The Antiviral Peptide Carbobenzoxy-D-phenylalanyl-L-phenylalanylglycine Changes the Average Conformation of Phospholipids in Membranes†

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ABSTRACT: The influence of the antiviral peptide, carbobenzoxy-D-phenylalanyl-L-phenylalanylglycine (ZfFG), on the average conformation of phosphatidylcholine in hydrated bilayers was investigated with multinuclear solid state magnetic resonance (NMR). Phosphatidylcholine was specifically deuterated (separately) in the choline *N*-methyls, the α and β positions of the choline, the C_2 carbon of the acyl chains, and at all the carbons of the acyl chains of the phosphatidylcholine. Phosphatidylcholine was also synthesized with the carbonyl carbons of the ester bonds between the glycerol and the hydrocarbon chains enriched in ^{13}C. ^2H NMR of the phosphatidylcholine perdeuterated in the acyl chains showed a loss of intensity from the deuteriums with the largest quadrupole splitting in the presence of ZfFG, while the remainder of the powder pattern was largely unaffected. The phosphatidylcholine specifically deuterated at the C_2 carbon (representative of the C–D bonds giving rise to the largest quadrupole splittings) showed the same loss of intensity suggesting changes in the phospholipid conformation and conformational dynamics near the glycerol. Analysis of the powder patterns in the ^{13}C NMR spectrum of phosphatidylcholine labeled with ^{13}C in the carbonyl carbons revealed a significant change in the average orientation of the *sn*-1 carbonyl due to the presence of the ZfFG and no change in the *sn*-2 carbonyl orientation. Changes in the headgroup conformation, as detected by ^2H NMR of the deuteriums in the α and β methylenes of the choline headgroup and ^{31}P NMR of the phosphate segment, reflected the electrostatic nature of the interaction of the carboxyl of ZfFG with phosphatidylcholine bilayers. No significant effect was observed from the deuteriums in the *N*-methyls of the choline. From these data it was concluded that phosphatidylcholine had access to more than one conformation around the glycerol segment of the molecule in a bilayer. In the absence of ZfFG, the two carbonyls are inequivalent in their orientation. The antiviral peptide ZfFG favored a conformation in which the average orientations of the two ester carbonyls (with respect to the axis of rotation diffusion) were approximately equivalent. This altered phospholipid conformation may be the source of the differences in phospholipid packing observed in the presence of the antiviral compound. The loss of apparent ^2H NMR intensity was likely due to the dynamics (on the time scale of 10^{-5} s) of the interchange between the (at least) two conformations adopted by the phosphatidylcholine in the presence of ZfFG.

The antiviral peptide, carbobenzoxy-D-phenylalanyl-L-phenylalanylglycine (ZfFG)[1] was observed to inhibit viral infection of the paramyxoviruses including Sendai, measles, CDV, and SV5 (Richardson et al., 1980). Recent studies revealed that this peptide inhibited viral infection of Sendai virus and measles by inhibiting membrane fusion, a required step in viral entry (Harrowe et al., 1990; Kelsey et al., 1990, 1991). Membrane fusion was inhibited by ZfFG in Sendai fusion with erythrocyte ghosts, Sendai fusion with large unilamellar vesicles (LUV) of *N*-methyldioleoylphosphatidylethanolamine (*N*-methyl-DOPE), and fusion of LUV of *N*-methyl-DOPE. The inhibition of fusion was specific for the structure of the antiviral peptide. The same structural specificity observed in inhibition of viral infection was also observed in the inhibition of membrane fusion by these antiviral peptides. More recently, the inhibition of membrane fusion by ZfFG was correlated with an inhibition of the formation of highly curved phospholipid surfaces (Yeagle et al., 1992), including those found in hexagonal II phases (Epand, 1986). Such observations suggested that the inhibition of membrane fusion was due to the inhibition of the formation of intermediates on the fusion pathway that incorporated highly curved phospholipid surfaces.

Studies of the interaction of small peptides (five amino acids or less) with lipid bilayers encompass two classes. Small charged peptides have an electrostatic component in their binding and bind to the surface of oppositely charged lipid bilayers (Epand, 1992). They may influence the dynamics of the headgroups of the lipids in the bilayer (Epand et al., 1988). They do not partition into the interior of the membrane because of the hydrophobic effect. Small hydrophobic peptides partition to the membrane due to the hydrophobic effect and also bind to the membrane surface (Jacobs & White, 1989). Such peptides do not partition into the interior of the membrane because they are too small to form secondary structures and thus the carbonyls and nitrogens of the peptide bonds must hydrogen bond to water molecules. The influence of small

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[1] Abbreviations: ZfFG, carbobenzoxy-D-phenylalanyl-l-phenylalanylglycine; NMR, nuclear magnetic resonance; di([1-^{13}C]oleoyl)phosphatidylcholine, 70% enriched in ^{13}C in the *sn*-2 position and 30% enriched in ^{13}C in the *sn*-1 position; $d_{2,2}$-DPPC, di([2,2-^2H]palmitoyl)phosphatidylcholine; d_4-PC, ([α,β-$^2H_2$]choline)palmitoyloleoylphosphatidylcholine; d_9-PC, ([γ-^2H]choline)phosphatidylcholine.

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hydrophobic peptides on the lipid properties of the bilayer has not been extensively studied.

This study examines how the antiviral peptide ZfFG (a small hydrophobic peptide) influences the behavior of the phospholipids of the membrane bilayer. The influence of the antiviral, antifusion peptide ZfFG on the conformation of phosphatidylcholine in membranes was examined by multinuclear solid-state nuclear magnetic resonance. The quadrupole splittings from ^{2}H NMR measurements of the variously deuterated phosphatidylcholines revealed a selective perturbation in the conformation of the phospholipids in the region of the hydrocarbon chain adjacent to the ester bonds of the hydrocarbon chains to the glycerol. ^{13}C NMR powder patterns of phosphatidylcholine enriched in the carbonyls with ^{13}C indicated a change in the average orientation of the *sn*-1 carbonyl, such that it became approximately equivalent in orientation to the *sn*-2 carbonyl (with respect to the director for axial diffusion of the phospholipid molecule in the membrane). ^{2}H and ^{31}P NMR indicate changes in the headgroup conformation that are typical for the introduction of a negative charge in the membrane surface from the carboxyl terminal of the peptide. The data obtained in this study suggested that phosphatidylcholine had access to more than one conformation in the lipid bilayer and that a change in average conformation of the phospholipid was induced by ZfFG in the membrane surface. It is possible that the change in average conformation might alter the most favored packing state of the phospholipids and thus provide a molecular basis for the inhibition of the formation of highly curved phospholipid assemblies by the antiviral compound, ZfFG.

MATERIALS AND METHODS

Di([1-^{13}C]oleoyl)phosphatidylcholine (70% enriched in ^{13}C in the *sn*-2 position and 30% enriched in ^{13}C in the *sn*-1 position), di([2,2-^{2}H]palmitoyl)phosphatidylcholine ($d_{2,2}$-DPPC), and ([α,β-^{2}H]choline)palmitoyloleoylphosphatidylcholine (d_4-PC) were synthesized by Avanti Polar Lipids. Carbobenzoxy-D-Phe-L-PheGly (ZfFG) and perdeuterated DPPC were obtained from Sigma (St. Louis, MO). Synthesis of ([γ-[^{2}H]choline) phosphatidylcholine (d_9-PC) from transphosphatidylated (from egg phosphatidylcholine) phosphatidylethanolamine was carried out using published procedures (Albert et al., 1985). Deuterium-depleted water was obtained from Aldrich.

Sample Preparation. Phospholipid conformation and dynamics were studied either in multilamellar liposomes (MLV) made by hydration of dry films of phospholipids followed by a freeze–thaw cycle or in large unilamellar liposomes (LUV) made by extrusion (Szoka et al., 1980) as described and characterized previously (Kelsey et al., 1990). The former membranes permit easier NMR measurements because of increased concentrations and are the systems used in previous reports on the NMR of these labeled lipids to which reference is made. In this preparation, ZfFG was mixed with the lipid prior to hydration of the membranes. ZfFG was mixed with PC in chloroform/methanol (2:1), the solvent was removed, and MLV were formed by addition of buffer (1 mM HEPES, pH 7.5, in deuterium-depleted H_2O).

The latter system (LUV) mimicked the vesicle systems used in the fusion experiments that revealed the ability of ZfFG to inhibit membrane fusion. ZfFG was added to the LUV after preparation of the vesicles in a small amount of methanol.

NMR Measurements. ^{2}H NMR spectra of the membrane samples with deuterated phospholipids exhibiting large quadrupole splittings (deuterium labels in the phospholipid hydrocarbon chains) were obtained at 61 MHz on a MSL400 NMR spectrometer in 7-mm sample tubes with a quadrupole echo sequence ($\pi/2$ pulse width of 5 μs) using a total delay of about 50 μs prior to recording the echo. All samples for ^{2}H NMR were hydrated in deuterium-depleted buffer.

^{2}H nuclear magnetic resonance (NMR) for membrane samples with deuterated phospholipids exhibiting small quadrupole splittings (deuterium labels in the phospholipid headgroup) were obtained at 41.4 MHz on a JEOL FX270 multinuclear Fourier transform NMR spectrometer in 10-mm sample tubes as a function of temperature and mol % ZfFG. For these ^{2}H NMR spectra, data were collected with the fully phase cycled (Rance & Byrd, 1983) Hahn echo sequence and repeat times of 100 ms ($\pi/2$ pulse width of 15 μs).

^{13}C NMR data were obtained at 67 MHz on the same instrument, using a 50-kHz spectral width and 5-mm sample tubes. A total of 2048 data points were collected in the time domain. ^{13}C NMR spectra were obtained with the fully phase cycled Hahn echo and a 1-s repeat time ($\pi/2$ pulse width of 8 μs).

^{31}P nuclear magnetic resonance (NMR) spectra were obtained on the same instrument in a broad band probe in 10-mm tubes at the indicated temperatures. A fully phase cycled (32 pulse) chemical shift anisotropy (CSA) echo was used with a 40-μs echo ($\pi/2$ pulse width of 15 μs). Gated proton decoupling (on only during acquisition) at a decoupling field of 9 kHz was employed to eliminate sample heating. A 50-kHz spectral width was used, with 50 Hz of line broadening in the Fourier transformation. A delay time of 1 s was used between pulses. The only ^{31}P nuclei in the preparation were in the phospholipid component of these membranes.

RESULTS

^{2}H NMR of γ-^{2}H-Labeled PC. ^{2}H NMR was used to probe the influence of ZfFG on the *N*-methyl region using γ-^{2}H-labeled PC (d_9-PC). ^{2}H NMR spectra were obtained as a function of temperature and as a function of mole ratio ZfFG:d_9-PC. Mole ratios of ZfFG:phospholipid of 0, 1:8, and 1:3 were explored at 25 and at 37 °C. A small quadrupole splitting was observed in the ^{2}H NMR spectra of the pure lipid in the MLV as reported previously (Albert et al., 1985) and similar to other reported spectra of this lipid (Sixl & Watts, 1983; Watts & vanGorkom, 1992). In the LUV, the quadrupole splitting was obscured by motional averaging on the surface of the LUV. No significant changes in line shape were observed due to the addition of ZfFG in either the MLV or the LUV (data not shown).

^{2}H NMR of α,β-^{2}H-Labeled PC. ^{2}H NMR was used to probe the influence of ZfFG on the phosphorus-to-nitrogen segment of the PC headgroup. The approach used was that described previously, with ^{2}H NMR spectra of α,β-^{2}H-labeled PC (d_4-PC). The ^{2}H NMR powder pattern of the d_4-PC bilayers in deuterium-depleted buffer was identical to that reported previously in MLV (Sixl & Watts, 1983; Seelig et al., 1987; Watts & vanGorkom, 1992). Within the uncertainty of the measurement, the same quadrupole splittings were observed in LUV. Two sets of quadrupole splittings were observed. The larger quadrupole splitting arose from the deuteriums at the α position of the headgroup, and the smaller quadrupole splitting arose from the deuteriums at the β position of the choline, according to previous assignments. These quadrupole splittings have been shown previously to be sensitive

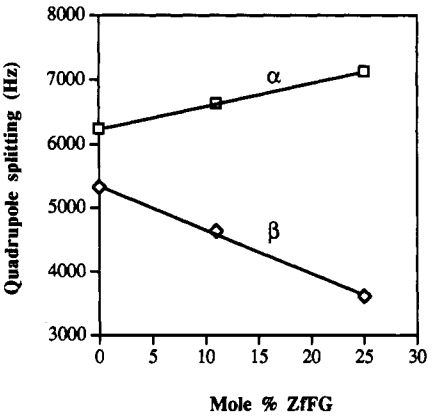

FIGURE 1: ^{2}H NMR quadrupole splittings of the α and β deuteriums in d_4-PC bilayers as a function of the mol % ZfFG in the bilayer, at 25 °C.

to the addition into the membrane surface of molecules carrying an electric charge (Seelig et al., 1987).

Addition of ZfFG, whether added to the exterior of preformed LUV of d_4-PC, or mixed with the d_4-PC prior to formation of MLV, led to a significant change in the deuterium quadrupole splittings observed. The results are presented in Figure 1 for 25 °C for LUV (qualitatively similar results were observed at 37 °C, except that all quadrupole splittings were proportionately smaller). An increase in the mol % ZfFG in d_4-PC bilayers caused an apparently linear increase in the difference in the ^{2}H quadrupole splittings between the α and the β positions. The increase in the quadrupole splitting arising from the α position corresponded to 0.3 kHz/0.1 mole fraction of ZfFG. This magnitude of change with mole fraction ZfFG was about one-third the magnitude of change in the same quadrupole splitting reported for the introduction of the negative lipid, dialkylphosphate, into d_4-PC bilayers (Seelig et al., 1987).

^{31}P NMR of PC. ^{31}P NMR has proven useful in probing phospholipid headgroup conformation (Seelig, 1978; Yeagle, 1978). Therefore the ^{31}P powder patterns of MLV and LUV of phosphatidylcholine were examined as a function of added peptide, from 0 to 33 mol %, at 25 and 37 °C. As above, peptide was mixed with egg PC, DPPC, or d_2PC prior to formation of MLV and was added to preformed LUV. In all cases, an apparently axially symmetric powder pattern characteristic of phospholipid rotational diffusion in bilayers was observed. The powder pattern of LUV was slightly modified by the diffusion of lipids over the surface of the LUV. A small reduction in the residual ^{31}P powder pattern occurred upon addition of peptide in both MLV and LUV. The chemical shift of the upfield maximum of the powder pattern , $\sigma_\perp$, was measured as a function of added ZfFG. Figure 2 shows this result at 25 °C for egg PC as a function of incorporation of ZfFG in MLV. Qualitatively similar results were obtained for ZfFG added to LUV and to MLV of DPPC or d_2PC. Similar magnitude effects were observed previously due to addition of amphipathic compounds to the membrane surface (Seelig et al., 1987).

^{2}H NMR of Perdeuterated DPPC. Next this study turned to an examination of the behavior of the lipid hydrocarbon chains in the presence of ZfFG. To do so, initially perdeuterated DPPC (all the hydrogens on the palmitoyl chains replaced with deuterium) was examined in the presence of a 3:1 mole ratio (DPPC:ZfFG) of antiviral peptide, a ratio at which all other physical effects of ZfFG that have been measured were maximal (i.e., the bilayer surface appeared to be saturated with ZfFG at this mole ratio). A fully saturated

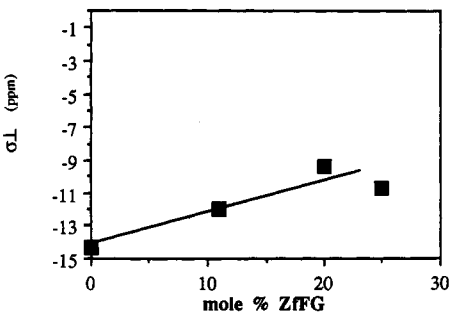

FIGURE 2: Chemical shift of $\sigma_\perp$ from the maximum in the ^{31}P NMR powder pattern in ppm of d_4-PC as a function of added ZfFG in the bilayer, at 25 °C.

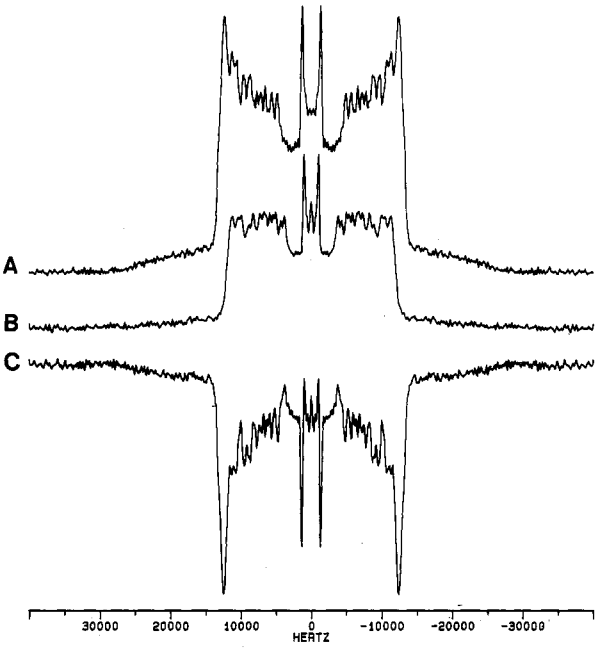

FIGURE 3: 61-MHz ^{2}H NMR spectra of perdeuterated DPPC at 45 °C. (A) MLV of DPPC. (B) MLV of DPPC with 25 mol % ZfFG; (C) difference spectrum A – B.

lipid was chosen for two reasons. First, the perdeuterated lipid was readily available and enabled an examination of the whole of the hydrocarbon chains in one series of experiments, since all carbons on the hydrocarbon chains, except the carbonyl carbon, would contribute deuteriums with observable quadrupole splittings in the ^{2}H NMR spectrum. Secondly, ZfFG had been previously shown to influence the radius of curvature of phospholipid assemblies made of DPPC (Yeagle et al., 1992). Therefore understanding how ZfFG influenced the conformation of DPPC might be expected to help explicate how the antiviral peptide influenced phospholipid packing and possibly how ZfFG thus inhibited membrane fusion.

Figure 3 shows the ^{2}H NMR spectra of DPPC in bilayers with and without ZfFG in MLV at 45 °C (because of the difficulties of handling LUV of DPPC and because no differences were observed in the other measurements reported here between MLV and LUV, MLV were used in this series of experiments). The spectrum of the pure lipid was the same as that reported previously. This spectrum contained the overlapping powder patterns of the deuteriums bonded to carbons 2–16 of the palmitoyl chains of the DPPC. The smallest quadrupole splittings arose from the terminal methyls. The remainder of the powder patterns lead to an envelope of quadrupole splittings which correspond to the plateau of order parameters in the middle of the hydrocarbon chain and the more highly ordered region of the chain near the glycerol of

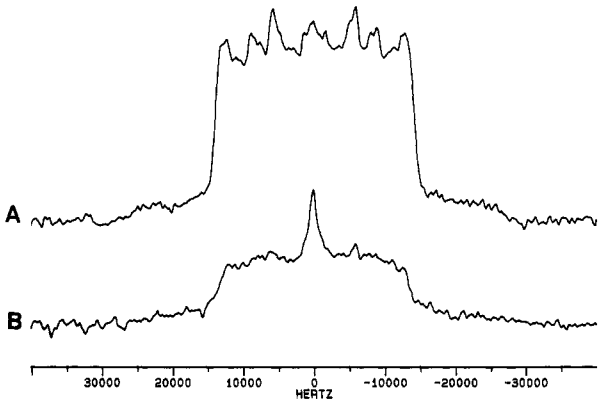

FIGURE 4: 61-MHz ^{2}H NMR spectra of perdeuterated $d_{2,2}$-DPPC at 45 °C. (A) MLV of $d_{2,2}$-DPPC. (B) MLV of $d_{2,2}$-DPPC with 25 mol % ZfFG.

the phospholipid (Seelig & Seelig, 1974; Davis, 1979). The spectrum of the DPPC containing ZfFG (ZfFG/DPPC, 1:3 mole ratio) was different than that of the pure lipid. Some of the powder pattern arising from the most highly ordered regions of the DPPC hydrocarbon chain was apparently absent from the spectrum, in the presence of ZfFG.

The difference spectrum showed that significant alterations in the ^{2}H NMR powder pattern arose predominantly from those deuteriums with the greatest quadrupole splittings. There was a loss of intensity from the most highly ordered deuteriums. These quadrupole splittings arise from deuteriums near the glycerol of the phospholipid and not from deuteriums in the middle of the hydrocarbon chain or near the terminus. The remainder of the ^{2}H powder pattern showed only moderate effects on spectral intensity and no significant alteration in transverse relaxation rate for these sites (T_{2e}, data not shown).

Only small changes occurred in the remainder of the spectrum. For example, the difference spectrum showed that there was a very small reduction in the quadrupole splitting arising from the terminal methyls due to the presence of ZfFG. However, this change was much smaller than the changes noted above.

^{2}H NMR of Specifically Deuterated DPPC ($d_{2,2}$-DPPC). The experiments on the perdeuterated DPPC indicated that the antiviral peptide affected only the deuteriums on the phospholipid hydrocarbon chain with the largest ^{2}H quadrupole splittings and thus exhibiting the greatest anisotropy. The deuteriums bonded to carbons in the region of the hydrocarbon chain near to the glycerol exhibit some of the greatest quadrupole splittings. Therefore, DPPC with deuteriums specifically on carbon 2 of the hydrocarbon chains was studied in the presence and absence of the peptide to examine specifically that region of the phospholipid bilayer that appeared to be most strongly affected by the addition of ZfFG. Figure 4 shows the ^{2}H NMR spectrum of bilayers of pure $d_{2,2}$-DPPC. The powder pattern observed was the same as that reported previously. Two quadrupole splittings are observed. One arises from the deuteriums on the *sn*-1 chain and the other arises from the deuteriums on the *sn*-2 chain. The differences in quadrupole splittings arise because of differences in conformation of the hydrocarbon chains in this region. This portion of the *sn*-1 chain is perpendicular to the membrane surface, whereas the corresponding portion of the *sn*-2 chain is approximately parallel to the membrane surface, according to ^{2}H NMR studies (Seelig & Seelig, 1975) and to X-ray crystal structures of the phospholipids (Hitchcock et al., 1974; Pearson & Pascher, 1979).

The ^{2}H NMR powder pattern of bilayers of the same phospholipid in the presence of 3:1 mole ratio (DPPC:ZfFG) was significantly different than in the absence of the ZfFG. A dramatic loss of resonance intensity of the ^{2}H NMR powder pattern was induced by the antiviral peptide. A well-defined ^{2}H NMR spectrum of this specifically deuterated phospholipid in the presence of ZfFG could not be observed at 45 °C, with the same amount of lipid and the same spectral conditions as in the control sample without peptide. The spectral shape that could be observed was consistent with the spectral distortions arising from significant intermediate range motions in this portion of the phospholipid (see Figure 4). This observation was consistent with the results from the perdeuterated DPPC (in the presence of ZfFG) where spectral intensity was lost from the regions of the largest quadrupole splittings.

^{13}C NMR Data of Phospholipid Carbonyls. The above experiments indicated that only in the most ordered region of the phospholipid bilayer near the glycerol of the phospholipid (and to a lesser extent in the headgroup region) were significant changes observed in the conformation and also dynamics of the phospholipid molecule due to the antiviral peptide. The ester bond in DPPC connects the hydrocarbon chain to the glycerol. Therefore, ^{13}C NMR was used to investigate the carbonyls of the phospholipid in the presence and absence of ZfFG. Previously it was shown that the powder patterns arising from these carbonyls were very sensitive to the orientation of the carbonyl in the phospholipid (Lewis et al., 1984). In particular, for the *sn*-2 position, the carbonyl gives rise to an isotropic resonance because the principle axis of the carbon chemical shift tensor is oriented close to, or fluctuates about, the magic angle with respect to the director for axial diffusion of the phospholipid as a whole (the latter is normal to the bilayer surface). That is the time-averaged angle, θ, between the principle axis of the chemical shift tensor and the axis for axial diffusion approximately satisfies the relationship $3\cos^2\theta - 1 = 0$, which scales the observable in the cases of motional averaging, in this case the chemical shift anisotropy. Previous studies showed that this orientation for the *sn*-2 carbonyl was not significantly affected by the presence of the peptide (Yeagle et al., 1992).

The labeled carbonyl at the *sn*-1 position gave rise to an axially symmetric ^{13}C powder pattern (Figure 5). The powder pattern arising from this carbonyl in pure phospholipid bilayers lay underneath the isotropic resonance from the *sn*-1 position. The effects of ZfFG on this powder pattern have not been previously reported. Figure 5 shows the effect on di([1-^{13}C]-oleoyl)phosphatidylcholine of increasing amounts of ZfFG. Evaluation of these spectra in comparison with those published previously indicates that the axially symmetric ^{13}C powder pattern from the carbonyl at the *sn*-1 position decreases in width and collapses smoothly into the isotropic resonance observed from the carbonyl at the *sn*-2 position upon the addition of ZfFG. The final result at the highest levels of ZfFG indicated that the *sn*-1 carbonyl had a similar, but likely not identical, lineshape to the *sn*-2 carbonyl. Similar results were obtained in both MLV and LUV, though in the latter the differences in the powder pattern of the *sn*-1 carbonyl and the *sn*-2 carbonyl were less distinct due to some motional averaging from diffusion of phospholipid over the surface of the LUV.

DISCUSSION

The data presently available on the influence of the antiviral peptide ZfFG on phospholipid bilayers suggest that ZfFG

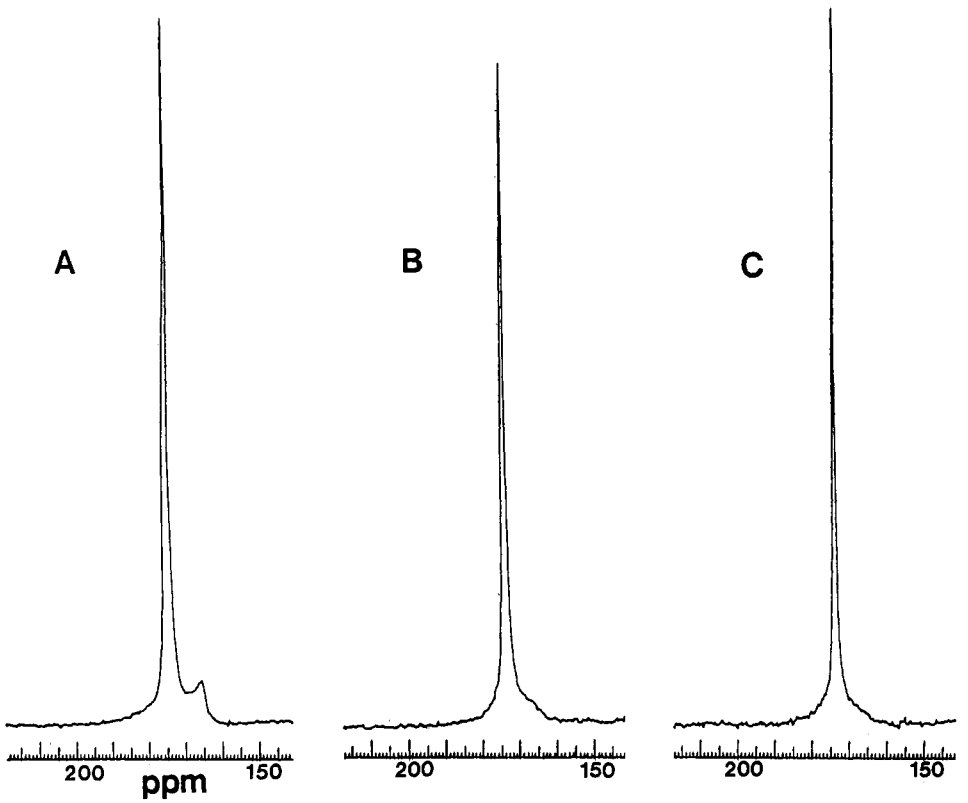

FIGURE 5: 67-MHz ^{13}C NMR spectra of the carbonyl region of di([1-^{13}C]oleoyl)phosphatidylcholine in MLV as a function of added ZfFG. (A) No ZfFG. (B) 25 mol % PC/ZfFG. (C) 33 mol % PC/ZfFG.

alters the conformation of the phospholipid molecule in the region of the molecule containing the glycerol. The moderate and small quadrupole splittings from the ^2H NMR powder patterns from the perdeuterated chains of DPPC were largely unaltered by the presence of ZfFG. These quadrupole splittings correspond to the portion of the acyl chain not including the region next to the ester bond. This observation was consistent with previous observations that the quadrupole splittings arising from the deuteriums at positions 9 and 10 of oleic acid esterified to phosphatidylcholine were largely unaltered by the presence of ZfFG (Yeagle et al., 1992). In contrast, the ^2H NMR of the DPPC with perdeuterated acyl chains showed a distinct loss of intensity from the resonances giving rise to the largest quadrupole splittings in the spectrum. The deuteriums exhibiting the large quadrupole splittings are located on the acyl chain near the ester bond to the glycerol and on the glycerol itself. Therefore this observation just referred to was tested by obtaining the ^2H NMR spectrum of a DPPC specifically labeled with deuterium on the 2 position of the acyl chains. The ^2H NMR spectrum of this specifically labeled phospholipid showed the same response as the large quadrupole splittings in the ^2H NMR spectrum of the DPPC with perdeuterated acyl chains. A significant spectral distortion and resonance intensity loss was observed.

These observations led to the examination of the behavior of the ester carbonyls which would have been expected from the ^2H NMR data to be centrally involved in the alterations in the phospholipid conformation induced by ZfFG. The ^{13}C NMR data on the carbonyls showed a significant effect of ZfFG on the orientation of the carbonyl at the *sn*-1 position. In the presence of the peptide, the ^{13}C NMR data showed that the orientation of the *sn*-1 carbonyl became approximately equivalent to the orientation of the *sn*-2 carbonyl. Such a change in orientation of the *sn*-1 carbonyl with no change in the orientation of the *sn*-2 carbonyl fairly closely defines a change in conformation of the phospholipid molecule in response to the antiviral peptide. In particular, both carbonyls in the presence of ZfFG showed an orientation of the principle axis of the carbonyl carbon chemical shift tensor approximately at the magic angle with respect to the axis for axial diffusion.

The crystal structures of several phospholipids show a consistent conformation of the glycerol region of the molecule. The *sn*-1 carbonyl is more buried in the bilayer than the *sn*-2 carbonyl, according to the crystal structures. Studies of well-hydrated membranes by ^2H NMR of specifically deuterated lipids have suggested that the conformation of this segment in the hydrated bilayer is similar to the conformation observed in the crystal structure (Seelig & Seelig, 1975). Furthermore, the ^{13}C NMR studies show a difference in orientation of the carbonyls (Lewis et al., 1984) and a difference in the exposure of the carbonyls to the aqueous phase (Yeagle & Martin, 1976), consistent again with the conclusions on the conformation of the glycerol and ester bond region of the phosphatidylcholine from the crystal structures.

To make the two carbonyls equivalent in their orientation with respect to the director for axial diffusion of the molecule, a conformational change of the glycerol region of the phospholipid is required. This would apparently necessitate a change in the orientation of the glycerol with respect to the director to allow the *sn*-1 carbonyl to reorient. Interestingly, the changes in orientation of the bonds within the phospholipid molecule appear confined to the region of the glycerol. In much of the hydrocarbon chains, away from the glycerol, little detectable change is observed in average conformation or dynamics. As discussed below, any changes in the headgroup are likely small.

These data suggest that phospholipids must have access to, minimally, two conformations with respect to the glycerol region of the phospholipid. In the presence of saturating amounts of ZfFG, the carbonyls are approximately equivalent

in orientation with respect to the director for axial diffusion. In the absence of ZfFG, the two carbonyls are inequivalent. As suggested below, rapid exchange likely occurs between these two conformations. The relative population of these two conformational states is determined by the ratio of peptide to phospholipid in the membrane. At the highest concenterations of peptide explored here, there was essentially a complete conversion of the phospholipid conformation to the state in which the carbonyls were approximately equivalent in orientation with respect to the director for axial rotation.

The axially symmetric ^{13}C powder pattern from the carbonyl at the *sn*-1 position decreased in width and collapsed smoothly into the isotropic resonance observed from the carbonyl at the *sn*-2 position upon addition of increasing amounts of ZfFG to the bilayer. These results were consistent with classic rapid chemical exchange phenomena as observed in NMR and implied in this case a rapid exchange between the unperturbed conformation and the perturbed conformation on the time scale of the ^{13}C NMR experiment. More specifically, an exchange rate faster than 10^3 s^{-1} between the two conformations would be consistent with these data.

More insight into this process was obtained from the ^2H NMR experiments. The spectra from perdeuterated DPPC bilayers showed, in the liquid crystalline state, that the largest quadrupole splittings were lost from the powder pattern in the presence of ZfFG. The ^2H NMR data from the DPPC specifically deuterated in position 2 of the palmitate showed this clearly. The effect appeared to be predominantly a loss in spectral intensity from the resonances. Such a loss of intensity could be explained by dynamics in the system with a time scale in the region of 10^5 s^{-1}. Thus if the exchange rate between the two conformations described above, one characteristic of the pure lipid bilayer and the other induced by the antiviral peptide, was on the order of 10^5 s^{-1} then a rapid loss of magnetization would have occurred during the time required to record the solid echos, leading to an apparent intensity loss as observed. This time scale was consistent with the above estimates of the rate of interchange between conformations from the ^{13}C NMR experiments.

The response of the phospholipid headgroup to the presence of the ZfFG was also a change in conformation. However, this change in conformation was not specific to the ZfFG. The change was that expected from the introduction of a negatively charged species in the surface of the bilayer, based on previous studies. In particular, deuteriums in the α and β positions of the choline showed changes in the values of the quadrupole splittings that quantitatively were about one-third the magnitude of changes in the same quadrupole splittings reported for the introduction of the negative lipid, dialkyl phosphate, into d_4-PC bilayers (Seelig et al., 1987). The pK_a of the carboxyl of the peptide is about 3.5 (manuscript submitted). Therefore at the pH of these experiments (about 7), the peptide should carry a full negative charge. The difference between the quantitative response of the choline segment (as measured by ^2H NMR) to the negatively charged dialkyl phosphate and the negatively charged peptide may be due to a different location of the negative charge in the membrane. In the former, the negative charge is most likely located at the level of the phosphate of the phospholipids. In the latter, recent data indicate that the negative charge is located farther from the bilayer midplane than the phospholipid phosphate and thus in a region of different dielectric (Dentino et al., manuscript submitted; Turner & Gaber, 1992). The ^{31}P NMR data show a small change in the breadth of the ^{31}P NMR powder pattern that could be consistent with a small alteration in the average orientation of the phosphate chemical shift tensor with respect to the director for axial diffusion (Thayer & Kohler, 1981), in response to the alteration in conformation of the glycerol and ester bonds (to the acyl chains). Alternatively, the observations could also be due to a small change in the extent of motional averaging due to a disordering of the headgroup in the membrane. The latter interpretation is not supported by the ^2H NMR data. Therefore, the most likely interpretation is a modest conformational change around the phosphate of the headgroup, likely in response to the change in conformation around the glycerol.

It is possible that the altered phospholipid conformation found in the presence of the antiviral peptide is responsible for the differences in phospholipid packing that inhibit the formation of highly curved phospholipid surfaces. However, the present data do not prove this hypothesis which awaits further testing.

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