Effect of chain-linkage on the structure of phosphatidyl choline bilayers

Hydration studies of 1-hexadecyl 2-palmitoyl-*sn*-glycero-3-phosphocholine

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ABSTRACT While hydrated dipalmitoyl phosphatidylcholine (DPPC) forms tilted chain $L_{\beta'}$ bilayers in the gel phase, the ether-linked analogue dihexadecyl phosphatidylcholine (DHPC) exhibits gel phase polymorphism. At low hydration DHPC forms $L_{\beta'}$ phases but at > 30% H_2O a chain-interdigitated gel phase is observed (Ruocco, M. J., D. S. Siminovitch, and R. G. Griffin. 1985. *Biochemistry.* 24:2406–2411; Kim, J.T., J. Mattai, and G.G. Shipley. 1987. *Biochemistry.* 26:6599–6603). In this study we report the behavior of a phosphatidylcholine (PC) with both types of chain linkage, 1-hexadecyl-2-palmitoyl-*sn*-glycero-3-phosphocholine (HPPC). HPPC has been investigated as a function of hydration using differential scanning calorimetry (DSC) and x-ray diffraction. By DSC, over the hydration range 5.1–70.3 wt% H_2O, HPPC exhibits two reversible transitions. The reversible main chain-melting transition decreases from 69°C, reaching a limiting value of 40°C at full hydration. X-ray diffraction patterns of hydrated HPPC have been recorded as a function of hydration at 20° and 50°C. At 50°C, melted-chain L_α bilayer phases are observed at all hydrations. At 20°C, at low hydrations (<34 wt% H_2O) HPPC exhibits diffraction patterns characteristic of bilayer gel phases similar to those of the gel phase of DPPC. In contrast, at ≥34 wt% H_2O, HPPC shows a much reduced bilayer periodicity, d = 47 Å, and a single sharp reflection at 4.0 Å in the wide angle region. This diffraction pattern is identical to that exhibited by the interdigitated phase of DHPC. Therefore, in the gel phase HPPC undergoes a hydration-dependent conversion from a regular bilayer structure to an interdigitated bilayer arrangement. Clearly, the presence of a single ether linkage (at the *sn*-1 position) is sufficient to allow formation of the chain-interdigitated phase in a hydration-dependent way essentially identical to that of DHPC.

INTRODUCTION

The lipid matrix in which mammalian cell membrane proteins operate is normally comprised of a variety of phospholipids, glycosphingolipids, cholesterol, etc. These lipids assemble to form bilayers in which protein receptors, channels, and enzymes are asymmetrically incorporated. In general, the lipid bilayer itself is also asymmetric with respect to the composition of its two component monolayers, containing lipids (e.g., phosphatidylcholine [PC], sphingomyelin, and glycosphingolipids) that prefer the outer monolayer with other lipids (e.g., phosphatidylethanolamine [PE] and phosphatidylserine [PS]) that favor the inner monolayer (Rothman and Lenard, 1977).

In some cases the lipids themselves have important functions. Examples include glycosphingolipids (e.g., gangliosides) for cell recognition and receptor functions (Hakomori, 1981), phosphatidylinositol (PI) as both a membrane anchor for extrinsic membrane proteins (Low and Saltiel, 1988), and as a precursor of inositol phosphate and diacylglycerol second messengers (Berridge and Irvine, 1984; Nishizuka, 1984), and some phospholipids as progenitors of arachidonic acid, prostaglandins, and leukotrienes (Smith and Borgeat, 1985).

For the glycerol-based phospholipids, the presence of multiple lipid classes dictated by the chemistry of the polar group (PC, PE, PS, PI, etc.) is further complicated by chemical variations in the hydrocarbon chains attached at the *sn*-1 and *sn*-2 positions of the glycerol moiety. These variations include chain length, chain unsaturation, chain branching, and chain linkage.

In recent years much information relevant to the lipid matrix of cell membranes has been derived from studies of model membranes formed from hydrated dispersions of isolated membrane phospholipids and synthetic phospholipids. A vast literature has evolved from physico-chemical and functional studies of model membranes of PC, PE, PS, etc., in which chain length, unsaturation, and branching have been systematically varied.

For PCs, our own studies have used differential scanning calorimetry, x-ray diffraction, and spectroscopic methods to probe the changes in structure and properties as the PC chain length is varied (Janiak et al., 1976, 1979; Ruocco and Shipley, 1982*a*, *b*; Mattai et al., 1987). More recently we and others have become interested in the effect of chain linkage, where the attachment to the glycerol is through either ester or ether linkages, or both. Replacement of the ester linkages of 1,2-dipalmitoyl PC

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(DPPC) with ether linkages as in 1,2-dihexadecyl PC (DHPC) has little effect on membrane properties such as the chain-melting temperature, T_m (Vaughan and Keough, 1974; Ruocco et al., 1985). Interestingly, Ruocco et al. (1985) concluded that the gel phases of DHPC present below T_m differ from those of DPPC. DPPC forms a series of bilayer structures with chain tilt ($L_{\beta'}$), chain crystallization (L_c), and bilayer rippling ($P_{\beta'}$) (Ruocco et al., 1982*a*, *b*), whereas DHPC forms interdigitated bilayers with the chains from both monolayers interpenetrating (Ruocco et al., 1985). Our own studies showed that in the gel phase DHPC converts between noninterdigitated and interdigitated bilayers at a specific hydration, ~30 weight% water (Kim et al., 1987*a*; see also Laggner et al., 1987); in addition, mixtures of DHPC and DPPC show eutectic behavior due to the formation of both DHPC-rich interdigitated and DPPC-rich noninterdigitated gel phase bilayers below T_m (see Kim et al., 1987*b*; Lohner et al., 1987). Given that alteration in chain linkage (ester-to-ether linkage) causes major structural alterations in DPPC and DHPC, we were interested in studying PCs (and other lipids) containing both ester- and ether-linked chains since these "mixed-linkage" species (e.g., plasmalogen-PC and -PE, platelet activating factor, etc.) are frequently present in membranes. Here we focus on the structure and properties of 1-hexadecyl-2-palmitoyl-*sn*-glycero-3-phosphocholine (HPPC), a chemical "intermediate" between DPPC and DHPC, with particular attention being paid to the structure of the gel phase.

MATERIALS AND METHODS

HPPC was synthesized according to the following method. 200 mg of 1-O-hexadecyl-sn-glycero-3-phosphocholine (R. Berchtold, Biochemisches Laboratory, Berne, Switzerland) was taken in anhydrous chloroform, freshly distilled from phosphorous pentoxide, and stirred with 1.0 g of palmitic anhydride (Nu Chek, Elysian, MN) in the presence of the base dimethylaminopyridine (60 mg) at room temperature under argon atmosphere for 48 h. The reaction mixture was evaporated and the residue was taken in 30 ml of methanol/chloroform/water (5:4:1, vol/vol/vol) and passed over a prewashed column of Rexyn-I-300 (Fisher Scientific Co., Fair Lawn, NJ). This treatment removes most of the base and some of the fatty acid. The eluate was evaporated, taken in chloroform, and passed over a silicic acid column (Unisil 100-200 mesh, Clarkson Chemical Company, Williamsport, PA). The column was eluted with chloroform containing increasing amounts of methanol (10, 20, 30, and 40%), and HPPC was eluted in chloroform containing 40% methanol. Yields were 60–65%. The HPPC was found to be pure by TLC using the solvent system chloroform/methanol/water (65:25:4, vol/vol/vol).

Samples were prepared for differential scanning calorimetry (DSC) by weighing anhydrous HPPC into stainless steel pans; adding doubly distilled, deionized water gravimetrically to bring the HPPC to the desired hydration; and hermetically sealing the pans. Calorimetry was performed between the temperatures of −10° and 50°C on a scanning calorimeter (DSC-2C; Perkin-Elmer Corp., Norwalk, CT) equipped with a Thermal Analysis Data Station (TADS). Immediately after sample preparation, or after periods of prolonged storage at −10°C, samples were heated and cooled until reproducible thermograms were obtained. Before a specific scanning protocol, samples were heated above the chain-melting transition temperature of HPPC. The transition temperatures were determined from the peak temperatures of the observed transitions and the transition enthalpies were determined from the areas under the peaks. The calorimeter was calibrated using a gallium standard.

Samples were prepared for x-ray diffraction by weighing HPPC and doubly distilled, deionized water into thin-walled glass capillary tubes. The tubes were flame-sealed, and coated with epoxy to ensure absence of leaks. The HPPC dispersions were centrifuged between tube ends at temperatures above the chain-melting transition temperature to ensure homogeneity. X-ray diffraction patterns were recorded on photographic film using nickel-filtered CuKα radiation (λ = 1.5418 Å) from a rotating anode generator (GX6; Elliot Automation, Borehamwood, UK). The x-ray beam was focused by using either double-mirror (Franks, 1958) or toroidal (Elliott, 1965) optics.

Microdensitometry was performed with a Joyce Loebl (Gateshead, UK) model III-CS scanning microdensitometer. The background curve was subtracted and intensities $I(h)$ were determined for each order h by measuring the peak height. The structure amplitude $F(s)$ was set equal to $[h^2I(h)]^{1/2}$. Structure amplitudes of the low angle reflections were normalized by choosing a reference diffraction pattern and using the ratio of the reference and sample high angle intensities as a normalization factor for the low angle intensities. From the set of normalized $F(s)$, and using the sampling theorem (Shannon, 1949), the continuous reciprocal-space amplitude functions were calculated and the phasing determined. The phased amplitudes for each HPPC hydration were Fourier transformed to give the electron density profiles across the layer.

Lamellar periodicities d were determined using Bragg's law. The maximum hydration was the hydration above which the bilayer periodicity, d, no longer changed. Values for lipid bilayer thickness, d_l, water layer thickness, d_w, and surface area per lipid molecule S were computed according to the formalism of Luzzati (1968). The sum of d_l and d_w is the lamellar periodicity d. Values for d_l, d_w, and s were calculated as a function of hydration until maximum hydration was reached; afterwards, d_l, d_w, and S were computed using d and the maximum hydration value.

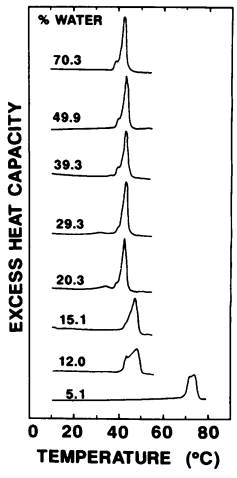

FIGURE 1 DSC heating curves of HPPC at different hydrations (wt.% water). Heating rate 5°C/min.

RESULTS

Scanning calorimetry of hydrated HPPC

DSC heating scans were recorded at eight different hydration values in the range 5.1–70.3 wt% H_2O. Heating curves following an initial heating and cooling cycle were recorded at 5°C/min and are shown in Fig. 1. At lower hydrations HPPC exhibits a complex transition. This complex transition has a prominent high temperature maximum with a second, less prominent maximum at a slightly lower temperature. As the hydration increases, the lower temperature peak decreases in enthalpy relative to that of the high temperature peak, until at hydrations >15 wt% H_2O only a low enthalpy shoulder on the main transition is observed.[1]

The thermodynamic behavior of HPPC is summarized in Fig. 2. At 5.1% H_2O HPPC has a transition temperature T_m of 69°C, a transition enthalpy (ΔH) of 7.7 kcal/mol, and a transition entropy (ΔS) change of 23 e.u. With increasing hydration, the transition temperature decreases, reaching a constant value of 40°C for hydration values $\gtrsim$20%. With increasing hydration, ΔH and ΔS increase, reaching limiting values of 10.1 kcal/mol and 32 e.u., respectively. For complex transitions the temperature of the highest peak is plotted in Fig. 2 *A*; to calculate enthalpies of complex transitions the area under the entire transition was used (Fig. 2 *B*).

X-Ray diffraction of hydrated HPPC

X-Ray diffraction patterns were recorded for hydrated HPPC at temperatures below T_m in the gel phase and above T_m in the liquid crystalline phase. Fig. 3 shows representative diffraction patterns for HPPC at 27.6 and 52.0% hydration, at 20° and 50°C. The low temperature diffraction patterns are typical of lipids in the gel phase. The strong wide angle reflections at 1/4.14 and 1/4.02 $Å^{-1}$ (*arrows*, Fig. 3 *A*) for HPPC at 27.6% hydration are characteristic of a gel phase with pseudo-hexagonal chain packing. At 27.6 wt% water the low angle region clearly shows lamellar reflections $1 < h < 6$ corresponding to a

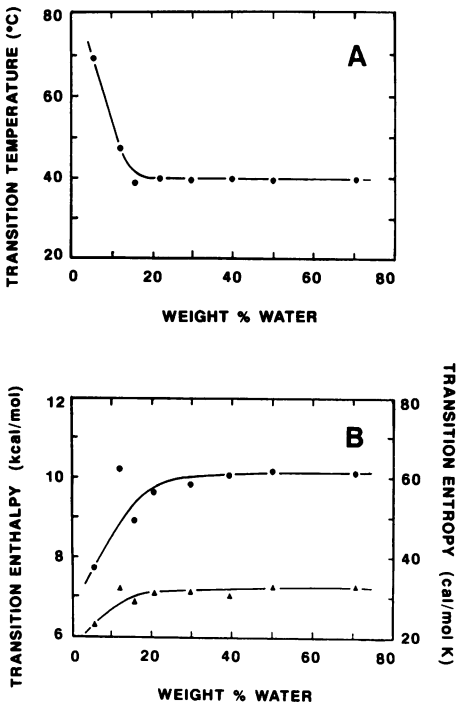

FIGURE 2 Hydration dependence of transition temperature (*A*) and transition enthalpy (●) and entropy (▲) (*B*) for HPPC.

bilayer periodicity, d = 59.1 Å, typical of a bilayer structure. The low angle region of the more hydrated (52.0 wt% H_2O) sample is unusual. More hydrated bilayers are expected to show an increase or no change in lamellar periodicity compared with less hydrated bilayers. However, the lamellar reflections $1 < h < 5$ correspond to a smaller bilayer periodicity, d = 46.3 Å. This small lamellar periodicity together with the single wide angle reflection at 1/4.00 $Å^{-1}$ (*arrow*, Fig. 3 *C*) is suggestive of an interdigitated bilayer structure as reported previously (Ruocco et al., 1985; Kim et al., 1987*a*). In contrast, at 50°C, at 27.6 and 52.0% H_2O, HPPC shows low angle lamellar periodicities of 51.8 and 60.3 Å, respectively. At both hydrations a diffuse wide angle reflection at 1/4.5 $Å^{-1}$ (*arrows*, Fig. 3, *B* and *D*) is obtained, characteristic of the melted-chain, liquid-crystalline L_α phase.

X-Ray diffraction data were recorded at 20° and 50°C from HPPC at 13 different hydrations varying from 11.8% H_2O to 52.0% H_2O. As shown in Fig. 4 *B*, HPPC in the L_α phase at 50°C shows an increase in d as the hydration increases, until the hydration maximum of 38.6% H_2O, after which d remains constant with d = 61.0 Å. Using the method of Luzzati (1968), the calculated lipid layer thickness decreases from d_l = 45.1 Å at 11.8% H_2O until a limiting value of d_l = 36.1 Å is reached at ~30% H_2O; the interbilayer water layer thickness

[1]Due to its proximity to the main transition, we have not been able to define the structural changes associated with this pretransition. By analogy with our previous studies of DPPC (Janiak et al., 1976, 1979; Ruocco and Shipley, 1982*a*, *b*) and, particularly, DHPC (Kim et al., 1987*a*, *b*) it probably represents a transition from the interdigitated $L_{\beta'}$-phase to a noninterdigitated $P_{\beta'}$-phase. However, this remains to be established. Based on preliminary low temperature incubation studies of HPPC, the broad, weak peak at ~30°C exhibited by HPPC samples at intermediate hydrations (see Fig. 1) appears to be associated with the formation of ordered-chain subgel phases (Haas, N.S., and G.G. Shipley, unpublished observations).

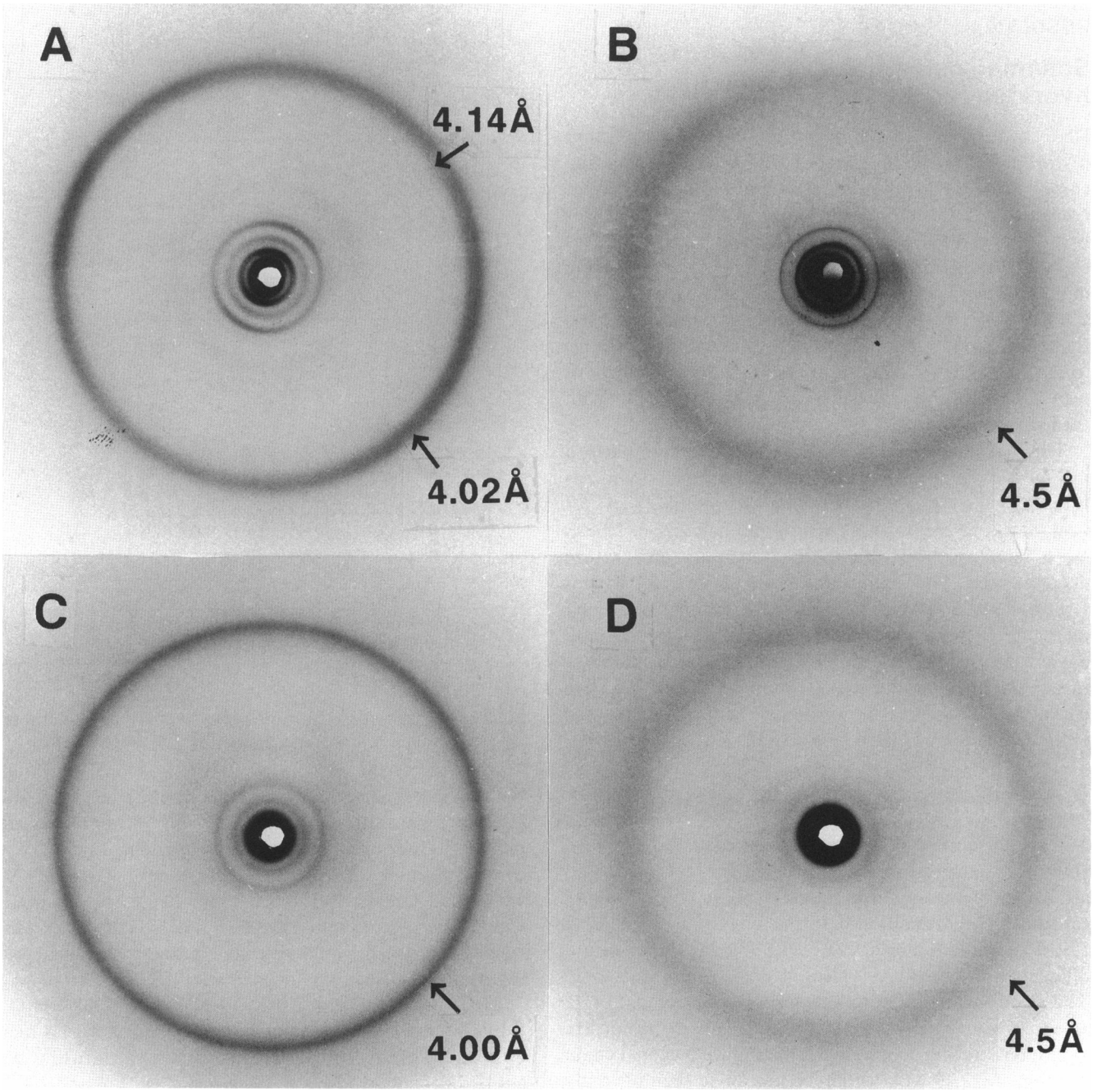

FIGURE 3 Representative x-ray diffraction patterns of hydrated HPPC. HPPC, 27.6% water at 20°C (*A*) and 50°C (*B*). HPPC, 52.0% water at 20°C (*C*) and 50°C (*D*).

increases with hydration from d_w = 6.5 Å at 11.8% H_2O to a limiting value of d_w = 24.5 Å at 38.6% H_2O. The calculated surface area per lipid molecule is 47.9 $Å^2$ at 11.8% H_2O, increasing to a limiting value of 58.6 $Å^2$ at ~30% H_2O.

The x-ray diffraction data of the gel phase of HPPC is shown in Fig. 4 *A*. The bilayer periodicity d increases from 60.0 Å at 11.8% H_2O to 62.5 Å at 31.0% H_2O. However, at 34.1% H_2O the bilayer periodicity decreases to 46.5 Å and remains essentially constant at d = 46.3 Å at higher hydrations. The calculated bilayer parameters d_l, d_w, and S, are shown in Fig. 4 *A*. Between 11.8% H_2O and 31.0% H_2O, d_l decreases from 52.0 to 43.1 Å; d_w increases from 8.0 to 19.3 Å; and S increases from 41.6 to

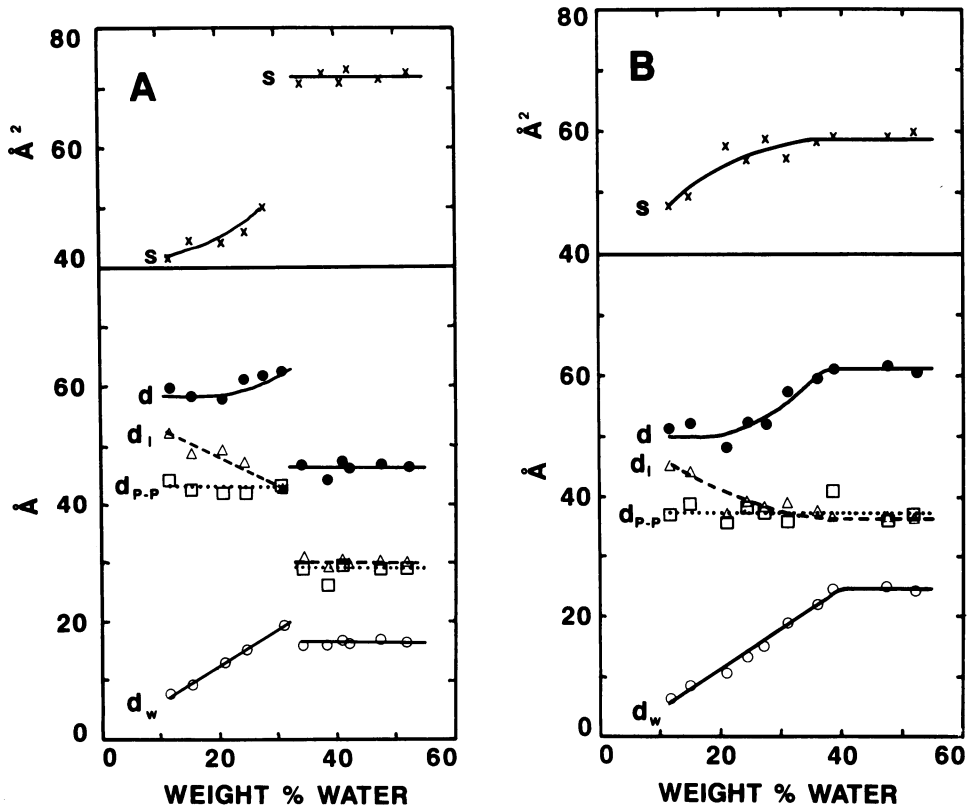

FIGURE 4 Structural parameters of HPPC as a function of hydration at 20°C (*A*) and 50°C (*B*): (●) bilayer periodicity *d*; (△) lipid thickness d_l; (○) water thickness d_w; (x) surface area per HPPC molecule *S*; (□) bilayer thickness d_{p-p}.

50.2 Å². At hydrations > 34.1%, the following average values are obtained: d = 46.3 Å, d_l = 29.9 Å; d_w = 16.3 Å, and S = 72.0 Å², assuming the hydration value is 34.1%. Obviously d , d_l, d_w, and S show major discontinuities between 31.0 and 34.1% H_2O (see Fig. 4 *A*).

The intensities of the low angle reflections $I(s)$ were measured and separated into three groups. These are a high temperature (50°C) L_α phase; a low temperature (20°C), low hydration $L_{\beta'}$ bilayer gel phase; and a low temperature (20°C), high hydration L_β interdigitated monolayer gel phase. Low hydrations are 11.8–31.0% H_2O, high hydrations are those above and including 34.1% H_2O. The normalized intensities $I(s)$ were corrected for Lorentz and polarization factors and converted to obtain $F(s)$. The amplitudes $F(s)$ were plotted as a function of reciprocal space coordinate s (= 2 sin θ/λ) as shown in Fig. 5. The nodes are suggested by the amplitude data and confirmed by application of the Shannon sampling theorem (Shannon, 1949). Based on the continuous $F(s)$ curve calculated using the Shannon sampling theorem for the $F(s)$ data for the 20.9% H_2O sample at 20°C (Fig. 5 *A*), nodes occur at s = (0.007, 0.041, 0.059, and 0.087 Å^{-1}) and the phasing for the observed amplitudes for h = 1–6 is −, −, +, −, 0, +; for the higher hydration series the continuous transform for the 34.1% H_2O sample and 20°C (Fig. 5 *B*) shows nodes at s = (0.014, 0.052, and 0.087 Å^{-1}) and the phasing for h = 1–5 is −, −, +, 0, −. For the 31.0% H_2O sample at 50°C (Fig. 5 *C*), the nodes occur at s = (0.008, 0.045, 0.070, 0.104 Å^{-1}), and the phasing for h = 1–5 is −, −, +, 0, −.

Representative electron density profiles $\rho(X)$ are shown in Fig. 6. At 50°C (Fig. 6 *B*) all profiles show a well-defined trough at X = 0 Å corresponding to the center of the bilayer and two high electron density peaks at X = ±18.5 Å defining the location of the electron-rich phosphate groups of HPPC. The separation of these two peaks provides an additional measure of the bilayer thickness d_{p-p} which, for the L_α phase at 50°C, stays essentially constant, 37 Å, as the hydration varies. Values of d_{p-p} are plotted as a function of hydration in Fig. 4 *B*. At 20°C, between 11.8% H_2O and 31.0% H_2O (Fig. 6 *A*, *bottom*), the profile is typical of that of a bilayer structure, once again showing a pronounced trough at X = 0 and electron-rich peaks at X = ±21.5 Å, corresponding to a bilayer thickness d_{p-p} = 43 Å (see also Fig. 4 *A*). However, at hydrations > 34.1% marked changes in $\rho(X)$ are observed (Fig. 6 *A*, *top*). Although the electron-rich peaks corresponding to the polar groups are still present

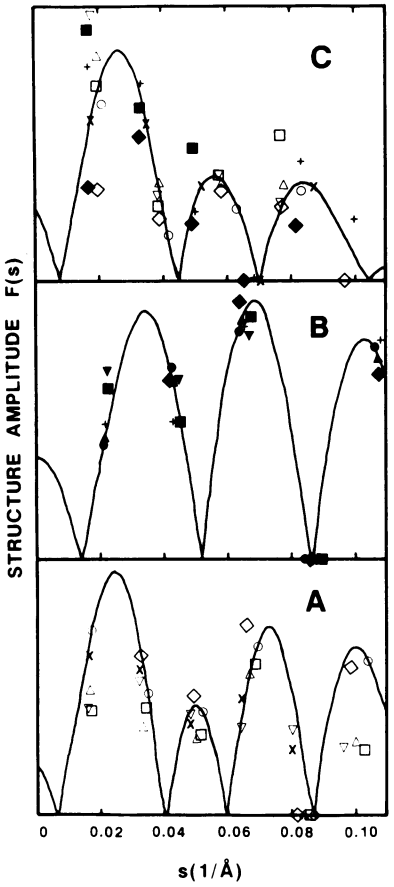

FIGURE 5 Structure amplitudes of HPPC bilayers. (*A*) 20°C, hydrations ≤31.0% water: (△) 11.8% water; (□) 15.3% water; (○) 20.9 water; (▽) 24.4% water; (◇) 27.6% water; (x) 31.0% water. (*B*) 20°C, hydrations ≥34.1% water: (▲) 34.1% water; (■) 38.6% water; (●) 41.0% water; (▼) 42.2% water; (◆) 47.4% water, (+) 52.0% water. (*C*) 50°C, all hydrations: (△) 11.8% water, (□) 15.3% water; (○) 20.9% water; (▽) 24.4% water; (◇) 27.6% water; (x) 31.0% water; (■) 38.6% water; (◆) 47.4% water; (+) 52.0% water. Continuous $F(S)$ curves calculated using the Shannon sampling theorem are shown for the following hydrations: (*A*) 20.9% water; (*B*) 34.1% water; (*C*) 31.0% water.

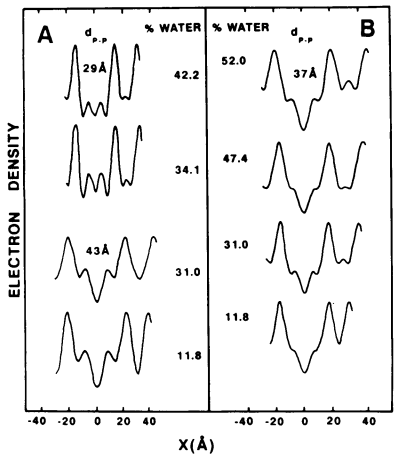

FIGURE 6 Representative electron density profiles $\rho(X)$ for hydrated HPPC at 20°C (*A*) and 50°C (*B*).

at $X = \pm 14.5$ Å, their separation has decreased to 29 Å and the trough in the center of the bilayer is no longer present. This small bilayer thickness $d_{p\text{-}p} = 29$ Å and the absence of the central trough is a characteristic of an interdigitated lipid phase.

DISCUSSION

A variety of physical methods have been used to study the ester-linked DPPC and all of its phases have been well characterized (see, for example, Chapman et al., 1967; Tardieu et al., 1973; Janiak et al., 1976, 1979; Chen et al., 1980; Füldner, 1981; and Ruocco and Shipley, 1982*a*, *b*). Upon heating DPPC, its crystalline (L_c) phase converts to gel ($L_{\beta'}$), rippled gel ($P_{\beta'}$), and liquid-crystalline (L_α) bilayer structures. These structures differ in chain packing and chain tilt, but all form well-defined bilayer structures.

The diether-linked phosphatidylcholine DHPC exhibits its calorimetric behavior similar to DPPC, but exhibits some marked hydration-dependent structural differences in the gel phase (Ruocco et al., 1985; Kim et al., 1987*a*; Laggner et al., 1987). At low hydrations ($\lesssim$30% H_2O) DHPC exhibits structural parameters nearly identical to DPPC, indicating that both DHPC and DPPC form the conventional $L_{\beta'}$ bilayer gel phase. However, for DHPC a major structural change occurs at 30 wt% water, highlighted by a major decrease in the bilayer periodicity, *d*. Analysis of the structural parameters, electron density profiles, and spectroscopic data indicate formation of a chain-interdigitated bilayer structure. Thus, alteration of the mode of chain linkage to the glycerol moiety from ester-to-ether linkage at both the *sn*-1 and *sn*-2 positions results in a major structural alteration in the gel phase. To gain more insight into the molecular features responsible for chain interdigitation of phosphatidylcholines, the present study of HPPC with both ether-linked (at the *sn*-1 position) and ester-linked (*sn*-2) chains was reported. The results show that the presence of an ether linkage in *only* the *sn*-1 position of the PC is sufficient to permit a similar hydration-dependent, chain-interdigitation process as that shown previously for DHPC.

At temperatures below the main transition and at low hydrations, HPPC has a lamellar periodicity, bilayer thickness, and electron density profile characteristic of a bilayer gel phase. In the gel phase, as hydration increases, the lamellar periodicity increases to a maximum of 62.5 Å

at 31.0% H_2O, then undergoes a sharp decrease, to 46.5 Å at 34.1% H_2O. At >34.1% H_2O the electron density profiles show an absence of the central methyl trough, as well as a decrease in $d_{p\text{-}p}$ (29 Å). They show a decrease in the calculated lipid layer thickness from 43 to 30 Å, and an increase in area per lipid molecule from 50 to 72 $Å^2$ at this hydration-dependent transition. Thus, HPPC in the gel phase exhibits an identical structural transition (bilayer ⟹ interdigitated bilayer) as DHPC, with chain interdigitation occurring at ~30 wt% water (Fig. 7). While the calorimetric behavior of all DPPC, HPPC, and DHPC are similar,[2] the hydration-dependent structural behavior of DHPC and HPPC in the gel phase are very similar and both display marked differences to DPPC.

A variety of factors have been shown to cause chain interdigitation in phospholipid bilayers. For PC, these include alteration of the molecular geometry as in β-DPPC (Serrallach et al., 1983), removal of the *sn*-2 chain to produce lyso-PC (Mattai and Shipley, 1986), and for DPPC, increased pressure (Braganza and Worcester, 1986), and addition of solutes such as alcohols (Simon and McIntosh, 1984; McDaniel et al., 1983) and surface active drugs (McIntosh et al., 1983). It is clear that many of these manipulations produce alterations at the critical lipid chain–aqueous layer interface. The comparative studies of DPPC, DHPC, and HPPC in which replacement of ester linkages with ether linkages (replacement at the *sn*-1 position alone being sufficient, as in HPPC) leads to chain interdigitation, add to the argument that molecular alterations at this interfacial region are critical. It remains to be seen what combination of changes in molecular conformation, molecular packing, hydrogen bonding, etc., at this interfacial region trigger the bilayer-interdigitated bilayer transition. Further, studies involving spectroscopic methods (NMR, FTIR, Raman spectroscopy) should be able to address these issues directly. Our own approach will be to examine the structural behavior of the positional isomer of HPPC, i.e., 1-palmitoyl 2-hexadecyl PC; this should allow us to define whether the position of the ether linkage at *sn*-1 or *sn*-2 is critical in allowing the chain-interdigitated structures to form.

[2]By deconvolution, we estimate the pretransition enthalpy of HPPC at full hydration to be ~1 kcal/mol, a value similar to that of the pretransitions of DPPC (Ruocco and Shipley, 1982*a*, *b*) and DHPC (Kim et al., 1987*a*, *b*). This results in an enthalpy of ~9 kcal/mol for the chain-melting transition, a value in reasonable agreement with that of the chain-melting transition of both DPPC (~8 kcal/mol) and DHPC (~9 kcal/mol). This suggests that similar structural changes are associated with the chain-melting transition of HPPC, DHPC, and DPPC, i.e., $P_{\beta'} \rightarrow L_\alpha$, and that, as observed previously with DHPC (Kim et al., 1987*a*, *b*) the interdigitated L_β-phase of HPPC converts to a noninterdigitated $P_{\beta'}$-phase at the pretransition.

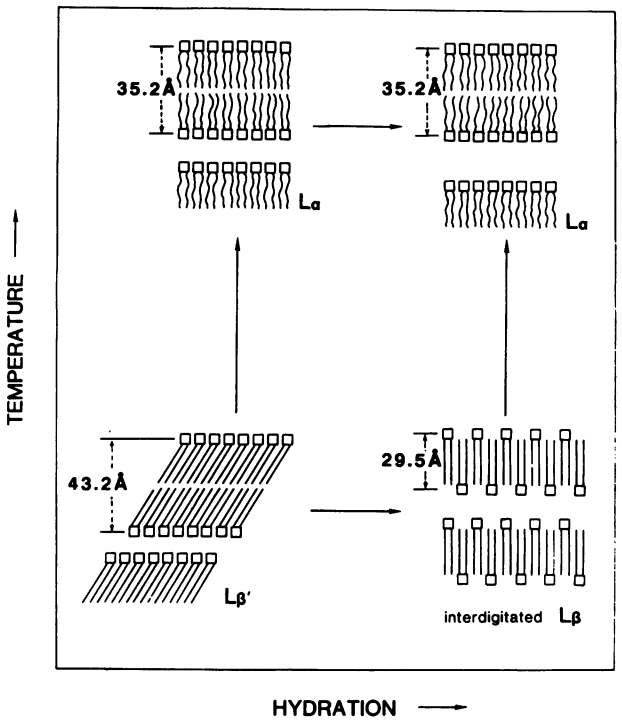

FIGURE 7 Structural behavior of HPPC as a function of hydration and temperature. It should be noted that at ≥30% water, the interdigitated L_β-phase of HPPC actually goes through an intermediate phase (perhaps ($P_{\beta'}$) associated with the pretransition before formation of the L_α-phase.

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