Two Homologous Apolipoprotein AI Mimetic Peptides

RELATIONSHIP BETWEEN MEMBRANE INTERACTIONS AND BIOLOGICAL ACTIVITY*

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Two related 18-amino acid, class A, amphipathic helical peptides termed 3F-2 and 3F14 were chosen for this study. Although they have identical amino acid compositions and many similar biophysical properties, 3F-2 is more potent than 3F14 as an apolipoprotein AI mimetic peptide. The two peptides exhibit similar gross conformational properties, forming structures of high helical content on a membrane surface. However, the thermal denaturation transition of 3F-2 is more cooperative, suggesting a higher degree of oligomerization on the membrane. Both 3F-2 and 3F14 promote the segregation of cholesterol in membranes containing phosphatidylcholine and cholesterol, but 3F-2 exhibits a greater selectivity for partitioning into cholesterol-depleted regions of the membrane. Magic angle spinning/NMR studies indicate that the aromatic residues of 3F-2 are stacked in the presence of lipid. The aromatic side chains of this peptide also penetrate more deeply into membranes of phosphatidylcholine with cholesterol compared with 3F14. Using the fluorescent probe, 1,3-diprenylpropane, we monitored the properties of the lipid hydrocarbon environment. 3F-2 had a greater effect in altering the properties of the hydrocarbon region of the membrane. The results are consistent with our proposed model of the effect of peptide shape on the nature of the difference in peptide insertion into the bilayer.

There is growing evidence that certain apo1 A-I mimetic, class A amphipathic helical peptides can be used to inhibit atherosclerosis (1). The oral administration of peptide 4F synthesized from all-D amino acids (D-4F) protects mice from diet-induced atherosclerosis (1). The oral administration of peptide 4F also penetrates more deeply into membranes of phosphatidylcholine with cholesterol compared with 3F14. Using the fluorescent probe, 1,3-diprenylpropane, we monitored the properties of the lipid hydrocarbon environment. 3F-2 had a greater effect in altering the properties of the hydrocarbon region of the membrane. The results are consistent with our proposed model of the effect of peptide shape on the nature of the difference in peptide insertion into the bilayer.

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1 The abbreviations used are: apo, plasma apolipoprotein; LDL, low density lipoprotein; PC, phosphatidylcholine; PO, 1-palmitoyl-2-oleoyl; SO, 1-stearoyl-2-oleoyl; PC, P, 1,3-diprenylpropane; MAS, magic angle spinning; NOESY, nuclear Overhauser enhancement spectroscopy; LUV, large unilamellar vesicle; DSC, differential scanning calorimetry; HPLC, high pressure liquid chromatography; PIPES, 1,4-piperazinediethanesulfonic acid; Iex, intensity of excimer emission; Im, intensity of monomer emission.

MATERIALS AND METHODS

Lipids—The lipids used in this study were obtained from Avanti Polar Lipids (Alabaster, AL). The purity of the phospholipids was verified by measuring the cooperativity and temperature of the phase transition using DSC.

Peptide Synthesis—The peptides were synthesized by the solid phase method with a Protein Technologies PS-3 automatic peptide synthesizer using the procedures described previously (2, 8). The peptides were purified using a preparative HPLC system (Beckman Gold), and the purity of the peptides was ascertained by mass spectral analysis and analytical HPLC.
Membrane Interactions of 3F Analogs

Concentrations of Peptide and Lipid—The concentrations of peptide solutions in buffer were determined spectrophotometrically using the absorbance at 280 nm and an extinction coefficient of 6970 cm\(^{-1}\) m\(^{-1}\), calculated from the Tyr and Trp content. Phospholipid concentration was determined by phosphate analysis (9).

Preparation of Samples for DSC and NMR Experiments—Phospholipid vesicles were dissolved in chloroform/methanol (2/1, v/v). For samples containing peptide, an aliquot of a solution of the peptide in methanol was added to the lipid solution in chloroform/methanol. The solvent was then evaporated under a stream of nitrogen with constant rotation of a test tube so as to deposit a uniform film of lipid over the bottom third of the tube. The last traces of solvent were removed by placing the tube under high vacuum for at least 2 h. The lipid film was then hydrated with 20 mM PIPES, 1 mM EDTA, 150 mM NaCl with 0.002% NaN\(_3\), pH 7.40, and suspended by intermittent vortexing and heating to 50 °C for 2 min under argon. The samples used for NMR analysis were hydrated with the same buffer made in \(\text{H}_2\text{O}\) adjusted to a pH meter reading of 7.0 (pD = 7.4). The samples used for NMR were incubated 24 h at 4 °C to allow conversion of anhydrous cholesterol crystals to the monohydrate form. For the NMR measurements, the samples were spun in an Eppendorf centrifuge at room temperature. The resulting hydrated pellet was transferred to a 12-μl capacity Kel-F spherical insert of an 18-mm ZrO\(_2\) rotor, attempting to pack the maximal amount of lipid into the rotor while maintaining it wet.

Differential Scanning Calorimetry—Measurements were made immediately after sample preparation using a Nano Differential Scanning Calorimeter (Calorimetry Sciences Corporation, American Fork, UT). The scan rate was 2 °C/min, and there was a delay of 5 min between sequential scans in a series to allow for thermal equilibration. DSC curves were analyzed by using the fitting program, DA-2, provided by Microcal Inc. (Northampton, MA) and plotted with Origin, version 5.0.

Centrifugation Assay for Membrane Binding of 3F Peptides—The fraction of the peptide bound to the lipid after three heating and cooling cycles between 0 and 100 °C was determined by separating the free peptide by centrifugation. The vesicles with bound protein were pelleted at 200,000 × g for 90 min at 25 °C. A clear supernatant was separated from the solid pellet and assayed for protein by absorption at 278 nm. The absorption of a blank was subtracted, and a base line, set immediately after sample preparation using a Nano Differential Scanning Calorimeter (Calorimetry Sciences Corporation, American Fork, UT). The scan rate was 2 °C/min, and there was a delay of 5 min between sequential scans in a series to allow for thermal equilibration. DSC curves were analyzed by using the fitting program, DA-2, provided by Microcal Inc. (Northampton, MA) and plotted with Origin, version 5.0.

RESULTS

DSC—Lipid mixtures of SOPC containing 0, 0.3, 0.4, and 0.5 mol fractions cholesterol were analyzed by DSC in the presence of 0, 5, 10, and 15 mol % 3F-2 or 3F-14. As examples, we present the results from mixtures containing 0 or 15 mol % of each of the peptides (Fig. 2). The DSC curves are presented as the excess heat capacity/mol cholesterol as in our earlier paper on 4F (7). Both peptides clearly promote the separation of chole-
terol into crystalline domains at higher mol fractions of cholesterol and of peptide (Table I). The cholesterol crystals formed are in a metastable state and disappear after sequential heating and cooling scans between 0 and 100 °C (Fig. 2). This has been found previously, albeit to a lesser extent, with the peptide 4F (7). Scanning only up to 50 °C can eliminate much of this loss (16), but the higher temperatures are required to observe the unfolding transition of the peptide. 3F14 causes

TABLE I

| Cholesterol in SOPC | 3F-2 | 3F14 |
|---------------------|------|------|
| 30%                 | 0    | 0    |
| 40%                 | 0    | 170  |
| 50%                 | 250  | 225  |

The enthalpy of the polymorphic transition of anhydrous cholesterol crystals is in cal/mol cholesterol determined from areas under the transition at ~36 °C on the first DSC heating scan. The numbers are the order in which the scans were carried out, with scans 1 and 3 being heating scans, each of which was followed by one of the cooling scans 2 or 4. Scans were displaced along the y axis for clarity of presentation.
separation of cholesterol crystals at lower mol fractions of cholesterol and peptide than is required with 3F-2. A more marked difference is observed in the peak at the lowest transition temperature that is ascribed to the gel to liquid crystalline transition of SOPC. The enthalpy of this transition can be reasonably estimated from cooling scans. For pure SOPC (data not shown), the transition occurs at 5.5 °C on cooling at 2 K/min with a transition enthalpy of 4 kcal/mol (16). With pure SOPC without cholesterol, the addition of 15 mol % 3F-2 lowers the enthalpy to 2.2 kcal/mol and to 2.4 kcal/mol with 3F14 (data not shown). With mixtures of cholesterol and SOPC the addition of increasing concentrations of 3F-2 eliminates this transition, whereas with 3F14 the enthalpy of this transition is slightly increased (Table II). The highest temperature transition observed in the DSC scans corresponds to that of the unfolding of the peptide. It occurs at about 65 °C for both peptides; with 3F14 the transition is readily observed only with SOPC in the absence of cholesterol (not shown), but the transition is quite prominent in scans with 3F-2 (Fig. 2). The transition temperature is slightly lower on cooling than on heating.

CD—The CD spectra in buffer of both 3F-2 and 3F14 exhibit some dependence on peptide concentration (5). The addition of SOPC or SOPC:cholesterol (1:1) to 3F-2 results in little change in the secondary structure of 3F-2 (Fig. 3) but slightly increases the magnitude of the CD for 3F14 (Fig. 3). The temperature dependence of the spectrum shows a substantial loss of secondary structure on heating to 95 °C with both 3F-2 and with 3F14 (Fig. 4). The thermal transition is broad with some hysteresis on heating and cooling.

Solubilization of Lipid and Peptide—Mixtures of SOPC, cholesterol, and the peptide 3F-2 or 3F14 were centrifuged after the DSC experiments. In the presence of peptide, lipid is partially solubilized (Fig. 5). A larger fraction of cholesterol is solubi-
lized, compared with SOPC in samples containing 0.3 mol fraction cholesterol. It was confirmed by PAGE that lipoprotein particles were formed after either a mixture of SOPC and cholesterol at a molar ratio of 1:1 or 7:3 and also containing 15 mol % of 3F-2 or 3F14 was incubated at room temperature for 3 h and then centrifuged. Particles of 100 Å size were seen (Fig. 6), confirming that soluble lipoprotein particles were formed with both peptides. The fact that the major fraction of both lipid and peptide is in the insoluble fraction at the low concentrations used for DSC indicates that in the case of the much higher concentrations used for NMR, the major fraction of peptide and lipid are found in the pellet and not in solubilized micellar form. This is in agreement with our finding of a bilayer shaped static 31P NMR powder pattern for this lipid mixture (see below).

**Tryptophan Fluorescence**—The fluorescence emission spectra of 3F-2 and 3F14 were measured in buffer and in the presence of lipid (Fig. 7). The emission maximum of 3F-2 in buffer is 336 nm compared with 338 nm for 3F14 in buffer. In the presence of lipid, either with or without cholesterol, the emission is close to 333 nm. The results indicate that the Trp residue inserts into the bilayer. These values are somewhat blue-shifted compared with our previous study, but in a different lipid system and at a somewhat higher peptide concentration (Fig. 7). Trp emission maximum is not sensitive to the presence of cholesterol, indicating that the polarity of the Trp environment is not greatly altered. This behavior is quite similar to that found with 4F (7). 3F14 has almost identical emission intensity in the presence of SOPC either with or without cholesterol (Fig. 7), suggesting that this peptide does not preferentially interact with cholesterol-depleted domains. The blue shift caused by the addition of the lipid is somewhat greater with 3F14 than with 3F-2, suggesting that the Trp of the former peptide is more deeply buried in the membrane.

We have also determined the effect of cholesterol on the red edge excitation shift. In accord with our previous findings (5), there is no red edge excitation shift with 3F-2, but there is with 3F14, indicating that Trp residues are more rigid with 3F-2 in presence of cholesterol containing membranes compared with 3F14. It should be noted that 3F-2 has Trp at the center of the nonpolar face, whereas Leu appears at the center of the nonpolar face in 3F14. This could be interpreted as a consequence of Trp tending to push the peptide up toward the lipid water interface, whereas Leu at the center of the nonpolar face increases interaction with the lipid acyl chain and hence results in a deeper penetration into phospholipid bilayer. Cholesterol has no effect on this phenomenon (Fig. 8).

**Excimer Formation in the Fluorescent Probe PC3P**—The Ie/Im ratio of PC3P (1% of lipid) was determined in LUVs of POPC and POPC:cholesterol (1:1) with and without the addition of 3F-2 or 3F14 at a 10:1 lipid to peptide ratio (Fig. 9). In agreement with previous results (12), in the present work we also find that cholesterol markedly lowers the Ie/Im ratio. This is likely a consequence of the lower rate and extent of molecular
motion in the presence of cholesterol decreasing the rate of conformational change in the fluorescent probe. The effect of the peptide is smaller and tends to increase with higher concentrations of probe, suggesting that there are both inter- and intramolecular formation of excimers. This ratio is insensitive to the presence of 3F14 but is affected by 3F-2, indicating that 3F-2 has a greater effect on hydrocarbon packing and/or dynamics.

**FIG. 5.** Solubilization of peptide and lipid in mixtures of 3F-2 (open bars) or 3F14 (striped bars) and multilamellar vesicle of SOPC with 0.3, 0.4, or 0.5 mol fraction cholesterol, each containing 5, 10, or 15 mol % peptide, subsequent to the series of DSC runs such as those shown in Fig. 2. *Top panel,* % peptide in supernatant. *Middle panel,* % lipid in supernatant. *Bottom panel,* % cholesterol in supernatant.

**FIG. 6.** PAGE of supernatant fractions after centrifugation of mixtures of cholesterol, SOPC, and peptide that had been incubated three h at room temperature. 3 µg were loaded on a 4–20% gel (PAGE) and run under native conditions for 20 h. The gel was stained with colloidal blue and destained with water. *Lane 1,* calibration markers; *lane 2,* SOPC:cholesterol (1:1) with 15 mol % 3F-2; *lane 3,* SOPC:cholesterol (1:1) with 15 mol % 3F14; *lane 4,* SOPC:cholesterol (7:3) with 15 mol % 3F-2; *lane 5,* SOPC:cholesterol (7:3) with 15 mol % 3F14.

We performed a similar analysis with the 3F14 peptide. The static 31P NMR powder patterns for this peptide were similar to those for 3F-2 showing a bilayer shape pattern with a significant isotropic component in the absence of cholesterol but only a minor isotropic peak in the equimolar mixture of POPC and cholesterol. The slices for the sample of 3F14 with POPC alone (Fig. 12) generally had cross-peaks of positive sign (i.e., negative NOE), opposite to that with 3F-2. In addition, only the peak on the diagonal was observed in the aromatic region, indicating that the aromatic groups are less stacked in 3F14 than in 3F-2, as might be anticipated on the basis of the larger cluster of aromatic residues seen in the helical wheel projection of 3F-2 (Fig. 1). The cross-peaks between the lipid and 3F14 are particularly weak in the presence of cholesterol (Fig. 13), suggesting that this peptide is largely excluded from cholesterol-containing membranes. In addition, the aromatic region of the one-dimensional spectrum is particularly well resolved compared with other cases.

Interaction of the peptide with lipid can also be assessed by monitoring the changes in the chemical shifts of the lipid resonances on introduction of the peptide (Table III). These changes in chemical shifts may arise from ring current effects as well as from changes in the polarity of the environment. In the case of 1H MAS/NMR, only changes in the spectrum of the phospholipid can be assessed, because resonances are not observed from protons of cholesterol in these lipid mixtures (13), and the lower concentration of peptide makes it difficult to discern its resonances. The addition of 3F-2 or 3F14 to either POPC or POPC:cholesterol (1:1) results in a small change in 1H chemical shifts of several resonances. The change is insensitive to the presence of cholesterol and does not indicate a preferen-
Because cholesterol resonances are not observed in $^1$H MAS/NMR, we also measured $^{13}$C MAS/NMR. The changes in chemical shift are generally small, and there is no large difference between 3F-2 and 3F$^{14}$. In mixtures containing cholesterol the changes in chemical shifts are comparable for POPC and for cholesterol (not shown).

**DISCUSSION**

Using *in vitro* assays expected to correlate with protection against atherosclerosis, 3F-2 and 3F$^{14}$ exhibit quite different potency (5); however, the differences in their biophysical properties have been found to be relatively small (this work and Ref. 5). Both peptides are class A amphipathic helices and therefore can fold into a helical structure resembling amphipathic helices contained in exchangeable plasma apolipoproteins, *i.e.* class A amphipathic helices (17). Although the position of the Trp residue in the two peptides is different, the nature and location of the other residues on the hydrophilic face of the helical conformation of these two peptides are identical, as is their total amino acid composition. The HPLC elution profile and monolayer collapse pressure indicate that the two peptides have similar hydrophobicities, but the 3F-2 is somewhat less hydrophobic (5). Both peptides can rapidly solubilize 1-palmitoyl-2-oleoyl phosphatidylcholine at an equimolar ratio of peptide and lipid (5). The CD spectra of the two peptides are similar, and we show in this work that the secondary structure is independent of the presence of cholesterol (Fig. 3). We also show that both peptides undergo a loss of secondary structure on heating (Fig. 4). However, the thermal denaturation of 3F-2 is more prominent in DSC scans of 3F-2 than of 3F$^{14}$ (Fig. 2). Because a similar amount of helicity is lost upon heating of the two peptides, we suggest that the transition of 3F-2 is more cooperative because this peptide has a higher degree of oligomerization on the membrane surface compared with 3F$^{14}$.

We have shown that another biologically potent class A helical peptide, 4F, promoted the formation of cholesterol-rich domains by preferentially interacting with the phospholipid component of cholesterol/SOPC mixtures (7). This finding provided an interesting contrast with the peptide LWYIK that

**FIG. 7.** Fluorescence emission spectra peptides with and without cholesterol. The left panel is for 3F$^{14}$, and the right panel is for 3F-2. Curve 1, 15 $\mu$m peptide in buffer. Curve 2, 15 $\mu$m peptide mixed with SOPC at a lipid to peptide ratio of 6; Curve 3, 15 $\mu$m peptide mixed with SOPC:cholesterol (1:1) at a lipid to peptide ratio of 6. Fluorescence intensities normalized to make the emission maximum for peptide in buffer equal to 1.0. The spectra were acquired using an excitation wavelength of 280 nm at a temperature of 25 °C.

**FIG. 8.** The red edge excitation shift. The effect of excitation on the emission maximum of the intrinsic fluorescence of Trp. Upper panel, 3F-2; lower panel, 3F$^{14}$. Peptide in PIPES buffer, pH 7.4 (30 $\mu$m); peptide in 1 mM SOPC with 15 mol % peptide; peptide in 1 mM SOPC:cholesterol (1:1) with 15 mol % peptide. The fluorescence was measured at 25 °C.

**FIG. 9.** Fluorescent properties of PC3P at 1 mol % in LUVs of POPC or POPC-cholesterol (1:1) (lipid to peptide ratio = 10). The peptide concentration was 24 $\mu$m. The experiments were done at room temperature using an excitation wavelength of 344 nm. Excimer emission intensity ($I_e$) was read at 478 nm, and monomer emission intensity ($I_m$) was read at 389 nm.
preferentially interacted with cholesterol (16). Rearrangement of cholesterol in membranes can result from preferential interaction of proteins with cholesterol-rich domains as well as with cholesterol-depleted domains (18). These observations are likely to be relevant to the mechanism of formation of “rafts” in biological membranes. The DSC shows that 3F-2 interacts preferentially with the phospholipid in mixtures of cholesterol and SOPC. This is indicated by the fact that this peptide is much more potent in lowering the phase transition of SOPC than is 3F14 (Fig. 2 and Table II). 3F-2 also promotes the formation of cholesterol crystals at mol fractions of cholesterol much lower than are required for their formation in the absence of peptide (Fig. 2 and Table I). The peptide 3F14 also promotes the formation of cholesterol crystals. However, 3F14 does not lower the enthalpy of the phase transition of SOPC in mixtures with cholesterol (Table II), indicating that it is not interacting preferentially with SOPC. 3F14 also does not greatly increase the enthalpy of SOPC by removing cholesterol, as we had shown for LWYIK (16). We suggest that a contributing factor to the promotion of cholesterol segregation in the membrane is through an increase in the lateral pressure of the membrane “squeezing out” cholesterol. The greater preference of 3F-2 for cholesterol-

![Fig. 10. One-dimensional slices from the MAS $^1$H NOESY spectrum at the chemical shifts of the aromatic protons of a sample of POPC containing 10 mol % 3F-2. The mixing times were 50 ms (left panel) and 300 ms (right panel). The top spectra are conventional one-dimensional proton spectra of the samples. The resonance assignments are indicated on the top spectrum.](image1)

![Fig. 11. One-dimensional slices from the MAS $^1$H NOESY spectrum at the chemical shifts of the aromatic protons of a sample of POPC:cholesterol (1:1) containing 10 mol % 3F-2. The mixing times were 50 ms (left panel) and 300 ms (right panel). The top spectra are conventional one-dimensional proton spectra of the samples. The resonance assignments are indicated on the top spectrum.](image2)
depleted domains is also common to another biologically potent peptide of this series, 4F (7). This correlation is not seen with the less biologically active peptide 3F14.

There is also a difference between the two peptides in how they interact with the membrane. The emission intensity from the Trp of 3F-2 is increased more by lipid, especially the lipid...
mixture containing cholesterol (Fig. 7), than is the case of 3F14. The behavior of the Trp fluorescence of 3F-2 is qualitatively similar to that of 4F (7), even though the Trp is in different positions for the two peptides. The similar behavior of the Trp fluorescence of two peptides, despite the different position of Trp in the helical wheel, suggests that 3F14 penetrates more deeply into the bilayer. The greater number of cross-peaks between protons of the aromatic residues of 3F-2 (Fig. 10) indicates that its side chains are stacked in the presence of lipid. In the presence of cholesterol particularly, 3F-2 exhibits several strong, negative cross-peaks with several lipid protons, including those from the terminal CH$_3$ group of the acyl chain. The peaks of negative sign of the NOESY suggests increased molecular motion that could allow transient access to the protons in the center of the bilayer. This conclusion appears opposite to that derived from red edge excitation shift, indicating a restricted motion of the Trp of 3F-2. We suggest that this difference is a consequence of the widely different time scale for the two measurements. Fluorescence decay occurs in nanoseconds, whereas proton relaxation occurs in milliseconds to seconds. Hence in the longer time scale of NMR, the relative position between the Trp and the lipid can change, but locally around the Trp the environment is rigid, and there is slow reorientation of the surrounding dipoles. According to the model we proposed (Fig. 14), 3F-2 would, because of its cylindrical shape, short length, and aromatic side chains pushing the peptide axis up closer to the polar-nonpolar interface, introduce a destabilization in the bilayer resulting in a decrease in the lipid order parameters. The decreased penetration of 3F-2 compared with 3F14 is also indicated by the small shift in Trp emission wavelength caused by lipid (Fig. 7). Again there is a difference between peptide penetration into the membrane as assessed by NOE effects and by fluorescence. It is possible that the greater bilayer disruption caused by 3F-2 allows some penetration of water into the membrane, resulting in a smaller effect on Trp emission, even though the NOE spectra indicate a greater penetration of this peptide. In comparison, 3F14 appears to be largely excluded from bilayers containing cholesterol. It shows only very weak cross-peaks between the aromatic resonances and the lipid protons (Fig. 11). This is also in accord with the smaller change in the intensity of emission from the Trp residue in the presence of cholesterol. However, when 3F14 incorporates into the bilayer, it may be able to pack with the acyl chains with less disruption of the hydrocarbon portion of the bilayer, as suggested in Fig. 14.

**Table III**

| Resonance        | POPC With 3F-2 | POPC/cholesterol (1:1) | POPC With 3F14 | POPC/cholesterol (1:1) |
|------------------|----------------|------------------------|----------------|------------------------|
| Glycerol C2      | 0.04           | 0.04                   | 0.05           | 0.02                   |
| Glycerol C3      | 0.03           | 0.03                   | 0.02           | 0.02                   |
| Choline α        | 0.03           | 0.03                   | 0.03           | 0.03                   |
| Glycerol C1      | 0.03           | 0.04                   | 0.03           | 0.03                   |
| Choline β        | 0.03           | 0.03                   | 0.02           | 0.05                   |
| Quaternary CH$_3$| 0.03           | 0.03                   | 0.02           | 0.02                   |
| CH$_2$CO         | 0.01           | 0.03                   | 0            | 0.03                   |
| CH$_3$CCO        | 0.03           | –0.04                  | –0.02          | –0.07                  |
| CH$_3$           | 0.03           | 0.02                   | 0.02           | 0.01                   |
| Terminal CH$_3$  | 0.03           | 0.02                   | 0.03           | 0.02                   |

*Chemical shift differences are in parts per million between that of lipid alone and in the presence of 10 mol% peptide. A positive charge corresponds to a shift in the resonance to a lower frequency caused by the peptide.*

The NMR parameters that we have monitored are not very sensitive to the hydrocarbon packing or molecular motion in the interior of the membrane. We have therefore also studied the properties of the fluorescent probe PC$_3$P that has been suggested to monitor changes in membrane motional properties (12, 19). We do not wish to use the probe as the basis for a model of the interaction of the peptide with lipid but rather as a demonstration that the more active peptide, 3F-2, has a much larger effect on the packing properties of the hydrocarbon region of the membrane, compared with the less active 3F14. The effects are consistent with the proposed model for the interaction of these peptides with bilayers based on molecular shape (Fig. 14). In this model the 3F-2 peptide would introduce greater destabilization of the bilayer as a consequence of its cylindrical shape, short length, and aromatic side chains pushing the peptide axis up closer to the polar-nonpolar interface. Accumulation of the probe in the bilayer defect would dilute the PC$_3$P and decrease intermolecular excimer formation and possibly also disfavor the conformation required for pyrene dimerization in the monomer. This is also consistent with the greater anti-atherogenic properties of 3F-2, because decreased membrane order has been suggested to be associated with increased risk for cardiovascular disease (20).

In summary, given the marked differences in biological activity between 3F-2 and 3F14, the nature of the interaction of these peptides with SOPC with or without cholesterol is remarkably similar. Nevertheless there are important differences between the two peptides that support our model of the difference in peptide “shape” (5). Although 3F-2 is less hydrophobic than 3F14 by the criteria of HPLC volume and monolayer exclusion pressure (5), 3F-2 has stronger NOESY cross-peaks with protons more in the center of the bilayer (Fig. 10). We suggest that this is a consequence of a greater disordering of the bilayer caused by this peptide. 3F-2 also has two features that are more in common with those of 4F (7). These are a preferential broadening of the chain melting transition in mixtures of SOPC and cholesterol (Fig. 2) and a more cooperative unfolding transition of the peptide. The latter suggests oligomerization that may also contribute to a greater disruption of the bilayer order by insertion of a larger peptide aggregate. The disordering of the lipid could allow for the transfer of

*Fig. 14. Biological activity of a Class A amphipathic helix depends on hydrophobic face-lipid acyl chain interaction. Top panel, a minimal effect on lipid acyl chain packing occurs in the wedge-shaped molecule 3F14. Bottom panel, the cylindrical shaped peptide, 3F-2, causes greater acyl chain perturbations, facilitating the entry of molecules such as water and lipid hydroperoxides into the hydrophobic milieu of the complex.*
oxidized lipids from the LDL surface to peptide-containing particles, thus rendering LDL less effective in inducing monocyte chemotaxis, an important step in the initiation of atherogenesis.

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