Peptide Models of Helical Hydrophobic Transmembrane Segments of Membrane Proteins. 2. Differential Scanning Calorimetric and FTIR Spectroscopic Studies of the Interaction of Ac-K_2-$(LA)_{12}$-K_2-amide with Phosphatidylcholine Bilayers†

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ABSTRACT: The interactions of the hydrophobic helical transmembrane peptide Ac-K_2-$(LA)_{12}$-K_2-amide [$(LA)_{12}$] with a series of *n*-saturated diacylphosphatidylcholines (N:0 PC) were studied by high-sensitivity differential scanning calorimetry (DSC) and Fourier transform infrared (FTIR) spectroscopy. The incorporation of $(LA)_{12}$ into these lipid bilayers results in a broadening of the chain-melting phase transitions of the lipids and progressive decreases in the characteristic temperatures and enthalpies of their gel/liquid-crystalline phase transitions. At low peptide/lipid ratios, the DSC thermograms exhibited by mixtures of $(LA)_{12}$ with the short chain PCs (13:0 and 14:0) and with very long chain PCs (21:0 and 22:0) appear to be a summation of sharp and broad components, the former diminishing in intensity with increases in peptide concentration. This behavior can be approximated by that of a macroscopic mixture of peptide-poor and peptide-rich lipid domains, the relative proportions of which change with changes in peptide concentration. For peptide mixtures with the medium-chain PCs, the hydrocarbon chain-melting phase transition endotherms are not clearly resolvable into similar sharp and broad components. Instead, at all finite peptide concentrations the DSC heating thermograms appear as broad and highly asymmetric endotherms, the transition temperatures of which decrease significantly with increases in peptide concentration. For mixtures of $(LA)_{12}$ with each of the lipids studied, the total hydrocarbon chain-melting transition enthalpy decreases with increasing peptide concentration but does not vanish at high peptide/lipid ratios. The FTIR spectra of $(LA)_{12}$ in these PC bilayers indicate that the peptide retains a predominantly α-helical conformation in both the gel and liquid-crystalline phases of the short to medium chain PCs studied ($N < 18$). However, when incorporated into bilayers composed of the longer chain PCs ($N \geq 18$), $(LA)_{12}$ undergoes a reversible conformational change at the gel/liquid-crystalline phase transition of the mixture. In the liquid-crystalline phase, the amide I regions of the FTIR spectra of these mixtures are indicative of a predominantly α-helical peptide conformation. However, upon freezing of the lipid hydrocarbon chains, populations and/or domains of $(LA)_{12}$ giving rise to a sharp conformationally unassigned band near 1665 cm^{-1} are formed. A comparison of the results of this calorimetric and FTIR spectroscopic study with a similar study of a polyleucine-based analogue of $(LA)_{12}$ [Zhang, Y.-P., Lewis, R. N. A. H., Hodges, R. S., & McElhaney, R. N. (1992) *Biochemistry 31,* 11579−11588] suggests that the thermodynamics of the interaction of hydrophobic transmembrane helices with lipid bilayers can be influenced by factors such as the polarity and topology of the helical surface, factors which are dependent upon the amino acid sequence of the helix. Also, possible adjustments to a hydrophobic mismatch between the protein (or peptide) and its host lipid bilayer covers a spectrum of possibilities which can include changes in the degree of conformational disorder in the lipid chains and/or significant conformational changes on the part of the protein or peptide.

Interactions between the intramembrane domains of transmembrane proteins and their host lipid bilayer are an integral part of the normal functioning of all biological membranes. For some membrane proteins, interactions between their transmembrane domains and the surrounding lipid probably only serve as the structural mechanism for tethering their functional water-soluble, extramembrane domains to the membrane [for examples, see Tomita *et al.* (1978), Arinc *et al.* (1987), and De Lemos-Chiarandini *et al.* (1987)]. However, for most other integral membrane proteins, such interactions are crucial to the maintainence their biologically active conformations and, indeed, may also be important facets of the overall mechanism by which their function(s) are regulated [for examples, see Sandermann (1978) and McElhaney (1982, 1995)]. At this time, however, the way(s) in which the intramembrane domains of membrane proteins affect and are affected by their host lipid bilayers is not fully understood. This fact has provided and continues to provide the impetus for many investigations of lipid−protein interactions in both model and biological membranes.

Most of the recent initiatives in the field of lipid−protein interactions are based upon synthetic model peptides which

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are designed to interact specifically with either the polar, the interfacial, or the hydrophobic domains of lipid bilayers [for examples, see Davis *et al.* (1983), Jacobs and White (1986, 1987), and Mclean *et al.* (1991)]. This approach has recently been adopted in this laboratory to model the interaction of helical hydrophobic transmembrane segments of integral membrane proteins (Zhang *et al.*, 1992a,b, 1995a) with phosphatidylcholine (PC)[1] and PE bilayers. Conceptually, the use of such peptide models should obviate many of the difficulties encountered in studies of real integral membrane proteins, where the data obtained are often very difficult to interpret because of the complexity of the interactions between the various domains of the protein and the host lipid bilayer [for examples, see McElhaney (1986) and George *et al.* (1989, 1990)]. The model peptide used in our previous studies, Ac-K_2-G-L_{24}-K_2-A-amide (P_{24}) (and some of its closely related analogues) has been studied by others and was shown to partition into lipid bilayers as single-stranded α-helices which traverse the lipid bilayer with the long axis of the peptide helix oriented along the bilayer normal (Huschilt *et al.*, 1985, 1989; Morrow *et al.*, 1985; Pauls *et al.*, 1985; Roux *et al.*, 1989; Bolen & Holloway, 1990). Our studies of the interaction of P_{24} with phospholipid bilayers have shown that this peptide forms a very stable, predominantly α-helical structure which alters the degree of conformational disorder in lipid hydrocarbon chains in a manner which is dependent upon the sign and extent of mismatch between its hydrophobic length and the hydrophobic thickness of the host lipid bilayer (Zhang *et al.*, 1992b, 1995a). Our studies also showed that despite the inherent stability of the helical structure of P_{24}, there is evidence of small distortions of its helical conformation when the thickness of its host bilayer changes. This observation is particularly interesting because it suggests a possible mechanism whereby information on the state of hydrocarbon chain conformational disorder within lipid bilayers can be transmitted to resident transmembrane proteins. To investigate this phenomenon further, we have begun a study of the interaction of lipid bilayers with helical hydrophobic peptides which form helices that are not as conformationally stable as those formed by P_{24} and its polyleucine-based analogues. Such peptides are expected to be more conformationally responsive to changes in the physical properties of lipid bilayers into which they are inserted. The peptide used in this study, $(LA)_{12}$, is of the same overall design as P_{24} but consists of a central repeating sequence of 12 Leu-Ala segments instead of 24 leucine residues. In the preceding paper [see Zhang *et al.* (1995b)], we describe the results of the conformation and amide exchangeability studies of this peptide and demonstrate that it forms helical structures in organic solvents, detergent micelles, and lipid bilayers. Also we demonstrate that $(LA)_{12}$ partitions into lipid bilayers with the long helical axis essentially parallel to the bilayer normal and that it is conformationally responsive to changes in the properties of the surrounding medium. In this paper we present the results of studies of the interaction of $(LA)_{12}$ with a homologous series of *n*-saturated diacyl-PC bilayers. A comparison of the results of this study with those of a similar study using the polyleucine-based analogue P_{24} indicates that the mutual interactions between hydrophobic peptide helices and lipid bilayers can vary significantly depending upon the amino acid sequence of the peptide and the physical properties of the lipid bilayer.

MATERIALS AND METHODS

Detailed procedures for synthesizing and purifying the peptide $(LA)_{12}$ are described in the preceding paper (Zhang *et al.*, 1995b). Samples of $(LA)_{12}$ destined for FTIR spectroscopic studies were subsequently lyophylized twice from 10 mM hydrochloric acid to replace the trifluoroacetate counterions with chloride. For such samples this procedure is essential because the trifluoroacetates give rise to a strong absorbance band (near 1670 cm^{-1}) which overlaps with the amide I band of the peptide (Surewicz & Mantsch, 1989). With the exception of 22:0 PC, all of the PCs used in this study were synthesized and purified in this laboratory as described previously (Lewis *et al.*, 1987). Samples of 22:0 PC were obtained from Avanti Polar Lipids (Alabaster, AL) and used without further purification. The $(LA)_{12}$/lipid mixtures were prepared and hydrated for DSC and FTIR spectroscopic studies by the same procedures used for preparing and hydrating P_{24}/lipid mixtures [see Zhang *et al.* (1992a)].

DSC thermograms were recorded with a computer-controled Microcal-MC2 high-sensitivity differential scanning calorimeter (Microcal Inc., Northampton, MA) operating at heating scan rates between 11 and 27 °C h^{-1}. Typically, samples exhibiting highly cooperative phase transitions (i.e., pure lipid samples or lipid/peptide mixtures of low R_p) were examined at scan rates near 11 °C h^{-1}, whereas those exhibiting broader thermotropic phase transitions (i.e., lipid/peptide mixtures of medium to high R_p) were studied at scan rates near 27 °C h^{-1}. Under the conditions used such scan rates were slow enough to ensure both sample and instrument equilibrium while being sufficiently fast to produce DSC thermograms with good signal-to-noise ratios. The data acquired were analyzed with DA2 software (Microcal Inc., Northampton, MA), Microcal Origin software (Microcal Software Inc., Northampton, MA), and other computer programs available in the laboratory. In cases where it was obvious that the observed DSC thermograms were the summation of overlapping peaks, curve-fitting methods were used to obtain estimates of the transition temperatures and enthalpies of the component peaks. The procedure used for the deconvolution of the DSC thermograms was based on the assumption that the observed thermogram can be described in terms of a linear combination of multiple independent transitions, each of which approximate a two-state transition. The lipid content of the samples used for DSC was quantified as described previously (Zhang *et al.*, 1992a).

Infrared spectra were recorded with a Digilab FTS-40 Fourier-transform infrared spectrometer (Digilab Inc., Cambridge, MA) using the standard methodology for these types of samples (Mantsch *et al.*, 1985). The data acquired were processed using DDS software (Digilab Inc., Cambridge,

[1] Abbreviations: $(LA)_{12}$, Ac-K_2-$(LA)_{12}$-K_2-amide; L_{24}, Ac-K_2-L_{24}-K_2-amide; P_{24}, Ac-K_2-G-L_{24}-A-K_2-amide; PC, phosphatidylcholine; PE, phosphatidylethanolamine; N:0 PC, *n*-saturated diacylphosphatidylcholine; N:0 PE, *n*-saturated diacylphosphatidylethanolamine; FTIR, Fourier transform infrared; CD, circular dichroism; NMR, nuclear magnetic resonance; DSC, differential scanning calorimetry; R_p, peptide/lipid molar ratio; T_m, gel/liquid-crystalline phase transition temperature; T_p, pretransition temperature; $\Delta T_{1/2}$, transition width measured at peak half height; ΔH, transition enthalpy. The hydrocarbon chains of the lipids used in this study are designated by the shorthand notation *N*:0 with "*N*" representing the number of carbon atoms on the chain and "0" indicating the absence of carbon−carbon double bonds.

Table 1: Hydrophobic Thicknesses of the Bilayers Formed by Various Phosphatidylcholines

PC	hydrophobic thickness (Å)[a]		
	gel phase	liquid-crystalline phase	mean[b]
13:0	31.5	21.0	26.3
14:0	34.2	22.8	28.5
15:0	36.8	24.5	30.7
16:0	39.4	26.3	32.9
17:0	42.1	28.1	35.1
18:0	44.7	29.8	37.3
19:0	47.3	31.6	39.5
21:0	52.5	35.0	43.8
22:0	55.1	36.8	46.0

[a] Hydrophobic thicknesses were calculated using the equations provided in Sperotto and Mouritsen (1988). [b] The mean of the hydrophobic thicknesses of the gel and liquid-crystalline phases.

MA) and other computer programs developed by the National Research Council of Canada. Prior to data analysis, water vapor spectra and spectra of the pure solvent, prepared with the same IR windows and recorded at the same temperature, were subtracted. In cases where the spectra obtained consisted of broad overlapping bands, data processing usually involved the use of Fourier deconvolution to obtain fairly accurate estimates of the frequencies of the component bands, followed by curve-fitting procedures (to the original spectra) as detailed previously (Zhang *et al.*, 1995b).

RESULTS

In this study the effect of the model helical transmembrane peptide $(LA)_{12}$ on the thermotropic phase behavior of a homologous series of *n*-saturated diacyl-PCs is examined by DSC and by FTIR spectroscopy. Thus, the hydrophobic thicknesses of the various host lipids used in this study as well as the probable hydrophobic length of $(LA)_{12}$ are important parameters to be considered in the interpretation of the data presented below. If it is assumed that the hydrophobic lengths of peptides such as P_{24} and $(LA)_{12}$ consist of "ideal" α-helices composed of a sequence of 24 hydrophobic amino acid residues, then an examination of molecular models of such peptides would suggest that their mean hydrophobic lengths[2] should be 31−32 Å. Given this value, and the hydrophobic thicknesses expected of the various PC bilayers used in this study (see Table 1), the following points should be noted. First, in the gel phase the matching of peptide hydrophobic length and lipid hydrophobic thickness should only occur with 13:0 PC (the shortest chain lipid used in this study). Thus, with all of the other lipids used, there should be a progressively greater mismatch between peptide hydrophobic length and bilayer gel phase hydrophobic thickness with increases in acyl chain length. Second, with the exception of 21:0 PC and 22:0 PC, the hydrophobic thickness of each of the lipid bilayers used in this study is shorter than the hydrophobic length of $(LA)_{12}$ when the lipids are in the liquid-crystalline phase. Third, the expected mean hydrophobic thicknesses of 15:0 PC is very close to the calculated hydrophobic length of $(LA)_{12}$. Thus, a mismatch between peptide hydrophobic length and bilayer mean hydrophobic thickness should occur with all of the other lipids used. Finally, for the long chain lipids, 21:0 PC and 22:0 PC, the bilayer hydrophobic thickness exceeds peptide hydrophobic length in both the gel and liquid-crystalline phases. However, the pattern of matching lipid bilayer hydrophobic thickness and peptide hydrophobic length described above applies only if the peptide adopts an ideal and invariant α-helical conformation. In our previous calorimetric and spectroscopic studies of P_{24}/PC interactions there was some evidence for small, lipid phase state-induced distortions of the α-helical structure of P_{24} (Zhang *et al.*, 1992b). Nevertheless, it was also evident that the thermotropic phase behavior of P_{24}/PC mixtures is consistent with the pattern of hydrophobic mismatch outlined above, suggesting that the P_{24} retains its predominantly α-helical structure regardless of lipid bilayer thickness. However, our studies of $(LA)_{12}$ indicate that this peptide may be more conformationally responsive to changes in the physical properties of the surrounding medium than is P_{24} and suggests that its α-helical domains may be subject to a greater degree of conformational distortion (Zhang *et al.*, 1995b). Since significant changes in conformation will inevitably change the effective hydrophobic length of the peptide, the effective hydrophobic length of $(LA)_{12}$ may differ significantly from that of an ideal α-helix and be subject to greater lipid-induced change than is observed with P_{24}. These considerations play an important role in defining the perspective from which our experimental data will be examined and interpreted.

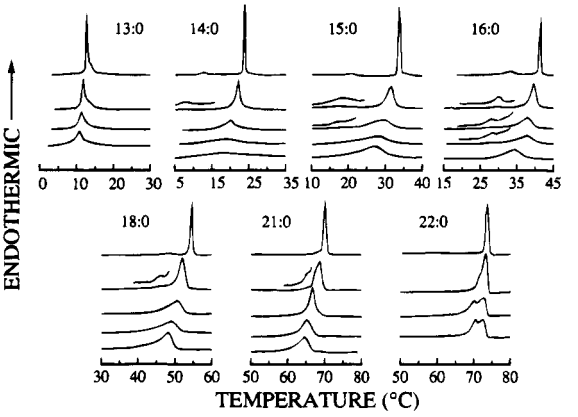

FIGURE 1: DSC heating thermograms of aqueous dispersions of mixtures of $(LA)_{12}$ with the *n*-saturated diacyl-PCs. Where possible, the pretransitions are shown on an expanded scale (×4). The R_p values of the thermograms shown are 0.0, 0.026, 0.056, 0.091, and 0.17 (top to bottom).

(a) Differential Scanning Calorimetric Studies

Thermotropic Phase Behavior of the Pure n-Saturated Diacyl-PCs. DSC thermograms of the pure *n*-saturated diacyl-PCs used in this study (i.e., $R_p = 0$) are shown in Figure 1. With the exception of 13:0 PC and 22:0 PC, the DSC heating thermograms shown consist of a cooperative, highly energetic chain-melting phase transition (the $P_{\beta'}/L_\alpha$ phase transition) which is preceded by the so-called pretransition (i.e., the $L_{\beta'}/P_{\beta'}$ phase transition) of these lipids. With 13:0 PC the pretransition occurs at temperatures below 0 °C (Lewis *et al.*, 1987) and is therefore outside the range of the Microcal MC-2 instrument used, whereas with pure 22:0 PC the single energetic phase transition observed near 74 °C has been shown to be a summation of overlapping $L_{\beta'}/P_{\beta'}$ and the $P_{\beta'}/L_\alpha$ phase transitions (Lewis *et al.*, 1987). The DSC thermograms exhibited by multilamellar dispersions of pure 13:0 PC also contain a distinctive high-temperature shoulder. The physical basis of this aspect of

[2] Note that this is different from the "end to end" distance of these types of α-helical peptides [≅35−36 Å; see Davis *et al.* (1983)].

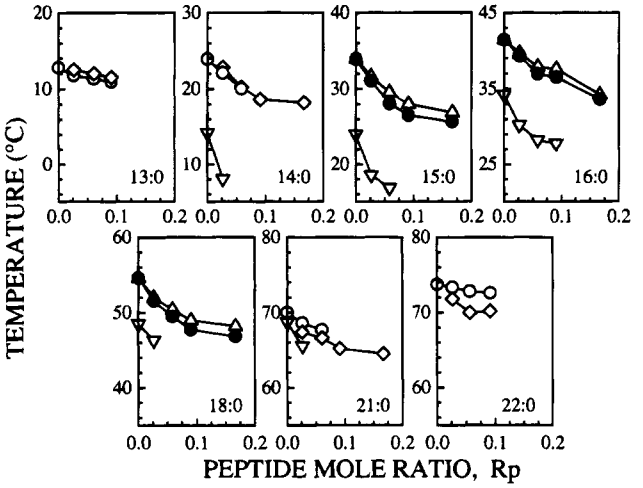

FIGURE 2: Effect of $(LA)_{12}$ on characteristic transition temperatures of $(LA)_{12}$/PC mixtures. (○) Sharp components of mixtures with short- and long-chain PCs. (◇) Broad components of mixtures with short- and long-chain PCs. (▽) Pretransitions. (△) Chain-melting endotherms of mixtures with medium-chain PCs (peak temperatures). (●) Chain-melting endotherms of mixtures with medium-chain PCs (temperatures at 50% area).

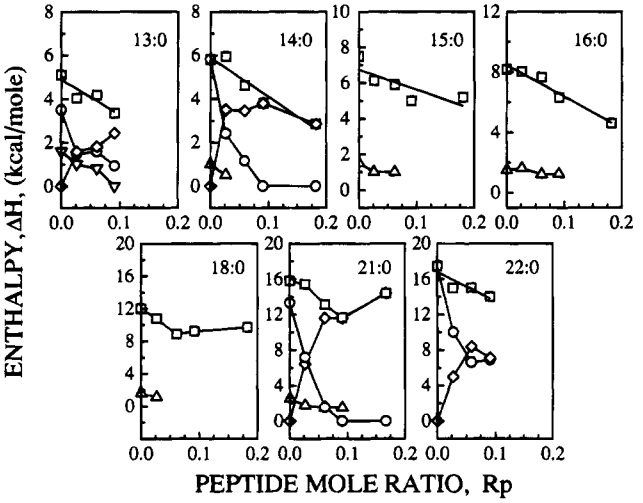

FIGURE 3: Effect of $(LA)_{12}$ on characteristic enthalpy changes associated with the phase transitions exhibited by $(LA)_{12}$/PC mixtures. The values shown are all normalized against the total amount of lipid present. (□) Total enthalpy of the main endotherm. (○) Sharp components of mixtures with short- and long-chain PCs. (◇) Broad components of mixtures with short- and long-chain PCs. (△) Pretransitions.

the behavior of 13:0 PC is currently unknown but it has been shown to be part of the hydrocarbon chain-melting process [see Morrow and Davis (1987)]. For a more detailed description of the thermotropic phase behavior of this homologous series of pure *n*-saturated diacyl PCs, refer to Lewis *et al.* (1987) and references cited therein.

Thermotropic Phase Behavior of Mixtures of $(LA)_{12}$ with the n-Saturated Diacyl-PCs. Figure 1 also shows representative DSC thermograms exhibited by mixtures of $(LA)_{12}$ with the various *n*-saturated diacyl-PCs studied. The effects of peptide concentration on the thermodynamic properties of the pretransition and gel/liquid-crystalline phase transitions of the lipids are summarized in Figures 2 and 3. The data shown therein clearly indicate that the thermotropic properties of the pretransition and the main or chain-melting phase transition of these lipids are both affected by the incorporation of the peptide $(LA)_{12}$. In cases where pretransitions are

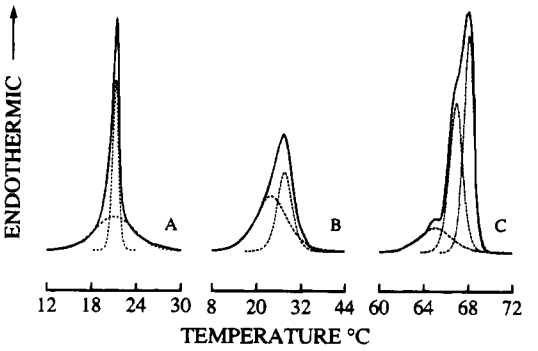

FIGURE 4: Illustrations of the deconvolution of the hydrocarbon chain-melting phase transition endotherms exhibited by the mixtures of $(LA)_{12}$ with representative short- (A), medium- (B), and long-chain (C) *n*-saturated diacyl-PCs. The solid lines represent the contours of the observed thermograms, and the dashed lines represent our estimates of the contours of the components. For the medium-chain lipids the DSC thermograms were not interpreted in terms of a summation of underlying components. The fitted subcomponents in B are shown merely to illustrate that it is feasible to fit these thermograms to such a description. The thermograms shown were obtained from (A) 13:0 PC/$(LA)_{12}$ mixtures (R_p = 0.056), (B) 16:0 PC/$(LA)_{12}$ mixtures (R_p = 0.017), and (C) 21:0 PC/$(LA)_{12}$ mixtures (R_p = 0.026).

resolvable from the chain-melting phase transition (i.e., 14:0 PC −21:0 PC), the incorporation of even small amounts of $(LA)_{12}$ results in a marked decrease in T_p coupled with a considerable increase in the width of the transition endotherm (see Figure 1). We also find that the magnitude of the decreases in T_p and the increases in $\Delta T_{1/2}$ increases with R_p and that pretransition endotherms of lipid/pepide mixtures made from many of the PCs studied cannot be resolved from the baseline at R_p values greater than 0.05 (see Figure 1). Presumably, the latter can be attributed to the marked increase in the $\Delta T_{1/2}$ of the pretransition which occurs at the higher R_p values. Our results also indicate that, at any given R_p value, the observed decrease in T_p gets smaller as the length of the hydrocarbon chain increases. Thus, for example, at R_p values near 0.03 decreases in T_p of some 6 °C are observed with 13:0 and 14:0 PC, whereas with the longer chain lipids (18:0−21:0 PC) decreases in T_p of some 2−3 °C are observed (see Figure 2). The physical basis of these latter observations is currently unknown.

The data shown in Figures 1−3 also indicate that the effect of $(LA)_{12}$ on the gel/liquid-crystalline phase transition of the diacyl-PCs studied varies with the length of the lipid hydrocarbon chains. Indeed, from an examination of the general shapes of the DSC thermograms obtained, it does appear that effects of $(LA)_{12}$ on the chain-melting phase transition of the shorter chain lipids (i.e., 13:0 and 14:0 PC) differ from those observed with the medium chain lipids (i.e., 15:0−19:0 PC) which, in turn, differ from those observed with the longer chain lipids (21:0 and 22:0 PC). With the shorter chain compounds, incorporation of low levels of $(LA)_{12}$ results in the appearance of endotherms which appear to be a superimposition of a sharp component upon a considerably broader underlying component (see Figure 4). For mixtures of $(LA)_{12}$ with either of these lipids, the sharp component exhibits characteristics which are similar to but not identical to those of the pure lipid. In both systems the sharp component exhibits broader endotherms and lower midpoint temperatures than does the corresponding pure lipid and, with increases in R_p, its width increases, its midpoint temperature decreases, and its contribution to the total

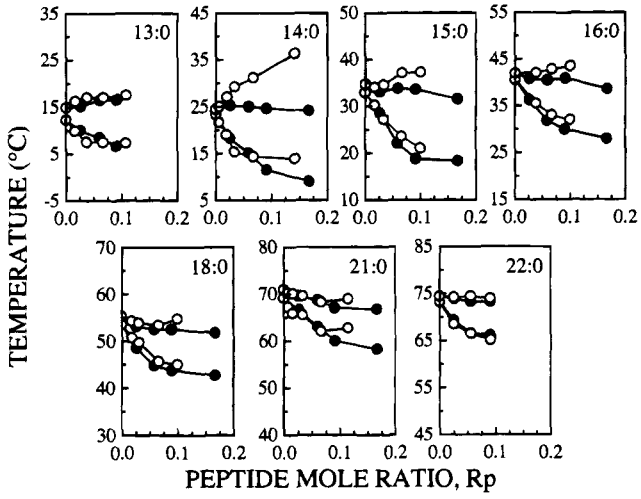

FIGURE 5: Temperature-composition pseudo phase diagrams constructed for mixtures of $(LA)_{12}$ with *n*-saturated diacyl-PCs hydrated in water. For comparison the phase diagrams for mixtures of P_{24} with the same lipid are also shown, and data for P_{24} mixture are taken from Zhang *et al.* (1992). (●) $(LA)_{12}$. (○) P_{24}.

enthalpy of the observed phase transition decreases. With the 13:0 PC mixtures, however, the sharp component seems to persist to very high R_p values (>0.1), whereas with 14:0 PC/$(LA)_{12}$ mixtures this component cannot be resolved at R_p values exceeding 0.06. Also, at any given R_p, the decrease in the midpoint temperature of the sharp component observed with the 13:0 PC/$(LA)_{12}$ mixtures is considerably smaller than that observed with the 14:0 PC/$(LA)_{12}$ mixtures. An analysis of the DSC thermograms of mixtures of $(LA)_{12}$ with either 13:0 PC or 14:0 PC also indicates that the underlying broad component observed at low to medium R_p is centered at lower temperatures than the chain-melting transition temperature of the pure lipid, but at a slightly higher temperature than that of the sharp component described above. Moreover, as was observed with the sharp component, increases in R_p result in an increase in the width of the broad component and a progressive decrease in the midpoint temperature. Also, as observed with the sharp component, the observed R_p-dependent decrease in the temperature of the broad component is greater in the 14:0 PC based lipid/peptide mixtures. Finally, with an increase in R_p, the broad component makes a progressively greater contribution to the total enthalpy change, and in the case of 14:0 PC/$(LA)_{12}$ mixtures it is the only component which can be resolved at high R_p values (see Figures 1−3).

With the medium chain lipids studied (15:0−20:0 PC), the incorporation of even small amounts of $(LA)_{12}$ results in an asymmetric endotherm which is skewed toward the low temperature end (see Figures 1 and 4), and the same general shape of these thermograms persists even to high R_p values. Unlike the DSC thermograms exhibited by the short chain lipids, it is not clear whether these endotherms are, in fact, the summation of two or more subcomponents, though, as illustrated in Figure 4B, it is possible to fit the observed DSC thermogram to such a description, even at very high R_p. For this group of lipids, an increase in R_p results in a decrease in the hydrocarbon chain-melting phase transition temperature, an increase in the width of the observed endotherm (see Figure 5), and an overall decrease in the enthalpy of the chain-melting phase transition (see Figures 1−3). The decrease in T_m appears to be dependent on R_p and independent of the length of the lipid hydrocarbon chain. Also, at any given R_p, the observed decrease in T_m is comparable to that observed with the $(LA)_{12}$/14:0 PC mixtures but considerably greater than that observed with the 13:0 PC/$(LA)_{12}$ mixtures.

Unlike the medium chain PC/peptide mixtures described above, the DSC endotherms exhibited by mixtures of $(LA)_{12}$ with 21:0 and with 22:0 PC seem to be summations of overlapping components. For the 21:0 PC/$(LA)_{12}$ mixtures, the DSC thermograms observed at low R_p seem to be a summation of a fairly sharp thermotropic event and two broader, lower-temperature thermotropic events (see Figure 5). With an increase in R_p the sharp component broadens significantly, its fractional contribution to the observed enthalpy change diminishes, and at R_p values above 0.05 its contribution to the total enthalpy change is negligible. In these respects the behavior of this sharp component is similar to those observed with mixtures of $(LA)_{12}$ with 13:0 and with 14:0 PC. At R_p values above 0.05, mixtures of $(LA)_{12}$ with 21:0 PC exhibit DSC thermograms that are essentially similar to those exhibited by mixtures of $(LA)_{12}$ with the medium chain PCs. In this range of R_p values the DSC endotherms broaden with increasing R_p (see Figure 5), and transition temperatures decrease to an extent comparable to those observed with the mixtures of $(LA)_{12}$ with the medium chain PCs. However, with $(LA)_{12}$/21:0 PC mixtures, the observed enthalpy change initially decreases with increasing R_p and then *increases* in the higher range of R_p values (see Figure 3).

Mixtures of $(LA)_{12}$ with 22:0 PC exhibit very complex DSC thermograms at all R_p values examined (see Figure 3). In this case the DSC thermograms are clearly definable in terms of a summation of overlapping peaks, and our data suggest that the resolution between these components improves with increasing R_p. Like the majority of the other lipid/peptide mixtures studied here, the DSC endotherms broaden and both transition temperatures and transition enthalpies decrease with increasing R_p. However, unlike the other lipid/peptide mixtures studied here, the 22:0 PC systems appear to be thermodynamically unstable because the shapes of the DSC thermograms change with succeeding runs, as does the partitioning of the observed enthalpy change between the resolvable subcomponents. A similar pattern of behavior was observed with 21:0 PC/P_{24} mixtures and was attributed to the phase separation of peptide-rich and peptide-poor domains (Zhang *et al.*, 1992b). The possibility that phase separation of peptide-rich and peptide-poor regions is also occurring with the 22:0 PC based lipid/peptide mixtures seems consistent with the observation that the widths of the DSC observed endotherms are smaller than those observed with all other lipid/peptide mixtures at all R_p values tested (see Figure 5).

(b) Fourier Transform Infrared Spectroscopic Studies

In these studies FTIR spectra of $(LA)_{12}$ mixtures were recorded in both the heating and cooling modes as a function of temperature and of the peptide mole ratio (R_p = 0.0, 0.091, and 0.17). FTIR spectroscopy was used here as a nonperturbing means of monitoring both the structural organization of the lipid bilayer and the conformation of the inserted peptide. The gel/liquid-crystalline phase transitions of the lipid bilayer were monitored by following changes in the frequency of the CH_2 symmetric stretching band near 2850 cm^{-1}, changes in solid-state hydrocarbon chain packing by

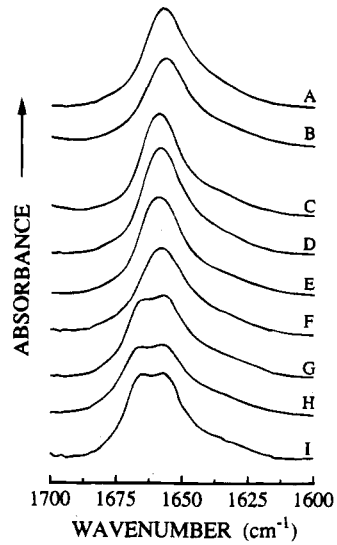

FIGURE 6: Amide I band contours of the FTIR spectra of $(LA)_{12}$/*N*:0PC mixtures. The spectra shown were under the following conditions. (A) Liquid-crystalline phase of mixtures with 13:0 PC (50 °C). (B) Liquid-crystalline phase of mixtures with 22:0 PC (75 °C). (C) Gel phase of peptide/13:0 PC (0 °C). (D) Gel phase of peptide/14:0 PC (0 °C). (E) Gel phase of peptide/16:0 PC (0 °C). (F) Gel phase of peptide/17:0 PC (10 °C). (G) Gel phase of peptide/18:0 PC (5 °C). (H) Gel phase of peptide/21:0 PC (25 °C). (I) Gel phase of peptide/22:0 PC (25 °C).

following changes in the CH_2 scissoring band near 1468 cm^{-1}, changes in the hydration and/or polarity of the polar/apolar interfacial region of the lipid bilayer by following changes in the contours of the ester carbonyl stretching bands near 1735 cm^{-1}, and changes in peptide conformation by following changes in the conformationally sensitive amide I band near 1655 cm^{-1} (Mendelsohn & Mantsch, 1986; Mantsch & McElhaney, 1991). We find that the incorporation of the peptide into all PC bilayers does not result in discernible changes in the hydration or the polarity of the polar/apolar interfacial regions of the lipid bilayer. However, peptide incorporation severely inhibits the formation of lipid subgel phases, and as a result the gel-phase frequencies of the methylene scissoring bands near 1468 cm^{-1} are always typical of rotationally disordered hydrocarbon chains even after prolonged, low-temperature incubation. Thus, the only spectroscopic feature of the lipid reported in any detail here is the CH_2 symmetric stretching band of the phospholipid hydrocarbon chains.

Illustrated in Figure 6 are a series of plots showing the amide I regions of the infrared spectra of samples in which $(LA)_{12}$ is dispersed in a range of PC bilayers of different hydrocarbon chain lengths. In the L_α phase of these mixtures the contours of the amide I band $(LA)_{12}$ are essentially independent of hydrocarbon chain length, being essentially similar for all of the lipids studied. As exemplified by the spectra A and B in Figure 6 (13:0 PC and 22:0 PC mixtures, respectively), the dominant feature of these spectra is the presence of a strong band centered near 1656 cm^{-1}, a frequency which is in the range expected of a peptide or protein adopting a predominantly α-helical conformation (Rabolt *et al.*, 1977; Dwivedi & Krimm, 1984). The other major features are less pronounced absorption bands centered near 1635−1640 cm^{-1} and near 1675 cm^{-1} which probably originate from non-α-helical domains (or populations) of the peptide (see Figure 10). However, the data also indicate that at temperatures where the lipids are in the gel phase the contours of the peptide amide I absorption band change with

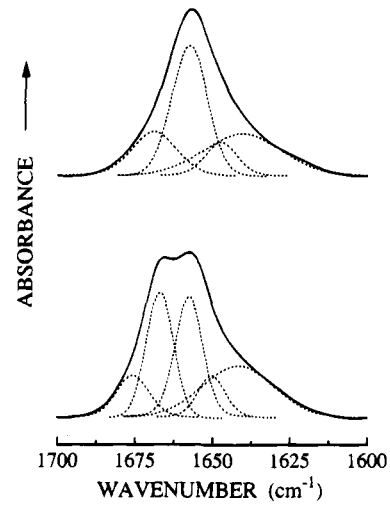

FIGURE 7: FTIR spectra showing the fine structure of the amide I band of $(LA)_{12}$ dispersed in 21:0 PC bilayers. The data shown were acquired at 25 °C (bottom) and 70 °C (top) corresponding to the gel and liquid-crystalline phases of the lipid/peptide mixture, respectively. Spectra are shown in the absorbance mode with the solid lines representing the observed spectra and the dashed lines representing our estimates of the contours of the subcomponents.

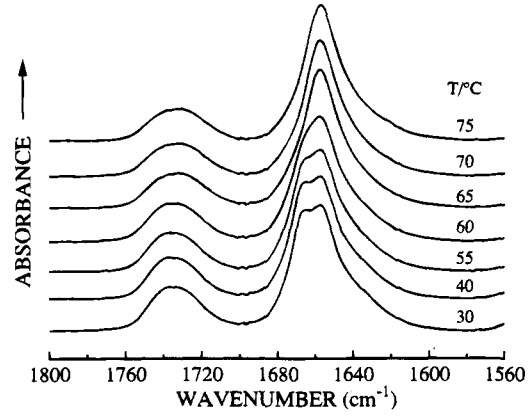

FIGURE 8: Temperature-dependent changes in the contours of the amide I band of $(LA)_{12}$ dispersed in 21:0 PC bilayers. Spectra were acquired at the temperatures indicated in the heating mode.

the length of the lipid hydrocarbon chain (see Figure 6, Spectra C-I). For lipid/peptide mixtures with PCs whose hydrocarbon chains range from 13 to 17 carbon atoms, the contours of the amide I absorption band of $(LA)_{12}$ are essentially similar to those observed in the liquid-crystalline phases of all mixtures. These spectra consist of a dominant absorption band centered near 1656 cm^{-1} with a smaller absorption shoulder centered between 1635 and 1640 cm^{-1}. However, with the longer chain lipids studied (i.e., 18:0−22:0 PC), the dominant features of the peptide amide I band observed at gel phase temperatures are a strong doublet with maxima near 1667 and 1656 cm^{-1} and an absorption shoulder near 1635−1640 cm^{-1} (see Figure 7). With these lipid/peptide mixtures, this doublet is observed at all R_p values examined, its appearance is a thermally reversible process, and, as illustrated in Figures 8and 9, its disappearance coincides with the gel/liquid-crystalline phase transition of the lipid/peptide mixture. Moreover, for those lipid/peptide mixtures which exhibit this phenomenon, a comparison of the substructures of the amide I bands observed at temperatures below and above the gel/liquid-crystalline phase transition of the mixture suggests that the peptide structure-(s) which give rise to the absorption band near 1667 cm^{-1}

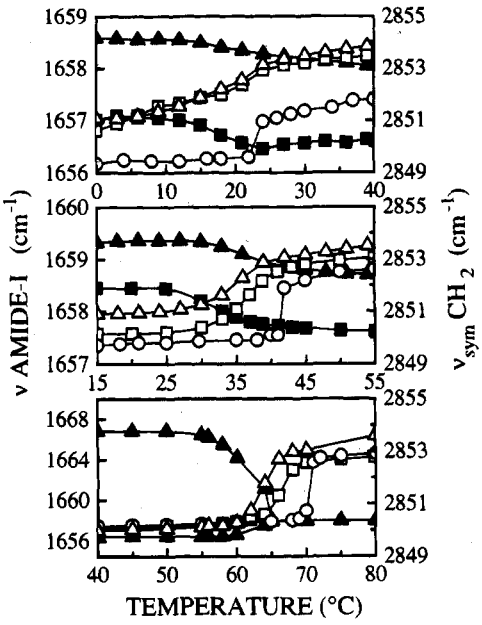

FIGURE 9: Combined plots of CH_2 symmetric stretch (open symbols) and peptide amide I band (solid symbols) as a function of temperature for mixtures of P_{24} with 14:0 PC (top), 16:0 PC (middle), and 21:0 PC (bottom). The CH_2 symmetric stretch (open symbol) for the pure lipid is also shown for comparison. The peptide ratios are 0.0 (circles), 0.091 (squares), and 0.17 (up triangles). For the amide I band of the peptide in 21:0 PC the α-helical component and the unassigned band (see text) are both shown.

are formed predominantly at the expense of the α-helical domains which give rise to the absorption band near 1656 cm^{-1}. Currently, the conformational disposition of the domains (or populations) of $(LA)_{12}$ which give rise to this absorption band near 1667 cm^{-1} is unknown. Nevertheless, our results clearly demonstrate that the conformational disposition of the peptide $(LA)_{12}$ can be changed by a sufficiently large phase state-dependent change in the thickness of the lipid bilayer in which it resides. The significance of this observation will be explored in the Discussion.

Figure 9 shows representative temperature dependencies of the frequencies of the peptide amide I band and of the lipid $\nu_s CH_2$ band for $(LA)_{12}$ mixtures with the short-, medium-, and long-chain PCs (14:0, 16:0, and 21:0 PC, respectively). Although the contours of the amide I band of $(LA)_{12}$ incorporated into bilayers composed of short to medium chain PCs (i.e., 13:0−17:0) are essentially unchanged upon lipid chain-melting transition, the frequencies of the amide I band do respond weakly to changes in lipid phase state. First, as exemplified in Figure 9, a small decrease ($\cong$1 cm^{-1}) in the frequency of the amide I band is observed at the chain-melting phase transitions of all of these lipid mixtures. Second, a comparison of the amide I frequencies of peptide/lipid mixtures of $R_p = 0.09$ and 0.17 indicates that the frequency of the amide I band increases slightly with an increase in R_p. Third, there also appears to be a small increase in amide I band frequency (<2.5cm^{-1}) as the lipid hydrocarbon chain length is increased up to 17 carbons. Clearly these changes are small, especially when compared with those which occur at the gel/liquid-crystalline phase transitions of the longer chain lipids (see above). These observations therefore suggest that when dispersed in bilayers composed of short to medium chain PCs, the α-helical domains of $(LA)_{12}$ are largely unaffected by changes in the phase state and physical properties of the lipids. Most probably, whatever conformational changes occur as a result of alterations in the phase state and physical properties of the short to medium chain PC bilayers are minor distortions of the peptide α-helix (Chirgadze *et al.*, 1976). Interestingly, however, such conformational changes always seem to occur whenever there is a decrease in the thickness of the lipid bilayer, however caused. Thus, small decreases in the amide I frequency are observed when the lipid chains melt and when the peptide is intercalated into thinner gel-phase lipid bilayers. Similar observations were observed when the peptide P_{24} was incorporated into *n*-saturated diacyl-PCs of similar hydrocarbon chain length (Zhang *et al.*, 1992b).

Figure 9 also shows that the hydrocarbon chain-melting phase transitions of the pure lipids are accompanied by discontinuous increases ($\cong$2−3 cm^{-1}) in the frequencies of their CH_2 symmetric stretching bands. These changes occur over a very narrow temperature range which coincides with the calorimetrically detected gel/liquid-crystalline phase transitions of the lipids. The increases in frequencies of the CH_2 symmetric stretching bands of these lipids are also accompanied by a discontinuous increase in their bandwidth (data not shown) and are typical of these types of hydrocarbon chain-melting phenomena [see Mendelsohn and Mantsch (1986) and Mantsch and McElhaney (1991) and references cited therein]. The incorporation of $(LA)_{12}$ into these lipid bilayers results in the following general changes in the absorption characteristics of the lipid CH_2 symmetric stretching band. First, with all lipid/peptide mixtures the heating endothermic transitions reported by DSC are accompanied by an increase in the CH_2 symmetric stretching band of some 2−3 cm^{-1} even at very high peptide concentrations. This result clearly indicates that at all peptide concentrations the endothermic transitions detected by DSC thermograms are indeed associated with lipid hydrocarbon chain-melting phenomena. Second, as was observed by DSC, the perturbing effect of $(LA)_{12}$ is manifest by an increase in the width of the spectroscopically detected hydrocarbon chain-melting transition and by a decrease of the midpoint temperature of that process. Third, for mixtures of $(LA)_{12}$ with the short- and medium-chain PCs, the perturbing effect of $(LA)_{12}$ is also manifest by an increase in the baseline frequencies of the CH_2 symmetric bands in both the gel and liquid-crystalline states, whereas for the longer chain lipids the presence of the peptide results in no obvious change in the frequencies observed in either the gel or liquid-crystalline states. Given that an increase in the frequency of the CH_2 symmetric stretching band is generally correlated with an increase in the degree of conformational disorder of hydrocarbon chains (Snyder, 1967; Maroncelli *et al.*, 1982, 1985), one can conclude that the incorporation of $(LA)_{12}$ into bilayers composed of the short- and medium-chain PCs results in a net conformational disordering of those bilayers, whereas the incorporation of the peptide into bilayers composed of the longer chain PCs has a considerably smaller effect on the degree of hydrocarbon chain conformational disorder.

DISCUSSION

A comparison of results of this study of $(LA)_{12}$/PC mixtures with those of similar studies of P_{24}/phospholipid mixtures (Zhang *et al.*, 1992b, 1995a) reveals a number of interesting similarities and differences in the thermodynamics of interactions between hydrophobic peptide helices and their lipid bilayers. Despite differences in the patterns of thermotropic phase behavior exhibited by the PC and PE-based

systems, the thermotropic phase behavior of mixtures of P_{24} with individual members of these two homologous series of phospholipids can be approximated by that of a macroscopic mixture of peptide-rich and peptide-poor domains (Zhang *et al.*, 1992b, 1995a). In this study it is not clear whether the same approximation holds for mixtures of $(LA)_{12}$ with the entire range of homologous PCs studied. Our data suggest that such an approximation does apply to mixtures with the short (13:0 and 14:0 PC) and long (21:0 and 22:0 PC) chain lipids studied. For example, with peptide mixtures with 14:0 PC or 21:0 PC (where the thermotropic phase behavior is not noticeably affected by lipid/peptide phase separation), the DSC thermograms observed at low R_p appear to be the summations of sharp and broad components. The sharp component exhibits properties that are similar to but not identical with those of the pure lipid and the relative contribution of the sharp component to the total enthalpy change decreases and eventually vanishes with increasing R_p. This behavior is essentially similar to that observed in our previous studies of P_{24}/PC and P_{24}/PE mixtures (Zhang *et al.*, 1992b, 1995a), and, similarly, we have tentatively assigned the sharp component to the melting of lipids whose behavior is not directly perturbed by interaction with the peptide (i.e., lipids in peptide-poor domains) and the broad component to the melting of lipids whose thermotropic phase behavior is directly perturbed by interaction with the peptide (i.e., lipids in peptide-rich domains). For mixtures of $(LA)_{12}$ with the medium-chain PCs, however, it is not clear whether the observed DSC thermograms are actually summations of broad and sharp components. Although these DSC thermograms can be fitted to such a description (see Figure 4), we find that at all finite R_p values the general shape of the DSC thermograms is essentially similar and the relative proportions of two fitted components do not change significantly with increases in R_p. This aspect of the behavior of mixtures of $(LA)_{12}$ with the medium-chain PCs differs from that previously observed with mixtures of P_{24} with the same lipids (Zhang *et al.*, 1992a). The physical basis for this difference in the pattern of thermotropic phase behavior of the P_{24}/PC and $(LA)_{12}$/PC mixtures is currently unknown.

Despite the similarities between the thermotropic phase behavior of P_{24}/PC mixtures and those of $(LA)_{12}$ with the short- and long-chain PCs, there are also some behavioral differences which may be of structural significance. Specifically, in all P_{24}/PC mixtures examined so far, the sharp component of the DSC thermogram is only observed at low R_p and usually disappears at R_p values near 0.05 (i.e., $\cong$20 lipids per peptide molecule). These R_p values are similar to that estimated for the formation of minimal unique solvation layers around each peptide molecule (Morrow *et al.*, 1985; Zhang *et al.*, 1992b, 1995b). With 13:0 PC/$(LA)_{12}$ mixtures, however, this sharp component, which presumably arises from domains of peptide-poor lipid, persists to fairly high R_p values (>0.1). We therefore suggest that this seemingly anomalous behavior of the 13:0 PC/$(LA)_{12}$ system is attributable to lipid/peptide phase separation which occurs at the higher R_p values examined. One should note that similar behavior is not observed with 14:0 PC/$(LA)_{12}$ mixtures, the behavior of which qualitatively resembles those of P_{24}/PC mixtures. Interestingly, in mixtures of 13:0PC/P_{24} peptide/lipid phase separation was not indicated (Zhang *et al.*, 1992b), while in mixtures of P_{24} with the shorter chain PEs, lipid/peptide phase separation was observed only when lipid hydrocarbon chain lengths approached 11 carbon atoms (Zhang *et al.*, 1995a). If lipid/peptide phase separation in mixtures of short chain lipids with either P_{24} or $(LA)_{12}$ is attributable to a mismatch of bilayer hydrophobic thickness and peptide hydrophobic length, then the above observations would suggest that $(LA)_{12}$ behaves as if its hydrophobic length is significantly longer than that of P_{24}. We suspect that this behavior is attributable to the rougher surface topology of the hydrophobic domain of $(LA)_{12}$ (see below). Interestingly, it was demonstrated that mixtures of P_{24} with 21:0 PC are thermodynamically unstable, presumably because lipid/peptide phase separation occurs when the mixture is cooled to temperatures below T_m (Zhang *et al.*, 1992b). In this study we have demonstrated that, unlike 21:0 PC/P_{24} mixtures, 21:0 PC/$(LA)_{12}$ mixtures show no discernible evidence for lipid/peptide phase separation and are, apparently, thermodynamically stable. However, evidence for lipid/peptide phase separation was observed with 22:0 PC/$(LA)_{12}$ mixtures. The suggestion that when dispersed in PC bilayers $(LA)_{12}$ behaves as if its effective hydrophobic length were longer than that of the polyleucine-based analogue P_{24} can also rationalize these particular observations.

Another interesting aspect of the behavior of PC/P_{24} and PC/$(LA)_{12}$ mixtures is that the former shows a continuous gradation of thermotropic phase behavior with increases in hydrocarbon chain length which is not apparent with the PC/$(LA)_{12}$ mixtures. Indeed, with the PC/P_{24} mixtures, the continuous changes in the relative T_m's of the sharp and broad components of the DSC endotherms were interpretable in terms of a continuous change in both the degree and the sign of the mismatch between peptide hydrophobic length and bilayer hydrophobic thickness (Zhang *et al.*, 1992b). In contrast, PC/$(LA)_{12}$ mixtures do not exhibit comparable graded changes in thermotropic phase behavior which one can attribute to incremental changes in the mismatch between peptide hydrophobic length and bilayer hydrophobic thickness. The effects of $(LA)_{12}$ on the thermotropic phase behavior of the PCs are essentially similar for lipid mixtures where there were small to moderate mismatches between peptide hydrophobic length and lipid bilayer thickness. Significant deviations from this general pattern are observed only with the very short and very long lipid hydrocarbon chain lengths where lipid/peptide phase separation seemed to occur. We suggest that the increased conformational plasticity of $(LA)_{12}$ in comparison to P_{24} may have resulted in a more pronounced mutual accommodation of potential differences in hydrophobic mismatch between the peptide and medium-chain PC bilayers.

We also found that, for $(LA)_{12}$ mixtures with PCs of hydrocarbon chain lengths ranging from 14 to 19 carbon atoms, the decrease in T_m (relative to the T_m of the pure lipid) observed at any given R_p is considerably greater than that observed with P_{24}/PC mixtures. In this respect, $(LA)_{12}$ could be considered to be more perturbing of lipid bilayer structure than is the polyleucine-based analogue P_{24}. The reasons why these two hydrophobic helical peptides should exhibit such markedly different effects on the chain melting phase transition temperatures of their host bilayers are also unclear. However, in considering some of the possible reasons for this phenomenon, the following points should be noted. First, because of its higher alanine content, the surface of an $(LA)_{12}$ α-helix should be intrinsically less hydrophobic than that of a P_{24} α-helix. Intuitively, one expects that the hydrophobicity of the surface of a transmembrane peptide must significantly

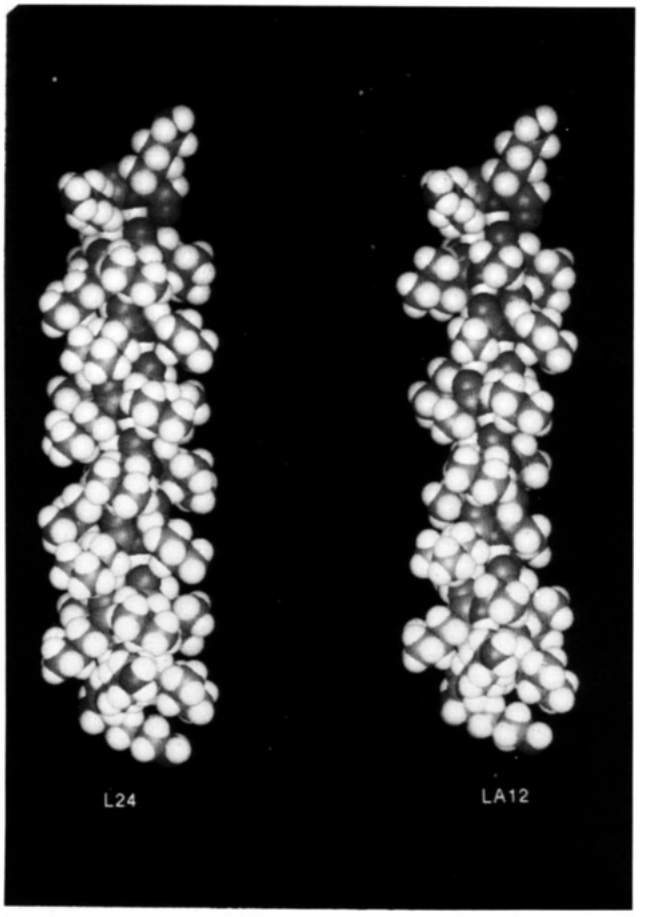

FIGURE 10: Comparison of space filling models of $(LA)_{12}$ and its polyleucine-based analogue, L_{24}. The computer drawn models are presented in the same orientation with both peptides in α-helical conformations.

affect the thermodynamic aspects of the interaction of the peptide with the surrounding lipids. Currently, very little information is available on this particular aspect of the interactions between transmembrane peptides and lipids, and it is difficult to determine, *a priori*, how the thermotropic phase behavior of any given lipid bilayer should change in response to changes in the surface hydrophobicity of the intercalated peptide helix. Clearly, this particular aspect of lipid−protein interactions should be investigated further. Second, an examination of molecular models of P_{24} and $(LA)_{12}$ indicates that even if one can assume that these two peptides adopt ideal α-helical conformations, the surface topologies of the two peptides would be significantly different (see Figure 10). Indeed, because of the differences in the sizes of the leucine and alanine side chains, P_{24} should present a smoother and more cylindrically symmetrical surface to the surrounding lipids. Thus, a hydrocarbon chain adjacent to the surface of $(LA)_{12}$ may well have a greater degree of conformational freedom than would a similar chain adjacent to the surface of P_{24}, resulting in a greater perturbation of hydrocarbon chain packing, particularly in the gel state. Third, it is clear from this work and the accompanying study (Zhang *et al.*, 1995b) that $(LA)_{12}$ is more conformationally responsive to changes in the physical properties of the host lipid bilayer than is P_{24}. Given that any conformational change in $(LA)_{12}$ in response to changes in the physical properties of the host lipid will concomitantly alter its effective hydrophobic length, it is also possible that the observed differences between the thermotropic phase behavior of $(LA)_{12}$/PC and P_{24}/PC bilayers may occur, in part, because the effective hydrophobic length of $(LA)_{12}$ is subject to greater variation than that of P_{24}. If this is indeed the case, such variations would essentially attenuate any pattern of graded change in thermotropic phase behavior when there is a small to moderate mismatch between peptide nominal hydrophobic length (i.e., the length the peptide would assume if it forms an ideal α-helix) and lipid bilayer thickness. This suggestion could offer a rationale for why we did not observe a graded pattern of thermotropic phase behavior comparable to that observed with the peptide P_{24}.

A significant result of this work is the demonstration that the conformational disposition of a hydrophobic peptide helix can be changed in response to changes lipid bilayer thickness. We have previously shown that, when dispersed in 16:0 lyso-PC, the conformation of $(LA)_{12}$ changes at the lamellar-coagel to micellar phase transition of the host lipid (Zhang *et al.*, 1995b). Here, our data clearly suggest that, when mixed with the longer chain PCs ($N \geq 18$), $(LA)_{12}$ undergoes a significant conformational change at the gel/liquid-crystalline phase transition of the lipid/peptide mixture. Evidence for such a conformational change was obtained from our FTIR spectroscopic studies which showed a major reversible change in the contours of its amide I absorption band at the gel/liquid-crystalline phase transition of the host lipid bilayer. This spectroscopic change was observed even at low R_p values, suggesting that it is the manifestation of a real conformational change instead of transitions between aggregated and disaggregated states of $(LA)_{12}$ at the gel/liquid-crystalline phase transition temperature of the lipid. Currently, the exact nature of this conformational change is unknown, though our data clearly suggest that it does involve some structural change to the α-helical domain(s) of the peptide. We believe that it is very unlikely that this conformational change involves significant folding/unfolding of the α-helical domains of $(LA)_{12}$ because this would expose the polar moieties of the peptide backbone to the hydrophobic domains of the lipid bilayer. We therefore suspect that the observed conformational change involves some distortion of the α-helical domains of the peptide and/or local transitions between α-helical and other helical folding motifs. Interestingly, the frequency of the anomalous amide I band observed in the gel phases of mixtures of $(LA)_{12}$ with the longer chain lipids is close to that reported for the amide I absorption bands of oriented purple membrane samples (Rothschild & Clark, 1979a,b). To account for the high frequency of the amide I band, Rothschild and Clark (1979a,b) suggested that the α-helical domains of bacteriorhodopsin (the major membrane protein of purple membrane) are probably distorted. Later, Krimm and Dwivedi (1982) suggested that the absorption band near 1665 cm^{-1} may be originating from α_{II} helices. Our evidence that the conformations of transmembrane helices can be altered by changes in the physical properties of the host lipid bilayer is particularly significant because it offers a possible mechanism by which information about the physical properties of a lipid bilayer can be transmitted to integral transmembrane proteins and possibly affect their biological activities. It is also noteworthy that although the transmembrane helical structure of $(LA)_{12}$ may well be less stable to conformational distortion than is that of P_{24}, it may well be more stable to such distortions than the compositionally complex transmembrane helical segments of many transmembrane proteins. Thus, the transmembrane helical domains of natural integral membrane proteins may be more conformationally sensitive to changes

in the physical properties of their host lipid bilayer membranes than is the case with the peptide models studied here.

The issues raised by this work underscore many of the inherent difficulties encountered in applying current theoretical descriptions of lipid–protein interactions to the interactions of even simple hydrophobic transmembrane peptides with lipid bilayers. Currently, virtually all theoretical descriptions of the effects of hydrophobic transmembrane proteins on their host lipid bilayers simplify the description of the transmembrane protein or peptide to that of an inert rigid body of invariant hydrophobic length and with a smooth, cylindrically symmetrical hydrophobic surface. Although the behavior of the essentially homogenous hydrophobic helical peptides such as P_{24} may well be approximated by such simplifications, our results suggest that those simplifications may not hold with a hydrophobic helical peptide such as $(LA)_{12}$, a peptide which is still compositionally simple relative to the transmembrane helices of natural proteins. Given this, it is difficult to envisage how theoretical approaches based on such simplifications can provide a reasonable framework for understanding the interactions between lipid bilayers and real membrane proteins. Indeed, our results suggest that the development of an adequate theoretical framework for understanding lipid–protein interactions must involve explicit considerations of factors such as surface topology, surface hydrophobicity, the potential for changes in peptide conformation, the possibility that the hydrophobic length of any given peptide may not be constant, as well as effects arising from variations in the polar headgroup and/or the hydrocarbon chain composition of the host lipid bilayer.

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