length of the peptide or thickness of the bilayer due to mixing of the two components, a term depending on the amount of hydrophobic surface exposed to the aqueous medium by mismatch in component lengths, and an adhesive term associated with van der Waals interactions between molecules. They choose not to treat the curvature of the bilayer surface near the peptide, but we should be aware that such an effect might also contribute to the free energy of any real system we attempt to model. It is also likely that the free energy is influenced by changes in the enthalpy and entropy of lipids whose motions are perturbed by the presence of peptide molecules. If the perturbation is extended into the bilayer, then the possibility exists for peptide molecules to interact without coming into direct contact. If this is the case, then the usual result for ρ^α in terms of nearest-neighbor interactions (Guggenheim, 1952) does not hold. We will, however, assume the validity of the qualitative interpretation for the ρ^α term as a concentration-dependent correction to the solute chemical potential. We will also assume that the explicit concentration dependence for this term reflects the amount of peptide/lipid contact.

For lipid/lipid mixtures, the two components are of comparable size, and it is reasonable to follow Lee (1978) in using a regular solution-type free energy with the excess free energy expressed in terms of mole fractions. The peptide, however, spans the bilayer and has a cross-sectional area approximately 3 times that of the lipid. The different sizes of peptide and lipid are expected to influence both the entropy and enthalpy of the mixture. For the entropy, we have chosen to use Flory's expression (eq 3) with $\beta = q_2/q_1 = 6$. Hildebrand & Scott (1950) suggest that the excess enthalpy should be proportional

to the product of the effective volume fractions of the two components but recognize that for a mixture of molecules of different shapes, the q_i should be an effective surface of the molecule rather than its volume. We have estimated the effect of size difference on the excess enthalpy of mixing by applying the quasi-chemical treatment presented by Guggenheim (1952) to a planar triangular lattice on which lipids occupy sites singly and peptides occupy triangular triplets of sites. The effect of size enters through the ratio of effective molar perimeters, γ. On the basis of the number of "contacts" each "molecule" can make with surrounding lattice sites in this model, we take $\gamma = 4$ and choose the following expression for the free energy of the peptide/lipid mixture at low peptide concentration:

$$G^\alpha = n_1^\alpha \mu_1^\circ(\alpha,T) + n_2^\alpha \mu_2^\circ(\alpha,T) + \frac{1}{\beta} RT \ln\left(\frac{n_1^\alpha}{n_1^\alpha + \beta n_2^\alpha}\right) + n_2^\alpha RT \ln\left(\frac{\beta n_2^\alpha}{n_1^\alpha + \beta n_2^\alpha}\right) + \frac{\rho^\alpha}{\gamma}\left(\frac{n_1^\alpha n_2^\alpha}{n_1^\alpha + \gamma n_2^\alpha}\right) \quad (8)$$

One application of eq 8 is to write the free energy change for converting a mixture at a given composition from the gel phase to the liquid-crystal phase. For a mixture of n_1 moles of lipid and n_2 moles of peptide, the free energy change is

$$\Delta G = n_1[\mu_1^\circ(l,T) - \mu_1^\circ(g,T)] + n_2[\mu_2^\circ(l,T) - \mu_2^\circ(g,T)] + \frac{\rho^l - \rho^g}{\gamma}\frac{n_1 n_2}{n_1 + \gamma n_2} \quad (9)$$

where we assume β and γ to be independent of phase. Employing eq 6 and 7 and the expression $\Delta H = \partial(\Delta G/T)/\partial(1/T)$, we find the enthalpy change for the transition to be

$$\Delta H = n_1 \Delta H_1(T_{c_1}) + n_2 \Delta H_2(T_{c_1}) + \frac{\rho^l - \rho^g}{\gamma}\frac{n_1 n_2}{n_1 + \gamma n_2} \quad (10)$$

We identify this ΔH with the integrated enthalpy of a DSC scan and see that, for small peptide concentration, a plot of $\Delta H/n_1$ vs. X_2 should display an intercept of $\Delta H_1(T_{c_1})$ and a slope of $\Delta H_2(T_{c_1}) + (\rho^l - \rho^g)/\gamma$. The slope of such plots has often been interpreted as reflecting the number of lipids prevented from participating in the transition due to their being perturbed by solute molecules (Akhrem et al., 1982; Ruppel et al., 1982; Gomez-Fernandez et al., 1980; Boggs et al., 1980; Boggs & Moscarello, 1978; Chapman et al., 1977). On the time scale of ^{2}H NMR (10^{-6}–10^{-3} s), however, there is little evidence for a distinct class of lipids (Davis, 1983). As we discuss below, for the current peptide/lipid system, such an interpretation is entirely untenable. For the purpose of describing phase behavior, it is thus preferable to invoke only one class of lipid and to interpret the slope of $\Delta H/n_1$ vs. X_2 as a measure of the extent to which the presence of the solute affects the lipid chain-melting transition by altering the change in enthalpy of the system.

In order to explore the phase behavior for this system, we obtain the chemical potentials for the two components from $\mu_i^\alpha = \partial G^\alpha/\partial n_i^\alpha$. The condition for the gel and liquid-crystal phases to be in equilibrium is that, for each component, the chemical potential in the two phases be equal. The resulting equalities are

$$-\frac{T - T_{c_1}}{T_{c_1}}\Delta H_1(T_{c_1}) + \frac{RT}{\beta}\ln\left[\frac{1 - X_2^l}{1 - X_2^g}\frac{1 - X_2^g(1-\beta)}{X_2^l(1-\beta)}\right] + \rho^l\frac{X_2^{l\,2}}{[1 - X_2^l(1-\gamma)]^2} - \rho^g\frac{X_2^{g\,2}}{[1 - X_2^g(1-\gamma)]^2} = 0 \quad (11)$$

and

$$\Delta H_2(T_{c_1}) - T\Delta S_2(T_{c_1}) + RT\ln\left[\frac{X_2^l}{X_2^g}\frac{1 - X_2^g(1-\beta)}{1 - X_2^l(1-\beta)}\right] + \frac{\rho^l}{\gamma}\frac{(1 - X_2^l)^2}{[1 - X_2^l(1-\gamma)]^2} - \frac{\rho^g}{\gamma}\frac{(1 - X_2^g)^2}{[1 - X_2^g(1-\gamma)]^2} = 0 \quad (12)$$

where we have divided n_i^α by $n_1^\alpha + n_2^\alpha$ to obtain the results in terms of mole fractions, X_i^α. The boundaries of the two-phase region for a given set of parameters are then found by numerically solving eq 11 and 12 for X_2^l and X_2^g. An iterative technique is used in which the updated pair X_2^l, X_2^g is the average of the two points found by using the gradients, evaluated at the previous point, of the left-hand sides of eq 11 and 12.

In writing eq 11 and 12, we have implicitly restricted the system to two phases and have failed to take into account the possible intervention of other phases. As such, we cannot expect to be able to extrapolate very far beyond the region of the phase diagram for which data are available. For example, there is no reason to believe that a particular set of parameters will yield simultaneous solutions to eq 11 and 12 at all temperatures. The implications of such failures to find solutions will be discussed below. It can be seen that for small X_2^g, eq 11 is independent of ρ^g. Thus, given ΔH_1 and T_{c_1} from calorimetric and NMR measurements, respectively, the behavior of the liquidus line, X_2^l, for small peptide concentration is uniquely determined by ρ^l. It is thus possible to explore the extent to which the remaining three parameters can be adjusted to fit the solidus line, X_2^g, in light of the constraint imposed by the slope of $\Delta H/n_1$ vs. X_2.

Simulation of Differential Scanning Calorimetry. Having modeled the phase diagram and its underlying thermodynamics, one is then able to calculate the change in enthalpy of a sample of given composition as its temperature is raised through the two-phase region. For a sample of overall peptide mole fraction X, at a temperature T such that $X_2^g(T) < X < X_2^l(T)$, the fraction of the sample in the liquid-crystal phase, F, may be calculated by the lever rule (Swalin, 1972). Using eq 8, it is a relatively simple matter to show

$$\frac{dH}{dT} = \Delta H_1(T_{c_1})\frac{d}{dT}[(F-1)(1 - X_2^g)] + \Delta H_2(T_{c_1})\frac{d}{dT}[(F-1)X_2^g] + \frac{\rho^g}{\gamma}\frac{d}{dT}\left[\frac{(1-F)X_2^g(1 - X_2^g)}{1 - X_2^g(1-\gamma)}\right] + \frac{\rho^l}{\gamma}\frac{d}{dT}\left[\frac{FX_2^l(1 - X_2^l)}{1 - X_2^l(1-\gamma)}\right] \quad (13)$$

where H is the enthalpy of the sample. With this expression, it is possible to simulate a DSC experiment. This type of simulation has been carried out by a number of workers (Mabrey & Sturtevant, 1976; Tinker & Low, 1982; Heyn et al., 1981), but we have made use of a somewhat more realistic model for the calorimeter in an attempt to account for the effects of instrumental response. Our model is roughly based on the operating characteristics of the Microcal MC1 in our laboratory. We have attempted to include the quadratic response of the feedback circuity and the finite heat capacities of the cells and their holders (which contain the heaters and temperature sensors). We have also included a thermal resistance between the cells and holders.

The elements of our model are depicted in Figure 1. In this figure, C_c and C_H are the heat capacities of the cell and holder, respectively. R is the thermal resistance between the

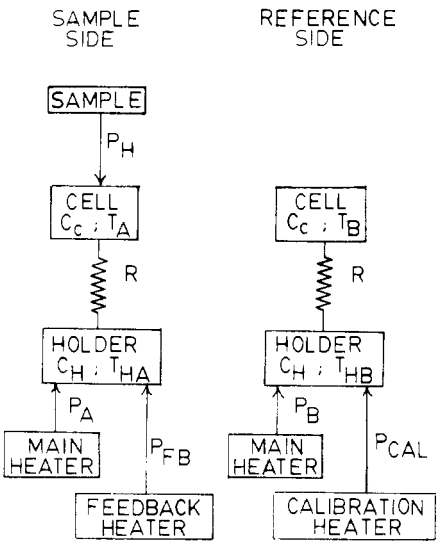

FIGURE 1: Block diagram of the calorimeter model. C_c and C_H are the heat capacities of the cells and holders, respectively. T_A and T_B are the temperatures of the sample and reference cell, respectively. R is the thermal resistance between the cells and holders. T_{HA} and T_{HB} are the temperatures of the sample and reference cell holders, respectively. The temperature is scanned by application of constant powers (P_A and P_B) to the holders. The temperature difference between the holders is minimized by feedback power $P_{FB} = k(T_{HB} - T_{HA})^2$. Heat absorbed by endothermic transitions in the sample is modeled by an effective power P_H.

cell and holder. On sample and reference sides, the cells have temperatures T_A and T_B, respectively, and the holders have temperatures T_{HA} and T_{HB}, respectively. A constant power, P_A or P_B, is applied to the sample or reference side holders to linearly raise the temperature. A feedback power, $P_{FB} = k(T_{HB} - T_{HA})^2$, is applied to the holder on the sample side. The proportionality constant, k, is estimated by directly measuring the output of the thermophile as a function of the calibration pulse power. The calibration power, P_{CAL}, is applied to the reference holder only during the calibration pulse. The sample, as it undergoes an endothermic transition, extracts heat from the sample cell at a rate $P_H = (dH/dT)(dT/dt)$ where dH/dT is given by eq 13 and dT/dt is the scan rate. P_A is chosen such that in the absence of P_H, the temperature difference $T_{HB} - T_{HA}$ is maintained at a bias offset ΔT_0 so that $P_A = P_B - k\Delta T_0^2$. For our apparatus, $\Delta T_0 \simeq 0.017K$ corresponds to a bias feedback current of 3 mA.

The heat capacity of the cells is estimated from the cell mass and an estimated specific heat of 0.1 cal/(g·°C) for a typical nickel alloy. The specific heat of the holder is estimated by considering the rate of temperature change for a given applied power with and without the cells mounted in their holders. In this way, we have chosen C_H to be $1.6C_c$. The magnitude of R is adjusted to provide a good approximation to the response for a calibration pulse. We have chosen to use R = 4.2 s °C/cal.

The simulation proceeds in a series of time intervals after each of which the temperatures are updated according to the four equations for the time derivatives of T_{HA}, T_A, T_{HB}, and T_B.

While this is obviously a highly simplified picture of the calorimeter, we are satisfied, from its ability to simulate the response to a step calibration pulse, that it is sufficient to allow us to estimate the effect of instrumental response on the DSC scans. As we shall see below, for larger peptide concentrations, the instrumental response does not appear to be the controlling influence on the shape of the DSC trace. We thus saw no justification for further refinement of the calorimeter model.

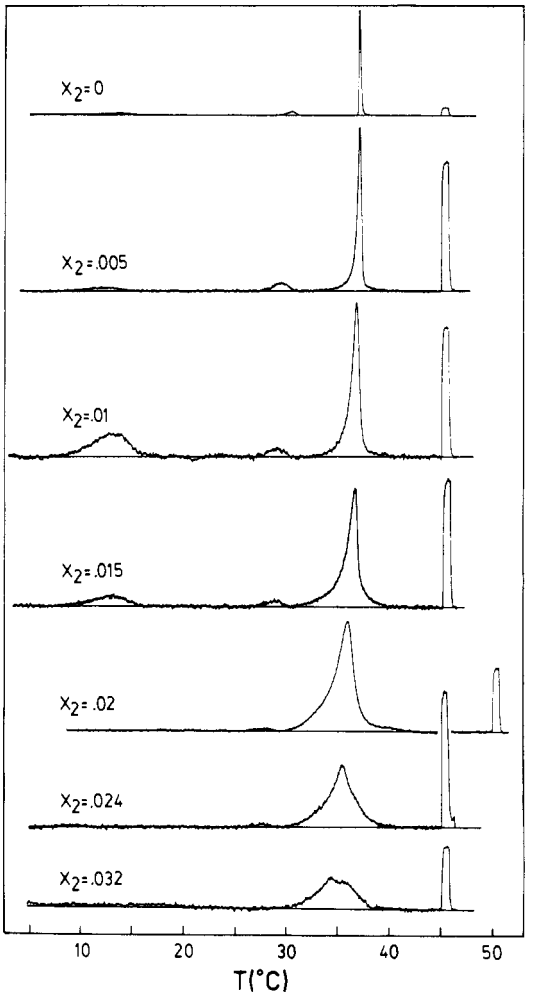

FIGURE 2: Selected DSC scans for peptide 16/DPPC-d_{62} mixtures. X_2 is the peptide mole fraction. Each scan finishes with a calibration pulse. In all cases shown, the scan rate was less than 10 °C/h. The base lines are flattened by the procedure described in the text.

RESULTS

Selected examples of DSC scans for the series of peptide 16 and peptide 24 mixtures in DPPC-d_{62} appear in Figures 2 and 3, respectively. The traces in Figure 3 for X_2 = 0.0149, X_2 = 0.0243, and X_2 = 0.0323 were recorded without computer data acquisition and have not had the base line subtracted. The remaining traces have had the base line subtracted as described above.

It is well-known that, for a two-component system at low solute concentration, the limiting slopes of the liquidus and solidus lines must have the same sign. This type of behavior is, in fact, displayed in the phase diagrams for these peptide/lipid systems reported by Huschilt et al. (1985) for which the two-phase region lies entirely below the pure lipid transition temperature. It can be seen, however, for the higher peptide concentrations shown in Figures 2 and 3, that the apparent transition extends to both sides of the pure lipid transition temperature. It would thus appear that the temperature limits of the DSC transition for higher peptide concentration cannot be directly identified with the boundaries of a two-phase region. Rather than being unprecedented, however, this type of behavior is reminiscent of the situation for DSC on mixtures of lipids with nonlipid molecules, particularly cholesterol (Mabrey et al., 1978), where the end points of the DSC transition are seen to straddle the pure lipid transition temperature even at fairly low solute concentration. It is thus of some interest to determine whether the high-temperature component of the

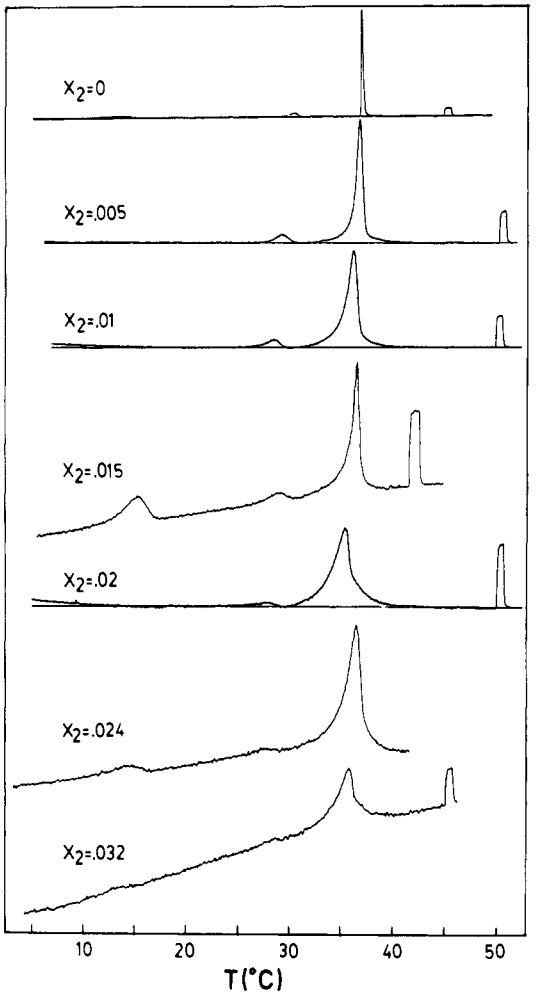

FIGURE 3: Selected DSC scans for peptide 24/DPPC-d_{62} mixtures. X_2 is the peptide mole fraction. In all cases shown, the scan rate was less than 10 °C/h.

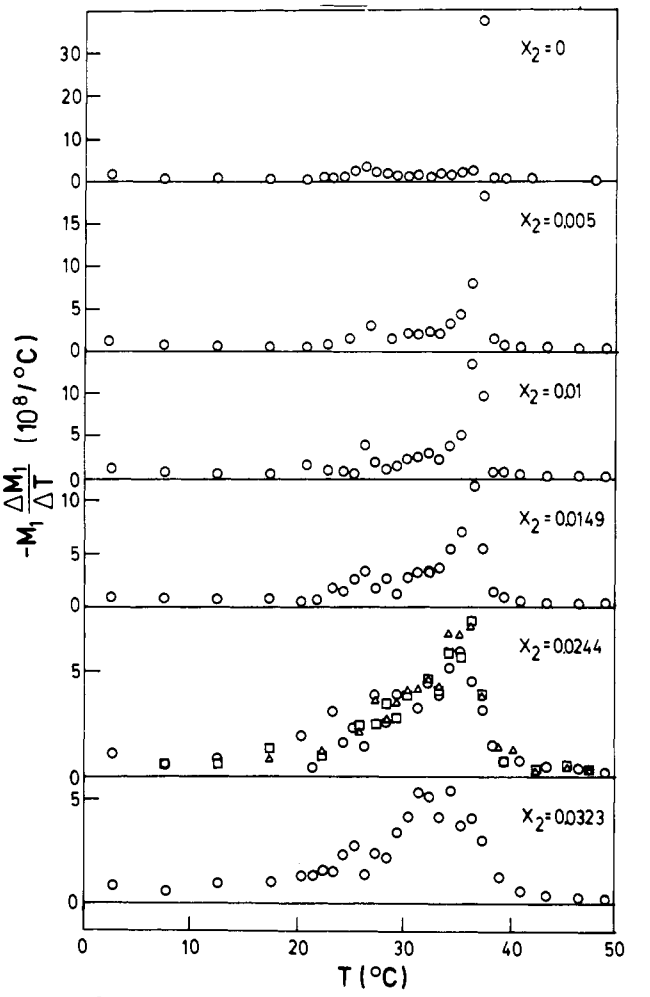

FIGURE 4: ^{2}H NMR first spectral moments for peptide 16/DPPC-d_{62} mixtures plotted as $-M_1\Delta M_1/\Delta T$ vs. temperature.

DSC trace, for higher peptide concentration, represents the temperature dependence of a true equilibrium heat capacity or if it is simply a nonequilibrium artifact of the finite scan rate of the DSC experiment. To this end, we have further examined some of the data from the ^{2}H NMR experiments by Huschilt et al. (1985) on these model peptide/lipid systems.

For a lipid bilayer in the liquid-crystalline state, it is well-known that the first moment, M_1, of the ^{2}H NMR spectrum is proportional to the S_{zz} component of the order parameter tensor where the z axis is taken perpendicular to the bilayer plane (Davis, 1983). Order parameters are of less use in characterizing the gel-state but the first moment continues to provide an internal probe of the lipid state. Jähnig (1978) has presented a molecular field model in which the energy of a layer of long chains is proportional to $-S_{zz}^2$. Accordingly, it is of some interest to examine the first moments of the ^{2}H NMR spectra collected by Huschilt. For the liquid-crystalline phase particularly, $-M_1 dM_1/dT$ should be analogous to the heat capacity recorded in a DSC experiment. For the purpose of this work, we will assume that such a relationship extends to the gel phase.

The NMR experiments were carried out at an effective rate of about 1 °C/h. They would thus be expected to more closely reflect the equilibrium properties of the system if there were any question of the observed DSC behavior having been distorted by the 10 °C/h scan rate. The temperature derivatives of the first moments were obtained by taking differences of moments for adjacent temperatures and dividing by the temperature interval. The plots of $-M_1\Delta M_1/\Delta T$ vs. T for peptide 16 are shown in Figure 4. The large scatter is due to the relative coarseness of the 1 °C temperature interval used in the NMR work. The analogy to the DSC results is apparent. As a further check, the ^{2}H NMR measurements for a peptide 16 concentration $X_2 = 0.0244$ were repeated for series of both increasing and decreasing temperatures. Both sets of measurements appear in Figure 4. The apparent lack of hysteresis further suggests that the high-temperature behavior of the DSC scans for the more concentrated samples reflects an equilibrium property of the system.

Finally, in Figure 5a,b, we have shown the integrated transition enthalpies, normalized to the lipid content of the sample, as a function of X_2 for peptides 16 and 24, respectively. Each point represents a separate preparation of the sample and is the average of up to three DSC runs on that sample. The enthalpies have been normalized to the gallium melting transition. On the basis of this calibration, we find the pure lipid transition enthalpy to be $\Delta H_1 = 8.7 \pm 1.2$ kcal/mol. The solid lines in Figure 5a,b are based on parameters used in the phase diagram simulation discussed below. The dashed lines are the best fit to the slope with the constraint that the intercept be ΔH_1.

Discussion

A linear decrease in transition enthalpy with increasing protein concentration has often been attributed to lipids being withdrawn from partcipating in the transition as a result of being strongly perturbed by the solute (Chapman et al., 1977).

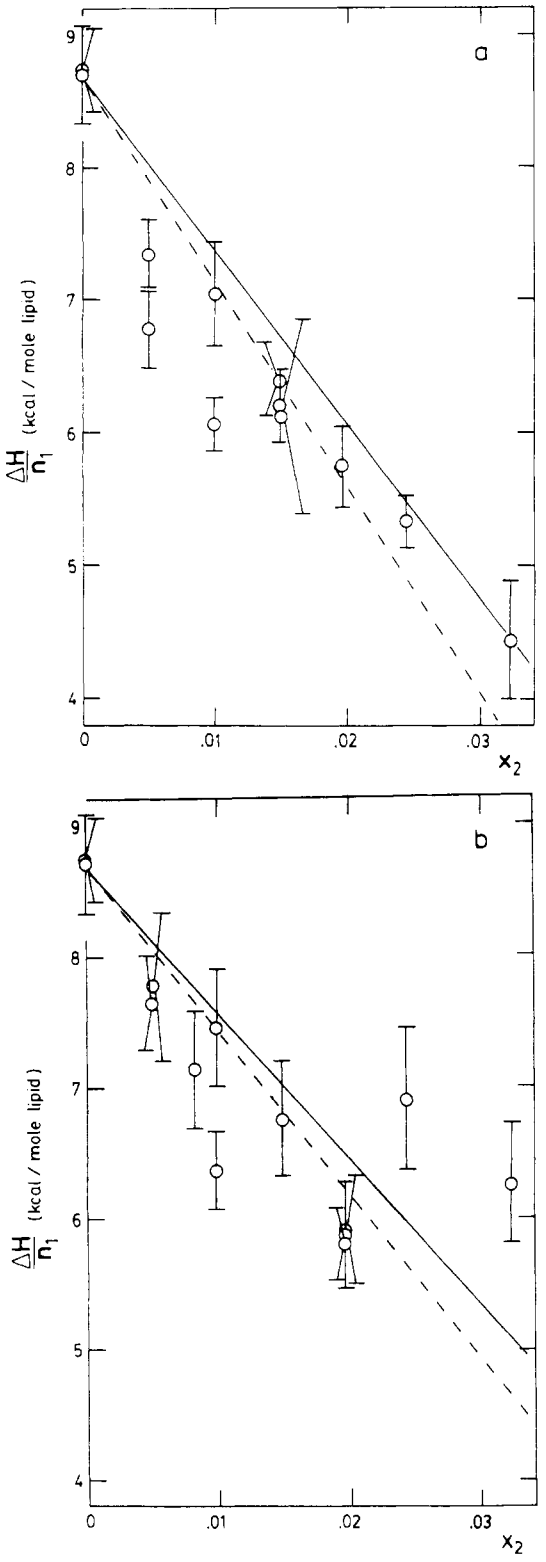

FIGURE 5: (a) $\Delta H/n_1$ vs. X_2 for peptide 16/DPPC-d_{62} mixtures. The solid line represents eq 10 using the parameters determined by simultaneous fitting of DSC and phase behavior. The dashed line represents the best fit for the slope of the integrated transition enthalpies considered alone. (b) $\Delta H/n_1$ vs. X_2 for peptide 24/DPPC-d_{62} mixtures. The solid line represents eq 10 using the parameters determined by simultaneous fitting of DSC and phase behavior. The dashed line represents the best fit for the slope of the integrated transition enthalpies considered alone.

In this "boundary lipid" picture, the melting enthalpy per mole of bulk lipid is independent of solute concentration, and the decrease in apparent molar enthalpy is simply due to the decrease in bulk lipid as solute is added. The idea that the bulk lipid melts with its full enthalpy is equivalent to postulating ideal mixing between the lipid and the peptide/lipid complex. There is little justification for such an assumption in so wide a range of systems.

An examination of the ^{2}H NMR spectra of Huschilt et al. (1985) and Huschilt (1984) for this peptide/lipid system fails to provide any evidence for a special class of lipid withdrawn from participating in the transition. The size of the peptide is such that one would expect it to be coordinated by about 18 lipids. For a peptide concentration as low as 3%, more than half the lipid would be involved in the annulus. It is found, however, that the observed spectra above and below the transition are distinctly liquid-crystal-like and gellike, respectively. It is interesting to consider the consequences of such results on the conjecture that the presence of the peptide gives rise to a large class of lipid which does not participate in the phase transition.

Above the phase transition, the spectra observed for the perdeuterated lipid display the sharp-edged power patterns characteristic of axially symmetric motion. On the time scale of the ^{2}H NMR experiment, about 10^{-4} s, there is only one class of lipid. The observed splittings are approximately 8% larger (at about 3% peptide) than found for the pure lipid. Roughly half of the lipid is close to or in contact with the peptide at any time. If exchange between this perturbed environment and the bulk lipid were slow, it would be possible to resolve spectra from the two environments. This is not the case, and it must be concluded that the exchange is fast enough that the spectra observed are the average of approximately equal contributions from both the perturbed and unperturbed environment. Since the resulting spectra are characteristic of axially symmetric motion, it must be concluded that the motion of the perturbed lipid is axially symmetric and similar to the motion for pure lipid in the liquid-crystal phase.

Similar arguments may be advanced regarding the spectra observed at temperatures below those of the main transition. These spectra are found to be similar to the pure lipid gel spectra but with a first spectral moment smaller by about 8%. The absence of an axially symmetric spectral component at low temperature effectively rules out the possibility that a large class of lipid molecules have maintained their high-temperature state while the rest of the sample has undergone the liquid-crystal to gel transition. For the peptide/lipid system described in this work, it can thus be asserted with confidence that there does not exist a significant class of lipid which is withdrawn, by contact with the peptide, from participation in the phase transition. The explanation for the peptide concentration dependence of the transition enthalpy is not to be found in a picture which refers to lipids withdrawn from the transition. It is thus useful to demonstrate the possibility of modeling the thermodynamics underlying the peptide/lipid phase diagram in a manner consistent with the transition enthalpies observed by DSC.

We have, accordingly, attempted to fit eq 11 and 12 to the boundaries of the two-phase region while simultaneously fitting eq 10 to the enthalpies. We have chosen β and γ to be 6 and 4, respectively, and are then faced with determining six parameters. We shall see, in fact, that for different choices of β and γ, we arrive at slightly different values for the rest of the parameters but that neither the quality of the fit nor the conclusions drawn are significantly affected.

Of the six parameters, ΔH_1 and T_{c_1} are directly measurable. We fix these at ΔH_1 = 8.7 kcal and T_{c_1} = 37.8 °C (temperature used in calculating the fits must, of course, be converted to kelvin). The only remaining parameter which may then

be varied to adjust the fit to the liquidus line, X_2^l, is ρ^l, and it is fixed accordingly. Varying ρ^l by 10% from its optimum value significantly decreases the quality of the fit to the liquidus. Since the choice of ΔH_1 fixes the intercept of $\Delta H/n_1$ vs. X_2, there remain three parameters with which we must fit the slope of $\Delta H/n_1$ vs. X_2 and the solidus line, X_2^g. If we define $\Delta Q = (\rho^l - \rho^g)/\gamma + \Delta H_2$, we see, from eq 10, that for small peptide concentration, the expected slope of $\Delta H/n_1$ vs. X_2 is ΔQ. As implied under Theoretical Procedures, the phase behavior at small peptide concentration provides no information about the partitioning of ΔQ between $(\rho^l - \rho^g)/\gamma$ and ΔH_2. It was found, however, that the behavior of the solidus below ~27 °C was sensitive to the parameters ρ^g, ΔH_2, and ΔS_2. Around this temperature, the liquidus and solidus approach each other. For some sets of parameters, there is a small temperature range in which this model fails to yield solutions describing gel/liquid-crystal equilibrium. For all parameter sets which fit the higher temperature phase behavior, there is a region below 25 °C in which the boundaries found indicate a melting of the mixture as temperature decreases and are thus unphysical. We have allowed ρ^g and ΔS_2 to vary while adjusting ΔH_2 to maintain a chosen value of ΔQ. By demanding that the solidus be well-behaved and extend to at least the highest peptide concentration for which it is observed experimentally, we are able to distinguish the contributions of ρ^g and ΔH_2 to ΔQ. Varying ρ^g by 10% above or below its optimum value, while adjusting ΔH_2 to maintain ΔQ and holding ΔS_2 constant, results in the solidus line missing its measured range at the highest X_2. If ΔS_2 is allowed to vary by less than 1% so as to compensate, the ρ^g can vary by ~25% before the calculated solidus line is in serious conflict with its observed range around $X_2 = 0.03$. Because of the relative sizes of ρ^g and ΔH_2, a change in ρ^g of 25% corresponds to a change in ΔH_2 of about 1%. We found, for the peptide 16 and peptide 24 systems, that an acceptable fit to the solidus could be achieved for ΔQ equal to 85% and 90%, respectively, of the apparent best slope for $\Delta H/n_1$ vs. X_2. For ΔQ closer to the best slope, the fit to the phase diagram was poorer. It is not known if this discrepancy is due to systematic error or to the inadequacy of the model. In either event, however, it seems clear that the thermodynamics of the phase behavior can account for the most of the slope of $\Delta H/n_1$ vs. X_2. It should be noted that for the data available, the fit to the phase diagram alone could not be used to place a lower limit on the magnitude of ΔQ.

The solid lines in Figures 5 and 6 were judged to represent the most acceptable simultaneous fits to the enthalpy and phase data for the two peptide systems. With β and γ set at 6 and 4, respectively, the parameters used for these fits for peptide 16 are

$$\rho^g = 7.65 \text{ kcal/mol} \qquad \rho^l = -94.41 \text{ kcal/mol}$$

$$\Delta H_2 = -105.95 \text{ kcal/mol} \qquad \Delta S_2 = -0.405 \text{ kcal/(mol·K)}$$

$$\Delta Q = -131.5 \text{ kcal/mol}$$

and for peptide 24

$$\rho^g = 7.65 \text{ kcal/mol} \qquad \rho^l = -76.48 \text{ kcal/mol}$$

$$\Delta H_2 = -91.3 \text{ kcal/mol} \qquad \Delta S_2 = -0.345 \text{ kcal/(mol·K)}$$

$$\Delta Q = -112.3 \text{ kcal/mol}$$

If β is chosen to be 1, corresponding to ideal entropy of mixing, the same fits are recovered by increasing ρ^g by about 20%. With both β and γ chosen to be 1, the fit is recovered by decreasing the magnitude of both ρ^l and ρ^g by about 22% and adjusting ΔS_2 by less than 0.5%. These changes numerically affect the detailed predictions of this model, but they do not qualitatively alter our interpretation of the results. For the rest of this discussion, we will refer only to the parameters found for $\beta = 6$ and $\gamma = 4$.

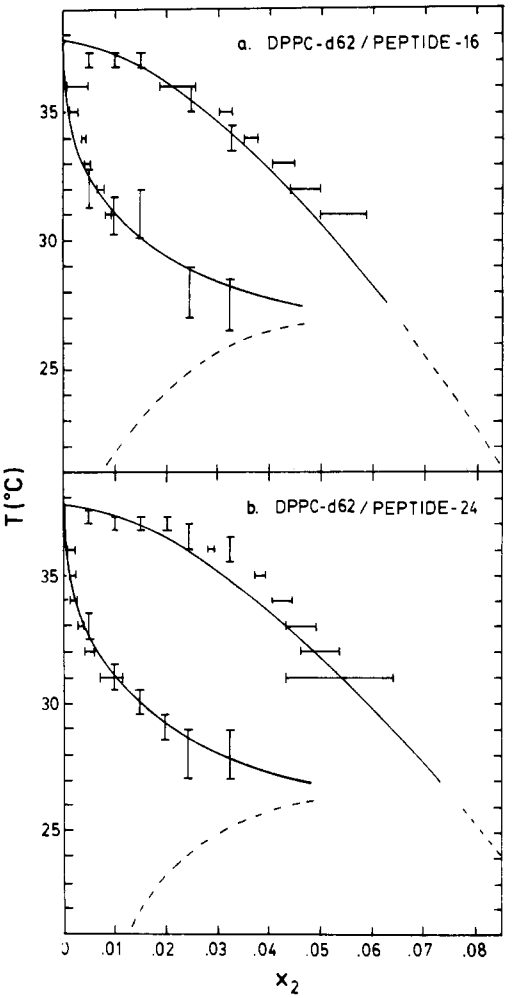

FIGURE 6: (a) Phase diagram for peptide 16/DPPC-d_{62} mixtures. X_2 is the peptide mole fraction. The horizontal error bars denote the determination of phase boundaries by ^{2}H NMR difference spectroscopy. The vertical error bars denote the phase boundaries determined by inspection of ^{2}H NMR spectra at fixed concentration. The solid line represents the calculated phase boundaries determined by simultaneous fitting of DSC and phase behavior. The dashed lines represent continuation of the solutions for the phase boundaries into regions of unstable equilibrium. (b) Same as (a) but for peptide 24/DPPC-d_{62} mixtures.

The most important consequence of this fitting exercise is simply the observation that it is possible to reproduce the phase diagram by using a simple model which can also accommodate the concentration dependence of the transition enthalpy. This is significant in that it raises the possibility of accounting thermodynamically for the concentration dependence of the enthalpy without having to postulate, in contradiction of the NMR evidence, a special class of lipid. While the boundary lipid picture has enjoyed some success, it has probably obscured the more detailed thermodynamics of the lipid/solute system. Of potentially more consequence is the possibility that the boundary lipid picture incorrectly interprets the source of the decrease in ΔH with increasing solute concentration. The boundary lipid picture would imply that for increasing solute concentrations, the distinction between gel and liquid crystal is lost as large amounts of lipid are prevented from undergoing

the transition. While the peptide certainly perturbs the lipid, the NMR results suggest that the overall structure and dynamics of the lipid in the bilayer remain recognizably gel- or liquid-crystal-like. The results of the current modeling imply that while the phases may retain their characteristic behavior, they do so at the expense of a lowered enthalpy difference between the two phases.

It must be emphasized that although we have based this model on regular solution theory, the likelihood of long-range peptide/peptide and peptide/lipid interactions raises the possibility that this is not the most appropriate form into which to cast the mixture free energy. If only direct contact were important, the interactions would be primarily accounted for by the terms in ρ^{α}, and one would expect ΔH_2 and ΔS_2 to be relatively unimportant. They are not, and this may be a result of the importance of bilayer-mediated long-range interaction. A more detailed microscopic theory taking explicit account of longer range interactions might yield a model with parameters more directly related to the relevant interactions in the peptide/lipid system. At present, we can only say that the effect, per peptide, on the free energy of the bilayer includes $\mu_2^{\circ}(\alpha,T) + \rho^{\alpha}(1 - X_2^{\alpha})^2$ with the concentration-dependent portion of the second term correcting for the solute/solute interaction. The signs found for ρ^{g} and ρ^{l} seem to indicate a slightly lower than random probability of peptide/lipid contact in the gel phase and the opposite in the liquid-crystalline phase.

In modeling the phase behavior and integrated enthalpies, we assume simple two-component phase behavior. We can, to some extent, test this assumption by using the model parameters to stimulate the DSC traces by the methods described above. Since we were interested more in comparing the features of such traces than in obtaining the best fit, we did not attempt to optimize the values of the parameters determining the response of the calorimeter. We have obtained acceptable simulations for the shape of the pure lipid response and the calibration pulse, and we are confident that our simulation does account for contributions to the shape of the trace due to instrumental response. It should be noted that for the parameters chosen, the response following the sharp pure lipid transition is primarily the result of the quadratic dependence of the feedback power on the temperature difference between the sample and reference sensors.

The results of the simulation for the peptide 16/lipid system are shown in Figure 7. The simulations for the peptide 24/lipid system are not significantly different. Particularly for $X_2 \leq 0.02$, the overall shape of the trace is reasonably well simulated. There are two major points on which the simulations deviate from the observed behavior. For all concentrations, the simulation predicts a small but abrupt increase in the heat capacity as the temperature crosses the solidus line. For concentrations above about $X_2 = 0.02$, the simulation fails to predict the tail on the DSC trace which extends to temperatures above the liquidus line and, for the highest concentrations studied, above the pure lipid transition temperature. We do not know whether these two failings are related, but for the purpose of this discussion, we will consider only the high-temperature edge of the transition. As we have discussed above, consideration of the ^{2}H NMR spectral moments leads us to believe that the high-temperature tail is not simply a kinetic effect. We also reject the possibility that it represents another well-defined phase in the same sense as the gel or liquid-crystal phases. Such an interpretation would imply a nonisothermal three-phase equilibrium, and this is prohibited in a strictly two-component system. The high-temperature

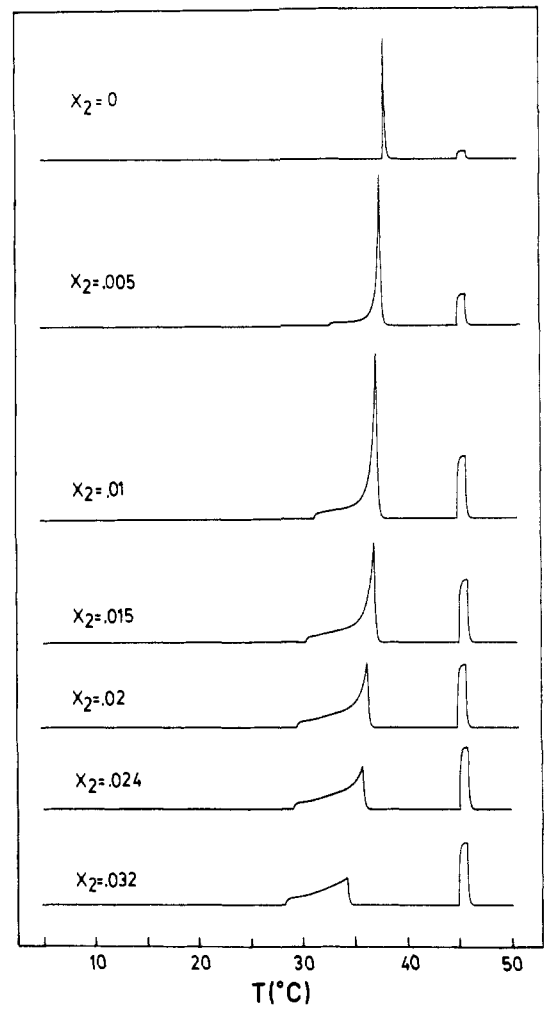

FIGURE 7: Simulations of DSC scans for peptide 16/DPPC-d_{62} mixtures using the parameters determined from the solid line fits in Figures 5a and 6a. The instrumental response of the calorimeter is included by use of the model of Figure 1.

tail may be an example of the behavior proposed by O'Leary (1983) to account for the broad component underlying the sharp DSC transition in lipid/cholesterol mixtures. He has suggested that the gel to liquid-crystalline transition is accompanied by a continuous lattice expansion. These two transitions would be strongly coupled in the pure lipid but are decoupled by the presence of the solute molecules. While our results do not shed much light on the nature of the broad component, it is clear that it is not described by a model, such as ours, which simulates the behavior of two phases separated by a first-order transition. The simple peptide/lipid system discussed in this work does, however, provide a situation where it may be possible to model the first-order behavior and perhaps distinguish contributions to the heat capacity from a closely coupled continuous transition.

The simulations do, however, reemphasize a number of points which bear repetition concerning the shape of the DSC trace. It can be seen that a simple two-component phase equilibrium, such as we have been considering, can give a rise to rather asymmetric traces in a DSC experiment. This simple observation calls into question a number of analyses of DSC results on two-component systems in which attempts have been made to analyze the shape of the DSC trace in terms of symmetric transitions. To do so without having first explored the contribution to the shape from any possible two-phase coexistence is clearly inappropriate. On the basis of O'Leary's work, an argument might be made for the resolution of a

transition into a first-order component and a continuous component. However, when faced with the prospect of describing a DSC trace for a two-component system in terms of overlapping first-order transitions consideration should be made of the phase rule which would, in general, prohibit such an interpretation.

Similar care should be taken when the van't Hoff equation is applied to the DSC results on a two-component system. This type of analysis for a pure lipid system treats the so-called cooperative unit as a quasi-macromolecule and then treats the phase equilibrium as a unimolecular chemical equilibrium characterized by a single parameter, the van't Hoff enthalpy. Such a simple picture is incapable of accounting for the temperature dependence displayed by the phase equilibria of a two-component system. Furthermore, as the temperature is changed through a two-phase region, the composition of the two phases changes. The van't Hoff picture assumes that the size of the quasi-macromolecule or cooperative unit is independent of temperature and this condition is not necessarily fulfilled in a two-component equilibrium. Indeed, since the compositions of the two phases in equilibrium are different, it is not clear that an analogy to a unimolecular process is appropriate even at a fixed temperature.

Conclusions

We have performed DSC experiments on the amphiphilic peptide/DPPC-d_{62} system studied by Huschilt et al. (1985). Regular solution theory has been adapted to this system. It has been demonstrated that the peptide concentration dependence of the integrated transition enthalpies can be incorporated into a model which reproduces the observed phase behavior farily well without invoking the idea of a complexing annulus of lipid around the peptide. We have indicated how the excess enthalpy of mixing implies a slightly nonrandom distribution of peptide in each phase and how the standard chemical potential of the peptide in the dilute limit may be interpreted in terms of the perturbation of the bilayer by insertion of the peptide.

The thermodynamic parameters determined by the simultaneous fitting of the phase behavior and the integrated transition enthalpies have been used in a simulation of the DSC scan shapes. While it is found that the asymmetry of the calorimetric scans for $X_2 \leq 0.02$ is reproduced by the model, the broad component observed for higher concentration is not. It is postulated that this is an example of the situation described by O'Leary (1983) in which a solute molecule decouples the first-order melting transition from a continuous lattice expansion.

It is significant that the DSC results presented here display a number of characteristics which alternate analyses might approach differently. The broadening of transitions with increasing solute concentration is sometimes attributed to a loss of cooperativity (Heyn et al., 1981). The asymmetry of calorimetric transitions is sometimes described in terms of overlapping symmetric transitions (Spink et al., 1982). The decrease in transition enthalpy with increasing peptide concentration is sometimes attributed to the withdrawing of lipid from the phase transition by association with the peptide (Akhrem et al., 1982). We have demonstrated that for this system, all of these characteristics are consistent with and perhaps consequences of the existence of a region of two-phase equilibrium. The Gibbs phase rule demands that first-order transitions in binary mixtures necessarily proceed via a region of two-phase equilibrium. It is thus strongly implied by the results of this work that the calorimetric behavior of the two-component system should not be analyzed in terms of symmetric transitions without adequately taking into account the possible extent of a region of two-phase equilibrium.

It has been noted that the application of this model to the fitting of the two-phase region boundaries does not provide sufficient information, of itself, to unambiguously determine the slope of $\Delta H/n_1$ vs. X_2. This results largely from our lack of independent knowledge of the peptide standard chemical potential. It would be of interest to extend these measurements to higher peptide concentration, but it must be recognized that such data would aid in the determination of the fit only in the absence of an intervening third phase. The present fit is not consistent with the existence of a melting temperature minimum at which the liquidus and solidus meet with zero slope. If future measurements indicate such behavior, it will be necessary to reexamine this model or the assumptions made.

It is unlikely that smaller error bars on the measured phase diagram would improve the precision to which the fitting parameters are known. The model used here is obviously an approximation, and even with the large error bars currently present in the phase diagram, the fit is imperfect. The possibility of a continuous transition playing a role at higher peptide concentration also raises some question as to the utility of fitting to strictly first-order phase behavior.

This study leads to some general conclusions about the peptide/lipid system. We have seen that the peptide does not remove lipid from the main transition but rather influences the transition thermodynamics for the bilayer as a whole. We have also seen that the phase behavior contains information about possible clustering of the peptide. It would appear, however, that quantitative determination of peptide clustering from phase behavior will only become possible with the development of a treatment for the excess enthalpy of mixing incorporating the possibility of long-range peptide/peptide interaction.

Part of the initial motivation for this work was the development by Mouritsen & Bloom (1989) of a model for peptide/lipid interaction emphasizing the role of hydrophobic mismatch. The similarity of the phase diagrams determined by Huschilt et al. (1985) suggests that mismatch is not, in fact, the primary determinant of peptide/lipid phase behavior. It would appear, however, that without a better understanding of peptide standard chemical potential, the current analysis does not provide a basis for discussion of the relative importance of various possible contributions to the peptide/lipid interaction.

It is to be hoped that the advent of relatively simple and well-characterized model peptide/lipid systems will allow systematic studies of the thermodynamic basis underlying membrane phase behavior. Indeed, the apparent simplicity of these systems serves to emphasize interesting departures from anticipated behavior such as the broadening to high temperature of the high peptide concentration DSC scans.

Registry No. DPPC, 63-89-8; peptide 16, 94751-61-8; peptide 24, 86968-49-2.

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Incorporation and Translocation of Aminophospholipids in Human Erythrocytes†

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ABSTRACT: Cell morphology changes are used to examine the interaction of exogenous phosphatidylserine and phosphatidylethanolamine with human erythrocytes. Short-chain saturated lipids transfer from liposomes to cells, inducing shape changes that are indicative of their incorporation into, and in some cases translocation across, the cell membrane bilayer. Dioleoylphosphatidylserine and low concentrations of dilauroyl- and dimyristoylphosphatidylserine induce stomatocytosis. At higher concentrations, dilauroylphosphatidylserine and dimyristoylphosphatidylserine induce a biphasic shape change: the cells crenate initially but rapidly revert to a discocytic and eventually stomatocytic shape. The extent of these shape changes is dose dependent and increases with increasing hydrophilicity of the phospholipid. Cells treated with dilauroylphosphatidylethanolamine and bovine brain lysophosphatidylserine exhibit a similar biphasic shape change but revert to discocytes rather than stomatocytes. These shape changes are not a result of vesicle–cell fusion nor can they be accounted for by cholesterol depletion. The reversion from crenated to stomatocytic forms is dependent on intracellular ATP and Mg^{2+} concentrations and the state of protein sulfhydryl groups. The present results are consistent with the existence of a Mg^{2+}- and ATP-dependent protein in erythrocytes that selectively translocates aminophospholipids to the membrane inner monolayer engendering aminophospholipid asymmetry.

A variety of methods have been developed for examining the asymmetric distribution of lipids across the erythrocyte membrane bilayer and other lipid membrane bilayer systems [for recent reviews, see Schwartz et al. (1985), Van Deenen (1981), Etemadi (1980), Op den Kamp (1979), and Roelofsen & Zwaal (1976)]. These techniques have established the asymmetric distribution of erythrocyte membrane lipids;

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