Interfacial Conformation of Dipalmitoylglycerol and Dipalmitoylphosphatidylcholine in Phospholipid Bilayers†

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ABSTRACT: Diacylglycerols are minor constituents of membrane lipids, yet are essential in the activation and membrane association of protein kinase C. Solid-state ^{13}C NMR experiments have been used to characterize the orientation of the glycerol backbone of dipalmitoylglycerol (DPG) and dipalmitoylphosphatidylcholine (DPPC) in egg phosphatidylcholine (PC) bilayers. The ^{13}C NMR spectra of both DPG and DPPC specifically ^{13}C-labeled at the *sn*-2 chain carbonyl exhibit a single narrow resonance (~2 ppm) in liquid-crystalline egg PC bilayers. In contrast, specific ^{13}C-labeling of both the *sn*-1 and *sn*-2 chain carbonyls results in an additional broad component (24–32 ppm) with an axially symmetric line shape. These data reveal that DPG has a distinct motionally-averaged structure in PC bilayers that is similar to that of DPPC and is not significantly affected by the absence of the large polar PC headgroup. The NMR line shapes are roughly consistent with the results of previous FTIR and NMR studies that indicate the *sn*-1 chain extends from the C_1 carbon of the glycerol backbone into the hydrophobic interior of the bilayer, while the *sn*-2 chain first extends parallel to the bilayer surface and incorporates a bend at the ester linkage in order to keep the *sn*-1 and *sn*-2 chains parallel. However, the data suggest that the time-averaged orientation of the glycerol backbone is tilted from the bilayer normal, in contrast to the nearly parallel orientation observed in the crystal structures of phosphatidylcholines and phosphatidylethanolamines or the perpendicular orientation observed in the crystal structures of diacylglycerols.

Diacylglycerols (DAGs)[1] are key components in the signal transduction pathway from several cell-surface receptors to protein kinase C (PKC) (Bell, 1986; Nishizuka, 1984). These molecules are transiently produced in membranes from phosphatidylcholine and phosphatidylinositol by receptor-activated phospholipase C and serve as second messengers in the activation of PKC. PKC, a serine–threonine phosphotransferase, associates with membranes and is activated upon binding a single molecule of DAG along with phosphatidylserine and Ca^{2+}. There has been considerable interest in the conformation of DAGs in phospholipid bilayers and in how these molecules influence bilayer structure (Epand, 1985; Hamilton et al., 1991a; De Boeck & Zidovetski, 1992). In this paper, we use solid-state ^{13}C NMR methods to characterize the conformation of 1,2-dipalmitoylglycerol (DPG) and 1,2-dipalmitoylphosphatidylcholine in egg phosphatidylcholine (PC) bilayers.

The crystal structures of several different lipids have been determined and provide an important reference for studying the conformation of lipids in bilayers (Hitchcock et al., 1974; Pascher et al., 1981; Hauser et al., 1981). In the crystal structures of phosphatidylethanolamine (PE) and phosphatidylcholine, the lipid headgroup extends from the C_3 position of the glycerol backbone and is oriented roughly perpendicular to the long axis of the fatty acyl chains (Figure 1). The orientation of the glycerol backbone is parallel to the acyl chain axis in PC and PE, but *perpendicular* to this axis in the crystal structures of dilauroylglycerol (DLG) and 1,2-DPG (Pascher et al., 1981; Dorset & Panghorn, 1988). The large polar headgroups in phospholipids dominate their interfacial chemistry, but it is not known how they influence the orientation or conformation of the glycerol backbone. An intuitive picture is that the large polar or charged headgroup effectively pulls the end of the glycerol chain away from hydrophobic interior of the bilayer, thereby orienting the backbone parallel to the acyl chain axis as seen in the crystal structures of PE and PC.

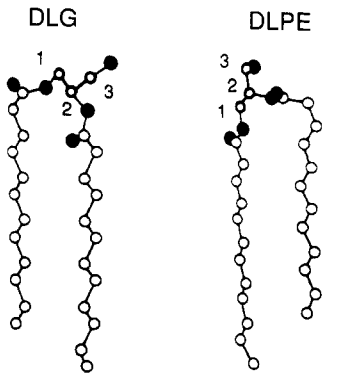

FIGURE 1: Crystal structures of DLG (left) and DLPE (right) illustrating the difference in glycerol backbone orientation (Hauser et al., 1981; Pascher et al., 1981). In DLG, the glycerol backbone is oriented roughly perpendicular to the acyl chain axis. The *sn*-2 chain extends directly down from the C_2 positin of the glycerol, while the *sn*-1 chain incorporates a 90° bend at the position of the *sn*-1 carbonyl. The molecular conformation of the β_1-crystal form of 1,2-DPG is essentially identical to that of 1,2-DLG (Dorset & Panghorn, 1988). In DLPE, the glycerol backbone is oriented roughly parallel to the chain axis, resulting in a trans *sn*-1 chain extending from the C_1 position of glycerol and a bend in the *sn*-2 chain.

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[1] Abbreviations: CSA, chemical shift anisotropy; DAG, diacylglycerol; DLG, dilauroylglycerol; DLPE, dilauroylphosphatidylethanolamine; DPG, dipalmitoylglycerol; DPPC, dipalmitoylphosphatidylcholine; FTIR, Fourier transform infrared spectroscopy; NMR, nuclear magnetic resonance spectroscopy; PC, phosphatidylcholine; PKC, protein kinase C.

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Solid-state ^{13}C NMR provides an approach for determining the interfacial conformation of membrane lipids in bilayer structures. Cornell (1981) originally observed that the carbonyl resonances of the *sn*-1 and *sn*-2 chains in dipalmitoylphosphatidylcholine (DPPC) had distinct NMR frequencies and line shapes. In the liquid-crystalline phase, the *sn*-2 carbonyl resonance has a relatively narrow (~2 ppm) Lorenztian line width, while the *sn*-1 resonance has a broader (24–32 ppm) axially symmetric line shape. The narrow line shape resulting from the *sn*-2 carbonyl has been interpreted in terms of a unique carbonyl chemical shift tensor lying near the magic angle ($\theta = 54.7°$) relative to the rotational diffusion axis of the lipid (Braach-Maksvytis & Cornell, 1985; Wittebort et al., 1981). The broader line shape of the *sn*-1 carbonyl results from the *sn*-1 chemical shift tensor having a non-magic-angle orientation which is incompletely averaged by lipid motion. If the crystal conformations of DPPC and DPG are retained in bilayers, then a comparison of the crystal structures would predict a reversal in the NMR line shapes for the *sn*-1 and *sn*-2 carbonyls since their orientations in the diacylglycerols have changed positions relative to those of the phospholipids.

In our study, the DAG was incorporated at low levels into a phospholipid bilayer to mimic the low levels required for biological activity and to preserve the bilayer structure in the heterogeneous mixture, since it is known that higher levels of DAG can disrupt the lamellar structure of phospholipids and induce hexagonal and cubic phases (Epand, 1985; De Boeck & Zidovetski, 1992). We compare the interfacial conformation of 10 mol% ^{13}C-labeled DPPC and DPG in egg PC bilayers. This study extends previous work on the interfacial conformation of DAG (Hamilton et al., 1991a,b) and PC (Braach-Maksvytis & Cornell, 1985; Wittebort et al., 1981), and reveals that the orientation of the glycerol backbone in these lipids is independent of the large polar headgroup.

MATERIALS AND METHODS

1,2-[1-^{13}C]DPPC was prepared by acylation of 0.26 g (0.6 mmol) of the cadmium complex of L-α-glycerophosphorylcholine (Sigma, grade I) with 0.93 g (3.6 mmol) of [1-^{13}C]-palmitic acid (Cambridge Isotopes) in chloroform. Optimized reaction conditions were used to maximize the yield based on the fatty acid. 2-[1-^{13}C]DPPC was prepared in a similar fashion by acylation of lysopalmitoylphosphatidylcholine with [1-^{13}C]palmitic acid. 1,2-DPGs with ^{13}C-enrichment at the *sn*-1 and *sn*-2 carbonyl positions (double labeled) or at the *sn*-2 position alone (single labeled) were prepared from the corresponding ^{13}C-enriched DPPC by treating with phospholipase C (*Bacillus cereus*) in moist ether medium (Mavis et al., 1972). DPG showed no detectable amounts of any impurities, especially the 1,3 isomer, either by TLC or by analytical ^{13}C NMR (Hamilton et al., 1991b). Lipid samples for NMR were prepared by mixing the appropriate amounts of lipids in chloroform, codrying the lipids under nitrogen, and removing trace amounts of organic solvent under high vacuum for at least 1–2 h. The lipid films were then dispersed in 0.3 mL of 0.1 M phosphate buffer in D_2O (pD ≈7) or H_2O (pH ≈7) by vortex mixing for 15–20 min and stored at ~5°C until use in the NMR experiments. In the case of pure DPPC, the hydration was varied from ~50 to 75 wt % H_2O to determine the effect of hydration on the observed line width. Following NMR measurements, samples were analyzed for isomer purity of the DPG and found to contain >95% of the 1,2 isomer.

The solid-state NMR experiments were performed on a Chemagnetics CMX spectrometer at a ^{13}C frequency of 90.4 MHz using single-pulse and Hahn echo pulse sequences with proton decoupling during acquisition. The ^{13}C 90° pulse lengths were typically 4 μs with a recycle delay of 3–5 s. Typically, 10 000–15 000 acquisitions were averaged for each spectrum. The temperature was controlled to ±2 °C by a constant flow of air across the sample, although the actual sample temperature may be a few degrees higher than the flow temperature because of high-power proton decoupling.

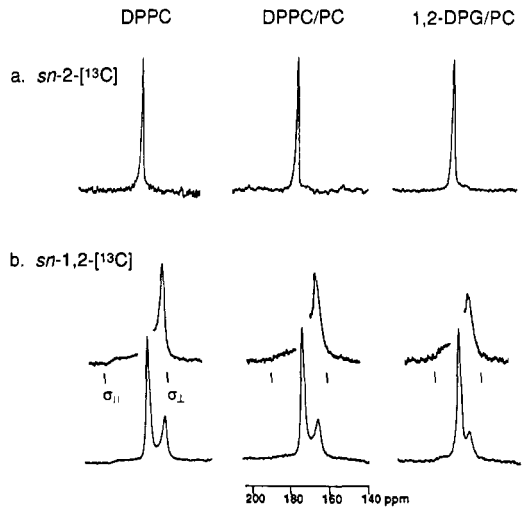

FIGURE 2: Solid-state ^{13}C NMR spectra of pure DPPC (left), DPPC in egg PC (middle), and 1,2-DPG in egg PC (right) specifically ^{13}C-labeled at the *sn*-2 carbonyl (a) and at both the *sn*-1 and *sn*-2 carbonyls (b). The broad components assigned to the *sn*-1 carbonyls are expanded vertically in (b) to highlight the axially symmetric line shapes. The CSA was obtained from the edges of the broad line shapes that are indicated in the expanded insets. The spectra of pure DPPC were obtained at 46 °C on ~50 mg of lipid, while the spectra of DPPC and DPG in egg PC were obtained at 24–28 °C on ~7 mg of ^{13}C-labeled lipid incorporated into egg PC at a 1:10 mole ratio.

RESULTS

Several previous structural studies based on phospholipid carbonyl line shapes have focused on *pure* DPPC at natural-abundance ^{13}C (Cornell, 1981) or with the *sn*-2 chain specifically ^{13}C-labeled (Wittebort et al., 1981). Our experiments differ from these previous studies in that we incorporated low amounts of the ^{13}C-labeled lipids into egg PC bilayers in the liquid-crystalline (L_α) phase. The components of the carbonyl spectrum were assigned by selective labeling of one of the carbonyls (Murari et al., 1987). In order to assess how incorporation into PC bilayers affects the carbonyl line shapes, ^{13}C NMR spectra were first obtained of pure DPPC and DPPC incorporated into egg PC. Figure 2 (left) presents the ^{13}C NMR spectra of DPPC specifically ^{13}C-labeled at the *sn*-2 carbonyl only (a) and at both the *sn*-1 and *sn*-2 carbonyls (b) in the L_α phase (above 42 °C). The single resonance in the 2-[1-^{13}C]DPPC spectrum has a broad (~110 ppm) axially symmetric line shape (data not shown) in the gel ($L_{\beta'}$) phase, where rotational motion is restricted, but collapses to the observed narrow resonance (1.8 ppm) in the L_α phase, where rotational motion is relatively free. These spectra correspond well with those obtained by Griffin and co-workers (Wittebort et al., 1981). The line width in the L_α phase is narrower than the 7 ppm width previously observed (Wittebort et al., 1981), but is close to the residual chemical shift anisotropy (1.5 ppm) for this position determined by Braach-Maksvytis and Cornell (1985). We found that the extent of hydration can influence the line width within this observed range; narrow resonances (<3 ppm) were obtained at high levels of hydration (>50 wt % H_2O). In contrast,

changing the temperature from 44 to 50 °C had no noticeable effect on the line width. The two resonances in the 1,2-[1-^{13}C]DPPC spectrum (Figure 2b, left) correspond closely with those in the natural-abundance DPPC spectrum obtained by Cornell (1981). Comparison of the NMR spectra obtained from single- and double-^{13}C-labeled DPPC clearly shows that the broad resonance in the double-labeled sample is due to the *sn*-1 carbonyl. A vertical expansion of the broad component is shown where the narrow *sn*-2 resonance has been edited for clarity. The broad component has a well-defined axially symmetric line shape with a residual chemical shift anisotropy (CSA) of −32 ppm defined by the difference between the downfield ($\sigma_{11} = \sigma_{\parallel}$) and upfield ($\sigma_{22} = \sigma_{33} = \sigma_{\perp}$) components of the chemical shift powder pattern. $\sigma_{\parallel}$ corresponds to the unique axis of the axially symmetric tensor. The full breadth of the chemical shift anisotropy is −148 ppm (Wittebort et al., 1981).

Figure 2 (middle) presents the ^{13}C NMR spectra of 10 mol% DPPC in egg PC in which the DPPC has been specifically ^{13}C-labeled at the *sn*-2 carbonyl only (a) and at both the *sn*-1 and *sn*-2 carbonyls (b). The *sn*-1 and *sn*-2 resonances each have line shapes similar to those for pure DPPC, although the residual CSA of the broad *sn*-1 resonance has decreased to −28 ppm. Consequently, the conformation and dynamics of DPPC in the region of the carbonyls do not change substantially in the mixed-lipid system compared to pure DPPC. The slight narrowing of the *sn*-1 carbonyl CSA pattern is most likely due to greater mobility in the mixed-lipid system owing to the presence of unsaturated acyl chains in egg PC.

Finally, the ^{13}C NMR spectra of 10% DPG in egg PC are shown in Figure 2 (right) for DPG specifically ^{13}C-labeled at the *sn*-2 carbonyl only (a) and at both the *sn*-1 and *sn*-2 carbonyls (b). It is readily apparent that the line shapes are similar to those in DPPC with the corresponding labels, in contrast to the prediction from the crystal structure of DPG that the line shapes would be reversed. The residual CSA of the *sn*-1 signal in DPG (−24 ppm) is similar to that for DPPC in a matrix of egg PC (−28 ppm).

DISCUSSION

^{13}C NMR spectra of lipids in solution exhibit carbonyl signals with narrow Lorenztian line shapes resulting from rapid isotropic motion and averaging of anisotropy in the chemical shift interaction. In contrast, in lipid multilayers below the phase transition temperature, the carbonyl resonances are extremely broad (100–150 ppm) and have characteristic shapes that reflect the chemical shift anisotropy (CSA). Above the lipid-phase transition, anisotropic motion of the lipid molecules (such as axial diffusion) results in *partial* averaging of the CSA. The extent of averaging depends on the orientation of the carbonyls relative to the axis of motion, or more precisely on how the carbonyl chemical shift tensor changes relative to the external magnetic field as a result of motion. For an axially symmetric tensor, the residual CSA ($\Delta\sigma_R$) is related to the static anisotropy ($\Delta\sigma_S$) by

$$\Delta\sigma_R = \Delta\sigma_S(3\cos^2\theta - 1)/2$$

where θ is the angle between the unique shielding tensor axis and the axis of motion (Wittebort et al., 1981; Mehring, 1983).

Solid-state ^{13}C (Cornell, 1981) and ^{2}H NMR (Seelig & Seelig, 1975; Seelig & Browning, 1978) studies of phospholipids have previously argued that the *sn*-1 and *sn*-2 carbonyls have different orientations in bilayers in the L_α phase. The *sn*-1 chain is thought to extend in a trans configuration from the C_1 position of the glycerol backbone into the hydrophobic interior of the bilayer, while the *sn*-2 first extends parallel to the bilayer surface and incorporates a bend at the ester linkage in order to keep the *sn*-1 and *sn*-2 chains parallel. However, accurate orientations for the *sn*-1 and *sn*-2 carbonyls have been difficult to determine since the interpretation of the NMR data relies on an accurate model for the lipid *dynamics*.

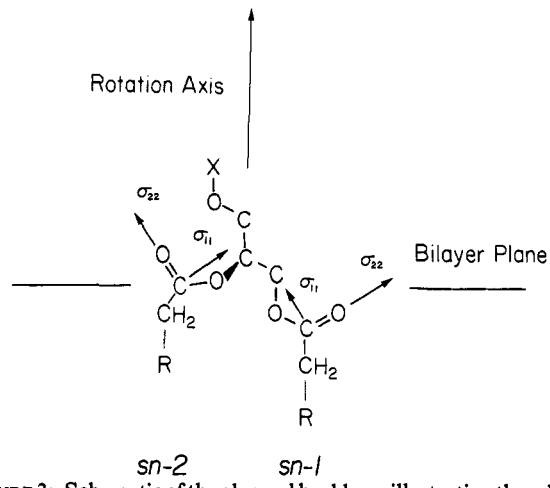

FIGURE 3: Schematic of the glycerol backbone illustrating the relative orientations of the σ_{11} ($\sigma_{\parallel}$) and σ_{22} ($\sigma_{\perp}$) tensor elements for the *sn*-1 and *sn*-2 carbonyls. In model compounds (see text), σ_{11} lies in the plane of the sp2 carbonyl, while the σ_{22} element is aligned within 10° of the C=O bond at 90° from σ_{11}. The σ_{33} element (not shown) is oriented out of the C=O plane perpendicular to σ_{11} and σ_{22}. The axis of rotational diffusion is along the long axis of the lipid molecule and perpendicular to the plane of the bilayer. X denotes a hydrogen atom in DAG and a PC headgroup in DPPC. R represents the hydrophobic acyl chains.

Orientation of the sn-2 Carbonyl. The narrow line shapes observed in the solid-state NMR spectra of 2-[1-^{13}C]DPG and 2-[1-^{13}C]DPPC argue that the unique axis of the chemical shift tensor for the *sn*-2 carbonyl is oriented at the magic angle relative to the axis of lipid motion. Few studies have been undertaken on the dynamics of DAGs in phospholipid bilayers. Spin–lattice relaxation time (T_1) and line-width measurements on DAGs in small unilamellar vesicles and in multilayers have suggested motions generally similar to those of PCs (Hamilton et al., 1991a,b). For DPPC, simple axial diffusion about the long axis of the lipid is thought to be the predominant motion responsible for averaging the ^{13}C shift tensors above the T_m (Wittebort et al., 1981). In addition, ^{2}H NMR studies of the glycerol backbone in several glycolipids indicate that there is a dramatic jump in the axial diffusion rate in going to the liquid-crystalline phase (Auger et al., 1990). On the basis of the assumption that fast axial diffusion is responsible for narrowing the ^{13}C line shapes from −148 to ~−2 ppm in DPG and DPPC, it is possible to calculate the residual CSA (using the equation above) if the orientation of the carbonyl chemical shift tensor is known. Although there are no single-crystal studies of the carbonyl chemical shift tensor orientation in diacylglycerols or phospholipids, in model compounds the unique tensor element (σ_{11}) is in the plane of the sp2 carbonyl, while the σ_{22} element is aligned within 10° of the C=O bond and 90° from σ_{11} (Griffin & Ruben, 1975; Cornell, 1986) (Figure 3). For the *sn*-2 carbonyl, only a magic-angle orientation of 54.7° ± 1° for the σ_{11} element would give the dramatic narrowing that is observed since $(3\cos^2\theta - 1)/2 = 0$ and $\Delta\sigma_R = 0$ for $\theta = 54.7°$.

Recent FTIR studies on oriented multilayers provide an important comparison for conclusions drawn from the NMR data since interpretation of the FTIR data is not subject to

the same motional constraints. For example, an orientation for the C(O)–O single-bond transition moment of 61° relative to the bilayer normal has been obtained from oriented FTIR measurements of liquid-crystalline DMPC and DPPC (Hubner & Mantsch, 1991), close to the 55° orientation predicted from NMR based on an axial diffusion model. The FTIR and NMR results should be directly comparable since the orientation of the σ_{11} tensor element roughly corresponds to the transition moment orientation for the C(O)–O single-bond stretch. Both lie approximately along a line connecting the –C(O)–O–C– carbons.

Orientation of the sn-1 Carbonyl. The residual CSA for the *sn*-1 carbonyl of –24 ppm (DPG) and –28 to –32 ppm (DPPC) corresponds to an orientation of ~45° for the unique tensor axis relative to the bilayer normal if axial diffusion is the only motion responsible for the observed line-narrowing. This result is relatively close to an FTIR-determined orientation of 40°. In contrast, the X-ray structures of PC and PE lipids indicate that the C=O bond is oriented at ~90° to the bilayer normal, thereby giving an orientation for the σ_{11} tensor element or the C(O)–O transition moment of ~0°. In this geometry, simple axial diffusion would not lead to narrowing of the ^{13}C line shape since $(3\cos^2\theta - 1)/2 = 1$ and $\Delta\sigma_R = \Delta\sigma_S$ for $\theta = 0°$.

One possible explanation for the discrepancy between the NMR and X-ray data is that the glycerol backbone is not parallel to the bilayer normal in PC liquid-crystalline multilayers. A motional model based on fast axial diffusion would argue that the glycerol backbone may be oriented *at most* ~45° (NMR) or 40° (FTIR) from the bilayer normal since both the σ_{11} tensor element and the C(O)–O transition moment lie roughly along the axis of the glycerol backbone when the torsion angles along the backbone and through the *sn*-1 carbonyl are all ~180°. The occurrence of torsion angles less than 180° would yield an orientation of the glycerol backbone *closer* to the bilayer normal and a corresponding NMR estimate for the glycerol orientation of <45°. An orientation of <45° is suggested by the preferential hydrogen-bonding of the *sn*-2 carbonyl with water (see below). On the basis of their FTIR results, Hubner and Mantsch (1991) proposed that the glycerol backbone is oriented at ~30 ± 10° to the bilayer normal, and argued that the C(O)–O single-bond stretching frequency at 1175 cm^{-1} provided evidence for gauche conformers near the *sn*-1 C(O)–O single bond. The time-averaged orientations of the *sn*-1 and *sn*-2 acyl chains are still thought to be parallel to the bilayer normal.

Since lipid dynamics are likely to be complex, motions other than axial diffusion may be responsible in part for averaging the *sn*-1 and *sn*-2 carbonyls and must be considered. Auger et al. (1990) and others have proposed fast internal motion about the glycerol C_2–C_3 bond in both the L_α and L_β phases of phospholipids and glycolipids. They have simulated C_3-^2H deuterium spectra with a three-site exchange model and C_2-^2H spectra with a two-site exchange model. The greater motion at the C_3 position is consistent with restrictions in the motion of the C_2 and C_1 positions imposed by the attached acyl chains (Weiner & White, 1992).

One specific model for conformational changes in the glycerol backbone of phospholipids is that of Hauser and coworkers (Hauser et al., 1988). They proposed that the C_1–C_2 bond has a motionally-averaged structure in micelles that is derived from four single-crystal conformations; two conformations have a parallel orientation of the glycerol backbone relative to the bilayer normal, and two conformations have a perpendicular orientation. Interconversion of the two forms having parallel orientations does not significantly change the orientation of the carbonyl chemical shift tensor and consequently would have no effect on the ^{13}C line shape. Interconversion between the parallel and perpendicular orientations would yield a net orientation for the glycerol backbone that is *intermediate* between the interconverting conformations, and distinct from the parallel or perpendicular orientations seen in the crystal structures. As a result, models based on either axial diffusion or glycerol conformational changes would predict narrowing of the carbonyl resonances and indicate that the glycerol backbone is tilted relative to the bilayer normal. Cornell has modeled the lipid dynamics in DMPC and DPPC using a combination of these motions to account for the residual *sn*-1 carbonyl CSA (Cornell, 1981).

Summary. Comparison of the DPG and DPPC spectra indicates that the motionally-averaged DPG and DPPC structures are similar and independent of the large polar PC headgroup. Although the molecular interaction of PKC with DAG remains to be defined, this observation argues that conformational changes *in the interfacial region* are not important for DAG activity. These results confirm predictions of recent high-resolution and MAS NMR studies on several DAGs in egg PC bilayers (Hamilton et al., 1991a,b). In these studies, the isotropic carbonyl chemical shifts were correlated with a greater degree of hydrogen bonding at the *sn*-2 than at the *sn*-1 carbonyl. This suggested that the bilayer structure of the DAG molecule was more similar to the crystal conformation of DLPE (or PC in bilayers) than to the crystal structure of DLG. The present approach leads to generally the same conclusion and raises the intriguing question of why the glycerol backbone has a similar conformation in DPPC and DPG. In the DLG crystal structure, the C_3 hydroxyl group hydrogen-bonds to the *sn*-1 carbonyl of the neighboring molecule. In hydrated mixed DAG/PC bilayers, it is likely that hydrogen bonding with water replaces this intermolecular hydrogen bonding and promotes a conformational change in the glycerol backbone. In PC and PE crystal structures, where the glycerol backbone is aligned parallel to the bilayer normal, the *sn*-1 carbonyl is more deeply buried in the hydrophobic interior of the bilayer. For DPPC, both solid-state NMR and FTIR studies suggest that the time-averaged orientation of the glycerol backbone may be tilted relative to the bilayer normal, thereby allowing the *sn*-1 carbonyl to more closely approach the aqueous interface. This would seem to be a reasonable adjustment to the crystal structure following hydration of the interface since it would permit better hydrogen bonding to the *sn*-1 carbonyl. Although both NMR and FTIR studies of DPPC show that the *sn*-2 carbonyl is more highly hydrated than the *sn*-1 carbonyl, the difference may not be large. The *sn*-1 and *sn*-2 carbonyl groups are estimated to be ~35–45% (*sn*-1) and ~45–57% (*sn*-2) in a hydrogen-bonded state (Blume et al., 1988; Schmidt et al., 1977). A difference of ~10% in relative hydration would be consistent with high-resolution NMR data for DAG in PC bilayers (Hamilton et al., 1991a). Together with our results, these data on liquid-crystalline bilayers suggest that the glycerol orientation is determined by a balance between hydrogen-bonding interactions involving not only the hydroxyl group (DPG) or phosphatidylcholine headgroup (PC) at C_3 but also both the *sn*-1 and *sn*-2 chain carbonyls.

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CORRECTIONS

Hydroxyl Radical Mediated Damage to Proteins, with Special Reference to the Crystallins, by Purnananda Guptasarma, Dorairajan Balasubramanian,* Seiichi Matsugo, and Isao Saito, Volume 31, Number 17, May 5, 1992, pages 4296–4303.

Page 4296. The name and address of Dr. Seiichi Matsugo should read as follows: Dr. Seiichi Matsugo, Department of Chemical and Biochemical Engineering, Faculty of Engineering, Toyama University, Gofuku 3190, Toyama 930, Japan.

Page 4303. The Saito et al. (1990) reference should read as follows: Saito, T., Takayama, M., Matsuura, T., Matsugo, S., & Kawanishi, S. (1990) *J. Am. Chem. Soc. 112*, 883–884.