Mixtures of a Series of Homologous Hydrophobic Peptides with Lipid Bilayers: A Simple Model System for Examining the Protein–Lipid Interface

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ABSTRACT: The interactions of several members of a homologous series of peptides with the phospholipid bilayer have been examined by using fluorescence and deuterium NMR spectroscopy, differential scanning calorimetry, and measurements of water-to-bilayer partition coefficients. 1,2-Dimyristoyl-sn-glycero-3-phosphocholine (DMPC) bilayers and tripeptides of the form Ala-X-Ala-O-tert-butyl are used as a model system to probe the influence of amino acid side-chain substitution on the insertion of peptides into membranes and the behavior of peptide/bilayer mixtures. Tripeptides with X = Gly, Ala, Phe, and Trp have been examined. All of the tripeptides are water soluble, and all partition into DMPC bilayer vesicles to some extent. The Gly-containing peptide is the least soluble and the Trp-containing peptide the most soluble in the bilayer. The extent of perturbation of the bilayer structure induced by the peptides parallels their bilayer solubility: the Gly and Ala peptides act as simple impurities while peptides containing bulky aromatic rings cause a phase separation. Changes in the fluorescence properties of the Trp analogue upon incorporation into the bilayer indicate that the Trp side chain is probably immersed in the hydrocarbon region of the bilayer. Peptides of this form should serve as easily modifiable model systems with which to examine details of how the bilayer environment affects peptide conformation, as well as how hydrophobic peptides affect the bilayer structure.

Most physical models for the insertion and translocation of proteins and peptides into and across membranes invoke relatively simple partitioning processes that rely upon knowing the free energy of transfer of the hydrophobic portions of proteins from water into the lipid bilayer (Engelman & Steitz, 1981; Jahnig, 1983; Sabitini et al., 1982; von Heijne & Blomberg, 1979; Wickner, 1979). Thus, it is of fundamental importance to understand how the hydrophobic portions of proteins interact with their surroundings. Almost all analyses of the protein insertion/translocation problem make four assumptions: (1) The interior of the bilayer can be treated as a simple bulk hydrophobic phase. (2) Partitioning data for the transfer of hydrophobic amino acid side chains from water to bulk organic phases provide an adequate quantitative basis for the partitioning process. (3) All membrane-spanning proteins are comprised largely of either $\alpha$ or $\beta$ helices. (4) Protein/water interfacial area analyses which are useful for examining globular proteins in water (Chothia, 1976; Richards, 1977; Guy, 1985) are equally applicable to membrane proteins. These assumptions are not necessarily unreasonable, and some parts of the analyses based upon them are compelling. On the other hand, direct experimental data adequate for the evaluation of these assumptions are lacking. The complexity of intact biological membranes makes it difficult to use them for this purpose. One must therefore turn to simpler systems to examine questions of the solubility of portions of proteins (i.e., peptides) in the bilayer, lipid perturbation induced by the incorporation of peptides, location of the peptides in the bilayer, peptide conformational and motional changes induced upon transfer from H$_2$O to the bilayer, and the effects of changes in the primary structure of the peptide.

Several model systems aimed at elucidating one or more of these properties are being investigated in various laboratories. Davis and co-workers have employed $^3$H NMR and other techniques to examine leucine oligomers that span the bilayer (Huschilt, Hodges, & Davis, 1985; Davis et al., 1983).
Gierasch and co-workers have been examining the implications of relatively minor sequence alterations on the conformation and membrane binding properties of the signal sequence of the LamB protein (Briggs & Gierasch, 1984; Briggs et al., 1985). Amphipathic α helices represent a qualitatively different class of membrane-associated peptides which Kaiser and co-workers are studying in some detail (Moe & Kaiser, 1985; Kaiser & Kezdy, 1984). Several small to medium size naturally occurring membrane proteins are also being employed as model systems (Dettman et al., 1984; Wilson & Dahlquist, 1985; Braun et al., 1983; Feigenson & Meers, 1980; Weinstein et al., 1980; Eisenberg, 1984).

Work employing short hydrophobic peptides (three to six residues) has shown that manipulation of the amino- and carboxyl-protecting groups can alter the solubility properties of the peptide (Wallace & Blout, 1979; Sugihara et al., 1982). With these results in mind we have been led to develop a class of peptides that can be used to resolve the contributions of single amino acid residues in lipid–protein interactions. These peptides are three residues long with a free amino terminus and the carboxyl end block with a tert-butyl ester:

 Ala-X-Ala-O-tert-butyl

The charged amine serves to anchor that end of the peptide at the lipid/water interface, while the bulky hydrophobic tert-butyl group serves to increase peptide solubility in nonaqueous media. Alanine was chosen to be the initial and final residues because it is relatively small and hydrophobic; thus, they will enhance lipid solubility without overwhelming the central residue. Tripeptides are a natural starting point since they are the simplest moieties in which there exists an amino acid residue in a more or less natural setting, i.e., with the peptide bonds on both of its ends. We report here a study of model lipid membranes with admixtures of members of this homologous series of peptides where X is glycine, alanine, phenylalanine, or tryptophan. Buffer to 1.2-dimyristoyl-sn-glycero-3-phosphocholine (DMPC)1 bilayer partition coefficients of the peptides, the thermodynamic phase behavior of the lipid/peptide mixtures, changes in the optical properties of the X = Trp peptide upon partitioning into the bilayer, and 2H NMR spectra of the lipids in lipid/peptide mixtures are presented. We find that even these relatively simple peptides interact strongly with the bilayer and that the characteristics of the interactions are strong functions of the identity of the central residue.

**Experimental Procedures**

**Peptide Synthesis.** Peptides of the form Ala-X-Ala-O-tert-butyl and Z-Ala-X (Vega Biochemicals, Tucson, AZ) and subsequent clipping of the Z protecting group. Coupling was performed with dicyclohexylcarbodiimide via standard methods (Rich & Singh, 1979; Bodanszky et al., 1976; Stewart & Young, 1984). The Z group was then cleaved by hydrogenation over Pd/C catalyst at atmospheric pressure. The product was purified by preparative thin-layer chromatography on silica gel 60 (Whatman, 20 × 20 × 0.05 cm plates). Purity of the final product was checked by TLC in three solvent systems: 1-butanol–H₂O–acetic acid (8:2:1), chloroform–methanol–H₂O (65:25:5), and ethyl acetate–hexane–acetic acid (20:10:1).

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1 Abbreviations: NMR, nuclear magnetic resonance; Δν₁, deuterium residual quadrupole splitting; DSC, differential scanning calorimetry; DMPC, 1,2-dimyristoyl-sn-glycero-3-phosphocholine; Z, benzoyloxycarbonyl group; A, alanine; G, glycine; P, phenylalanine; K, lysine; L, leucine; W, tryptophan.

Visualization was accomplished with ninhydrin, I₂, and UV absorption on fluorescent plates (silica gel 60 F₂₅₄).

**Lipids.** Unlabeled DMPC was purchased from Avanti Polar Lipids (Birmingham, AL). Acyl chain perdeuterated DMPC was purchased from Cambridge Isotopes, Inc. (Cambridge, MA). Tritiated DMPC was purchased from New England Nuclear (Boston, MA).

**Vesicle Preparation.** Phospholipid vesicles were dispersed in the hydrosol suspension and fluorescence titration studies were prepared by the method of Huang and Thompson (1974). Briefly, lipid suspended in 50 μM phosphate buffer (pH 7.0) was sonicated to clarity (15–30 min) under argon atmosphere with a microtip probe attached to a 250-W Sonics & Materials (Danbury, CT) sonicator. Temperature was maintained at 10–15 °C above the lipid phase transition throughout the sonication. The sample was then centrifuged and decanted to remove titanium particles and large vesicles. Vesicle samples were used within 12 h of their production and maintained above the lipid phase transition temperature.

Peptide/vesicle mixtures were made by combining stock solutions of the peptide and vesicles, diluting to the appropriate volume with buffer, and then bath sonicated under nitrogen for 1/2 h at 30 °C immediately prior to filtration or recording of the fluorescence spectrum.

**Hydrosopic Desorption.** Bilayer/water partition coefficients were measured by the hydrosopic desorption method (Pjura et al., 1984; Conrad & Singer, 1981). Three filters were mounted in a 47-mm Swin-Lok Holder (Nuclepore Corp.). The top filter was polycarbonate with a nominal pore size of 0.03 μm (Nuclepore Corp. 111102). The middle filter was a Schleicher & Schuell 1-HV inert glass filter, while the bottom filter was a Schleicher & Schuell 470 cellulose pad. The polycarbonate filter was conditioned by washing in 10% acetic acid for about 30 min. The filter was then thoroughly rinsed with deionized H₂O, dried, and used immediately. Treatment of the polycarbonate filter with surfactant [soaking in poly(vinylpyrrolidone)] did not materially affect the filtration times or retention of the vesicle mixtures; thus, this treatment was not routinely incorporated into our experimental protocol.

Peptide/vesicle samples (0.2–0.5 mL, 1–5 mM lipid, 1–5 mM peptide) were filtered for 5–20 min under vacuum. The more concentrated samples took longer to filter, but no dependence of Kₚ on concentration was observed. The sample, filtering apparatus, and immediate environs were held at 33 °C before and during the filtration. The lipid/peptide mixture was extracted from the filter by washing in 4 mL of 2-propanol. The number of moles of peptide in the prefilterate sample (Cₚ) and in the sample remaining on the filter (Cₛ) was determined by fluorescein assay (Chen et al., 1978). The radioactivity of the lipid in the prefilterate sample and in the sample remaining on the filter was used to assay lipid content. Nonspecifically bound peptide was measured in each experiment by running a parallel sample without lipid. Typically, more than 95% of the lipid was retained on the filter, and nonspecifically bound peptide amounted to less than 10% of the total. Values of Cₛ used in the calculation of Kₛ were corrected for nonspecifically bound peptide.

The bilayer/water partition coefficient (Kₛ) was evaluated as the mole fraction of peptide associated with the bilayer divided by the mole fraction free to pass through the filter:

\[
Kₛ = \frac{Cₛ}{Cₛ + L}
\]

where Cₛ = Cₛ - Cᵢ is the number of moles of peptide in the supernatant, L is the number of moles of lipid retained, and
$W$ is the number of moles of water.

**Fluorescence Titration.** Fluorescence spectra were measured on a Spex Fluorolog spectrofluorimeter (Model 1902) interfaced to a Hitachi Equipment Corp. LSI-11 microcomputer where the spectra were stored and evaluated. Excitation and emission slits were set at 5 nm. The excitation wavelength was 280 nm, scan limits were 290 and 530 nm, and scan speed was 0.5 nm/s. All spectra were measured in the ratio mode by using 10-mm path-length cuvettes thermostatted at 33 °C.

The change in the integrated intensity of the Trp emission peak ($F$) as a function of lipid concentration was used to calculate $K_p$ (Surewicz & Epand, 1984; Bashford et al., 1979). In these calculations, $R$, the enhancement factor, is defined as

$$R = F/F_0$$  \hspace{1cm} (2)

where $F_0$ is the integrated intensity in the absence of lipid.

Following Bashford et al. (1979)

$$\frac{1}{R - 1} = \frac{[H_2O]}{(R_m - 1)K_p [lipid]} + \frac{1}{R_m - 1}$$  \hspace{1cm} (3)

Linear least-squares analysis of the data plotted in this double-reciprocal fashion yields $K_p$ as defined in eq 1 and $R_m$, the maximal enhancement factor. Peptide concentration was fixed at 20 μM while the lipid concentration was varied from 0.5 to 5 mM.

**Calorimetry.** All calorimetric work was performed on a Perkin-Elmer DSC 2B interfaced to a Digital Equipment Corp. LSI-11 microcomputer. DSC samples were prepared by co-dissolving the appropriate amounts of lipid and peptide in chloroform, evaporating the solvent under a stream of dry nitrogen, and then further drying in vacuo overnight. One to three milligrams of the dry sample was weighted into the DSC sample pan, an equal (or somewhat greater) mass of buffer was added, and then the pan was sealed. The sample was then mixed in the calorimeter by cycling the temperature from 275 to 310 K. Typically, no change was observed in the thermogram after the third cycle, although we routinely subjected each sample to seven cycles before recording the thermogram. All thermograms were recorded at a scan speed of 1.25 K/min.

**Deuterium NMR.** 1H NMR spectra were obtained at the NSF Southern California Regional NMR Center on a Bruker WM-500 spectrometer in the Fourier transform mode at 76.8 MHz ($H_2O = 11.7$ T). The quadrupole echo technique (Davis et al., 1976) was employed with a $r_{echo} = 40$ μs and a 90° pulse of 4-6 μs. The pulse repetition rate was 0.8 s. The data acquisition rate was 16666 Hz. An exponential multiplication factor of 40 Hz was applied to all spectra. Samples were prepared in essentially the same manner as described for the DSC experiments, except then a total mass of approximately 10 mg of dry lipid/peptide mixture and 500 mg of deuterium-depleted water (Aldrich) were sealed in the NMR tube, and the tube was agitated and equilibrated at 30 °C for 18 h before spectra were recorded.

**RESULTS**

**Hygroscopic Desorption.** A number of alternative definitions of the water-to-bilayer partition coefficients ($K_p$) are available (Katz & Diamond, 1974a; Pjura et al., 1984; Nozaki & Tanford, 1971), but the definition in terms of mole fractions has the advantage of coupling directly measured quantities (see eq 3) with thermodynamic quantities of interest [e.g., the free energy of transfer, $\Delta G_{transfer} = kT \ln (K_p)$] without intervening assumptions, e.g., no molecular volumes need be assumed (Pjura et al., 1984). $K_p$ is defined as

$$K_p = N_{bilinear}/N_{aqueous}$$  \hspace{1cm} (4)

where $N_{bilinear}$ is the mole fraction peptide associated with the bilayer and $N_{aqueous}$ is the mole fraction peptide in the aqueous phase. The results of experiments aimed at measuring $K_p$ for a homologous series of tripeptides are shown in Table 1. The temperature (33 °C) was chosen to ensure that the bilayer is in its fluid state. There is a large change in $K_p$ upon changing the central residue from glycine to tryptophan, while only a very small change is observed upon substitution of alanine for glycine. Figure 1 shows $K_p$ plotted vs. the calculated surface of the central amino acid residue (Chothia, 1976). A monotonous increase of $K_p$ with surface area is observed.

**Fluorescence Spectral Changes Induced by Lipid.** The spectral properties of the tryptophan indole ring system are known to be strongly dependent upon the local environment of the chromophore (Bell, 1981; Cogwill, 1967). In fluorescence emission spectra of the tryptophan containing tripeptide in the presence of increasing concentrations of DMPC vesicles, the fluorescence intensity increases and the wavelength of maximum intensity ($\lambda_{max}$) decreases from 354 nm in the ab-
The addition of peptide causes a general decrease in $\Delta H$ which is essentially the same for the methyl and all of the methylenes. The fractional change in $\Delta H$ induced by A-A-A-O-tert-butyl is 7%, while that induced by A-W-A-O-tert-butyl is 16%. In contrast, large amounts of hexane can be incorporated into fluid-phase DMPC bilayers with no appreciable change in the lipid ordering (Jacobs & White, 1984). Due to the large number of variables, no attempt was made to fit computer simulations to these spectra. Nevertheless, visual examination of the spectra in Figure 2 indicates that no gross changes in line width accompanies the introduction of large amounts of either of the tripeptides into the bilayer.

**Calorimetry.** DSC thermograms of pure DMPC liposomes and liposomes with 15 mol % A-G-A-O-tert-butyl or A-A-A-O-tert-butyl are shown in Figure 3. The addition of either of these peptides causes a small decrease in $T_m$ along with a decrease in intensity and concomitant broadening of the bilayer gel to liquid-crystalline phase transition. Little or no change in enthalpy is observed (see Table III).

Much more dramatic changes in the thermal properties of the bilayer are induced by the addition of the tripeptides that contain aromatic side chains. Figures 4 shows the effects of increasing amounts of tripeptide on the lipid bilayer phase transition. The effects of the phenylalanine- and tryptophan-containing peptide are qualitatively similar (data not shown...
for the glycyl peptide 2.8, 6.4, and 9.5 mol % are incorporated, for the alanyl peptide 2.5, 7.5, and 10.2 mol % are incorporated, for the phenylalanyl peptide 4.7, 9.2, and 14.3 mol % are incorporated, while for the tryptophan peptide 5, 10, and 15 mol % are incorporated into the bilayer.

**DISCUSSION**

We have examined some of the microscopic and thermodynamic properties of lipid bilayers with admixtures of relatively small and simple peptides. All of the tripeptides have the same amino-terminal alanyl and carboxy-terminal alanyl tert-butyl ester, differing only in the central amino acid residue. This initial study focuses on four uncharged residues that span the Tanford–Nozaki and other hydrophobicity scales (Tanford, 1962; Nozaki & Tanford, 1971; Charton & Charton, 1982; Eisenberg, 1984) and are of very different sizes: glycyl, alanyl, phenylalanyl, and tryptophan. Following Lahmig (1983) and Engelman and Steitz (1981), we express the total free energy change associated with the incorporation of a peptide into the membrane as

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\Delta G^\circ = \Delta G_e + \Delta G_h + \Delta G_w + \Delta G_p + \Delta G_t
$$

the sum of contributions from peptide conformational change (\(\Delta G_e\)), alterations in hydrogen bonding (\(\Delta G_h\)), the hydrophobic effect (\(\Delta G_w\)), the protein immobilization effect (\(\Delta G_p\)), and lipid perturbation effects (\(\Delta G_t\)). In this peptide series the only alteration in chemical structure occurs in the central amino acid side chain. Thus, one would hope to be able to attribute differences in the measured partition coefficients for these peptides in terms of free energy contributions arising from the central amino acid side chains. This is appropriate only if each of the peptides adopts the same conformation (or samples a similar portion of conformational space) in the aqueous phase and, similarly, if the conformation of the peptides is the same in the bilayer. This tantamount to making the assumption of group additivity (Tanford, 1980), i.e., that the different portions of the molecule make independent contributions to the free energy expression noted above. Making this assumption, we can directly compare the partition coefficients in Table I with various hydrophobicity scales of amino acid residues. As discussed by Eisenberg (1984) and Charton and Charton (1982), the different scales often reflect different properties of the amino acids. We emphasize that these scales are aimed at describing the folding process of globular proteins in which the peptide chain comes to interact with itself rather than the aqueous environment, whereas the process of interest here is the insertion of the peptide chain into a lipid-crystalline hydrophobic environment—the bilayer membrane. According to various hydrophobicity scales the Ala and Gly residues are similar and much less hydrophobic than the Trp and Phe residues. This rough ranking is reflected in the partition coefficients for the tripeptides (see Figure 1). Several of the scales rank tryptophan less hydrophobic than phenylalanine and some rank it less hydrophobic than glycine (Kyte & Doolittle, 1982). In the tripeptide series the Trp-containing peptide is clearly the most hydrophobic. Considering the variation among the hydrophobicity scales, there is reasonable agreement between them and the tripeptide partition data.

Several models describing protein incorporation into membranes make the simplifying assumption that \(\Delta G_e^\circ\) is directly proportional to the interfacial area (Jahnig, 1983; Engelman & Steitz, 1981; von Heijne & Blomberg, 1979). From analyses of amino acid and hydrocarbon partitioning between water and organic solvents, a free energy per area of 20–25 cal/mol\(^{-1}\)/A\(^{−2}\) is obtained (Richards, 1977; Reynolds et al., 1974). The two lines in Figure 1 are the \(x_1^*\)'s calculated by

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**FIGURE 4:** DSC thermograms of multilamellar suspensions of DMPC containing various amounts of Ala-Trp-Ala-O-tert-butyl: A, no peptide; B, 5 mol %; C, 10 mol %; D, 15 mol %.

**FIGURE 5:** Plots of the onset and completion temperatures of the high-temperature heat absorption event in the thermograms shown in Figures 3 and 4. Open circles: Ala-Phe-Ala-O-tert-butyl/DMPC mixtures. Closed circles: Ala-Trp-Ala-O-tert-butyl/DMPC mixtures. Heat absorption onset and completion temperatures were determined as described by Mabrey and Sturtevant (1976), except that no correction was made for the finite width of the pure DMPC–bilayer phase transition.

for the phenylalanyl peptide. The addition of small amounts of either peptide causes the pretransition to disappear and the main transition to broaden considerably with a shift to lower temperatures. At larger concentrations of peptide the endotherms become quite broad and exhibit a complex multipeak line shape. Taking the base-line intercept of the relatively sharp features at the low-and-high-temperature extremes of the endotherms (Mabrey & Sturtevant, 1976) yields the plot shown in Figure 5. The high-temperature completion points are essentially the same for both peptides. The low-temperature onset points occur at lower temperatures for the tryptophan-containing peptide than for the phenylalanyl-containing peptide.

It should be noted that all of the mole percent values quoted above are based on the total amount of peptide in the sample. Since there are physical differences between vesicular lipid bilayers and multilamellar lipid bilayers (Sheetz & Chan, 1972), it is not a priori certain that amphiphiles will partition into both systems to the same extent. Nevertheless, if the partition coefficients measured for vesicular DMPC bilayers are assumed to also apply to the liposome systems used in the NMR and DSC experiments, the calculation of the amount of peptide in the bilayer and in the aqueous phase is straightforward. In the NMR experiments the amounts of peptide in the bilayer become 11 and 14 mol %, respectively, for the alanyl- and tryptophan-containing peptides. In the DSC experiments with 5, 10, and 15 mol % peptide added,

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using these values for $\Delta G_m$. To facilitate comparison, the peptide $K_e$'s have been shifted so that the glycylic peptide value lies at the origin. The measured $K_e$'s are all significantly lower than those calculated. Taken at face value this indicates that the hydrophobic amino acid side chains have significantly less affinity for the bilayer than predicted by this range of $\Delta G_m$. Whether this is due to a breakdown in the group additivity assumption, the fact that the bilayer is a highly anisotropic solvent, or contributions from the other terms in the equation for $\Delta G^\circ$ is not clear at present.

Water to bilayer partition coefficients for a large number of amphiphiles have been measured (e.g., see Table 1). The partition coefficients for the glycylic and alanyl peptides are of the same magnitude as those found by Katz and Diamond (1974b) for medium to small size alcohols (see Table 1). $K_P$ for the trytophan-containing tripeptide is close to that found by Sureauwicz and Epaed (1984) for charged pentagastrin analogues, whereas the neutral analogue has a partition coefficient more than an order of magnitude larger. As expected, $K_P$ for fatty acids is much larger than that of the peptides—more than a factor of 2000 larger than the glycyl tripeptide.

The extent of peptide association deduced from the DSC thermograms parallels the partition coefficients measured via the hygroscopic desorption and fluorescence methods. Increasing effects are seen as the central residue is changed from Gly to Ala to Phe to Trp. At the same mole percent peptide the main DMPC phase transition is perturbed only slightly more by the alanyl than the glycyl tripeptide. Neither of these has the dramatic effects induced by the phenylalanyl and trytophan peptides. The trytophan peptide induces the formation of a slightly broader transition at a somewhat lower temperature than is observed upon addition of the same amount of the phenylalanyl tripeptide. In thermograms of some mixtures of short peptides (e.g., glucagon and pentagastrin) and lipid there is an endothermic attributable to undisturbed lipid (Epaed & Sturtevant, 1984). No undisturbed lipid melting endotherm is observed in these mixtures, indicating homogeneous lipid-peptide mixtures.

The heat absorption peaks in thermograms of two-component lipid bilayers can be interpreted in terms of the phase diagram for the mixture (Mabrey & Sturtevant, 1976). Figure 5 shows such a plot derived from thermograms of DMPC/A-F-A-O-tert-butyl and DMPC/A-W-A-O-tert-butyl mixtures. The liquidus curve delineates the boundary between the high-temperature single-phase fluid and lower temperature two-phase fluid plus solid region (Findley, 1951). This boundary (local of high-temperature points in Figure 5) is coincident for the phenylalanyl and trytophan peptides. The solidus curve (marking the two-phase and single solid-phase boundary) occurs at slightly lower temperatures for the trytophan than the phenylalanyl peptide. This implies that the larger trytophan peptide destabilizes the gel-phase lipid more than does the phenylalanyl-containing peptide. Thus, the Trp- and Phe-containing tripeptides induce a phase separation in the plane of the membrane in which a peptide-poor gel phase is in equilibrium with a peptide-rich liquid-crystalline phase. A similar two-phase region is seen in mixtures of DPPC and a leucine oligomer, $K_e$-G-L$_n$-K$_{A}$-amide, where $n$ is either 16 or 24, although in this system the phenomenon is shifted to much smaller peptide concentrations: 1–6 mol % (Davis et al., 1983; Huschilt et al., 1985). At the concentrations examined, the Gly- and Ala-containing peptides act as simple impurities—broadening and lowering the temperature of the phase transition.

It is useful to compare the tripeptide/bilayer mixtures with mixtures of lipids with more structurally similar molecules. Alkanes, fatty acids, and cholesterol are each a chetrapyl hydrophobic molecules that form characteristically different types of mixtures with lipid bilayers. The type of phase behavior, morphology, and structural characteristics of alkane/lipid bilayer mixtures are strongly dependent upon the alkane length and type of lipid (Jacobs & White, 1984; McIntosh & Costello, 1981; McIntosh et al., 1980). For example, in the hexane/DMPC mixture there is a solid–solid-phase separation of hexane-rich and hexane-poor solid phases at temperatures below the pure lipid $T_m$ (Jacobs & White, 1984), while the phase transition temperature of hexadecane/DPCC bilayer mixtures increased with increasing alkane concentration (McIntosh et al., 1980). Fatty acids also tend to raise the lipid bilayer phase transition temperature (Mabrey & Sturtevant, 1977). Mixtures of fatty acids in lipid bilayers appear to form sharp melting 1:2:lipid:fatty acid complexes where nonideal mixing is observed between the complex and free lipid when the fatty acid is the same length or longer than the lipid acyl chain (DPCC–palmitic acid and DPCC–stearic acid mixtures) while shorter fatty acids form nearly ideal bilayer mixtures (Schullery et al., 1981). Mixtures of cholesterol and phospholipids exhibit quite complex multiphase DSC thermograms which are difficult to interpret in terms of a phase diagram (Estep et al., 1978; Mabrey et al., 1978). Magnetic resonance experiments indicate that cholesterol has a general plasticizing effect on the lipid bilayer (Brown & Seelig, 1978; Oldfield et al., 1978). In terms of their phase diagrams, the tripeptide/bilayer mixtures we have examined exhibit relatively simple behavior: all appear to mix ideally in the bilayer, no stoichiometric complexes are observed, the less hydrophobic peptides act as simple impurities, and the more hydrophobic ones induce phase separations. Whether this is a general feature of bulky amphipholic molecules associated with the bilayer or a specific characteristic of these peptides in the DMPC bilayer we do not know. To address this question, we are examining mixtures of this series of peptides and longer model peptides (e.g. Ala$_n$-X-Ala$_m$-O-tert-butyl) in a variety of types of lipid bilayers (e.g., lipids with unsaturated acyl chains and different types of head groups).

Although this investigation does not directly address the question of where the bilayer-associated tripeptides are located (e.g., between the monolayers, aligned with the acyl chains, and/or adsorbed at the lipid/water interface), the fluorescence and $^2$H NMR spectroscopic data are suggestive. The blue shift in $\lambda_{max}$ and increase in intensity of the trytophan fluorescence upon lipid binding indicate that the fluorphore is being transferred to a solvent of low polarity (Cowgill, 1967; Bell, 1981), i.e., somewhere in the interior of the lipid bilayer. The $^2$H NMR lipid spectra undergo an overall contraction upon peptide binding, implying a general disordering of the bilayer induced by both the alanyl- and trytophan-containing peptides [see Davis (1983), Jacobs and Oldfield (1981), or Seelig (1977) for details concerning the interpretation of lipid bilayer $^2$H NMR spectra]. This overall disordering is, again, consistent with the peptides being located in the bilayer interior. These effects and the phase separation seen in the DSC thermograms are most easily explained by assuming that the peptides reside within the bilayer hydrocarbon core with the charged amino terminus at the bilayer/water interface.

CONCLUSION

Relatively simple peptides of the form Ala-X-Ala-O-tert-butyl partition into the lipid bilayer. The fashion and extent of the interaction are strongly dependent upon the identity of
the central amino acid residue. Fluorescence, $^3$H NMR, and DSC experiments indicate that the peptides are probably located in the bilayer interior. Thus, peptides of this form can serve as easily modifiable model systems with which to study the details of (1) how the bilayer environment affects amino acid side chain motion, (2) how the bilayer organization is affected by different side chains, and (3) how amino acid substitution affects peptide partitioning into the bilayer membrane. If one is interested in modeling the insertion of peptides into membranes (Engelman & Steitz, 1981; von Heijne & Blomberg, 1979; Sabitini et al., 1982; Wickner, 1979), quantitative information concerning the transfer of amino acid residues on peptides into the bilayer membrane is needed. Although assumptions concerning conformational changes need clarification, the use of a simple homologous series of peptides allows us to examine bilayer–amino acid interactions where the amino acid is in a “natural” environment (i.e., attached via peptide bonds to two adjacent amino acids). Detailed NMR and neutron diffraction experiments with specifically labeled peptides are under way which should unequivocally answer the question of the location(s) of these peptides in the bilayer and probe questions of peptide conformation in the bilayer vs. aqueous solvent systems.

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REFERENCES


