FT-IR and NMR Studies on the Conformational and Structural Properties of 1,2-Dipalmitoyl-*sn*-glycero-3-phosphatidyloligoglycerols

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The conformational behavior of membranes derived from a new class of phospholipids, 1,2-dipalmitoyl-*sn*-glycero-phosphatidyloligoglycerols $DPPG_x$, is studied for the first time by FT-IR spectroscopy in the liquid crystalline and gel state. The phospholipids examined here are characterized by oligoglycerol chains of variable length in the headgroup, ranging from one ($x = 1$, $DPPG_1 = DPPG$) to four glycerol units ($x = 4$, $DPPG_4$). The transition from the gellike to the liquid crystalline phase is monitored via CH_2 and CD_2 stretching bands as well as CH_2 wagging band progressions. CH_2 wagging bands are used to determine the relative amounts, i.e., integral values over the whole chains, of kink/gauche-trans-gauche, double gauche, and end gauche conformers in the acyl chain region. Information about the absolute amount of gauche conformers at a specific chain segment is obtained by a quantitative analysis of the CD_2 rocking band region for phospholipid samples with selectively deuterated acyl chains. These latter data are combined with the results from an independent ^2H NMR study on the same compounds, which allows a distinction between the overall chain order and the local conformational order of the phospholipid chains. In addition, results from solid state ^{31}P and ^{13}C NMR studies are presented which provide additional support for the conclusions based on the FT-IR data. The present work clearly shows that the conformational and structural properties strongly depend on the headgroup structure and hydrophilicity, sample composition as well as sample temperature. The derived data are discussed by considering related phospholipid systems, such as DPPC, to demonstrate the particular impact of the headgroup length and hydrophilicity on the ordering characteristics of the acyl chains.

Introduction

In recent years spectroscopic techniques, such as NMR[1−3] and IR spectroscopy,[4−7] have been extensively used in the field of biological membranes. In this connection it has been demonstrated that IR spectroscopy is particularly suitable to monitor the conformational properties in the acyl chain region of the phospholipid molecules along with their changes as a function of external parameters, such as temperature or sample composition. Here, several conformation sensitive vibrational bands are accessible, from which the desired information about the conformational state can be obtained.[4−8]

On the basis of these former IR investigations in the present work data will be provided, that are derived from the analysis of the following well-known conformation sensitive IR regions: the symmetric CH_2 stretching band at around 2850 cm^{-1} and antisymmetric CD_2 stretching band at around 2172 cm^{-1}, from which information about conformational order−disorder transitions can be received;[9] the CH_2 wagging progressions between 1100 and 1380 cm^{-1}, which reflect changes in the amount of all-trans chains upon temperature variation;[10,11] the wagging band region between 1315 and 1400 cm^{-1}, which yield quantitative information about the integral amount of various gauche conformers (kink/gtg (gauche-trans-gauche), double gauche (dg), and end gauche (eg) conformers) along the aliphatic chains in the liquid crystalline state;[12,13] the CD_2 rocking bands in the region from 620 to 670 cm^{-1} from which, using selectively deuterated compounds, data about the local conformational changes at a specific CH_2 segment as a function of temperature can be obtained.[14−17] These data can be further combined with those from supplementary ^2H NMR experiments. On this basis it is possible to distinguish between conformational (or segmental) order and overall chain order of the phospholipid molecules, as already demonstrated during an earlier work on other phospholipids.[16]

The variable temperature FT-IR and NMR investigations presented here were performed on a new class of model membranes that differ from the biologically relevant and widely studied[18,19] 1,2-dipalmitoyl-*sn*-glycero-phosphatidylglycerol DPPG (in the following: $DPPG_1$) in the length of the glycerol unit of the headgroup. Thus, we examine 1,2-dipalmitoyl-*sn*-glycero-phosphatidyl-oligoglycerols $DPPG_x$ ($x = 1-4$) with one to four glycerol units attached to the phosphate unit[20] (Figure 1). Like the "Stealth phospholipids",[21] liposomes on the basis of these $DPPG_x$ show a prolonged in *vivo* blood circulation time of up to 24 h[20] and are therefore of great interest as drug delivery systems. It has been demonstrated that this ability is more pronounced in $DPPG_3$ and $DPPG_4$ than that for membrane systems bearing two glycerol units ($DPPG_2$) or one glycerol unit ($DPPG_1$), respectively. It is therefore of interest in which manner the length and hydrophilicity of the headgroup affects the conformation and structure of the lipid and how that, in return, alters the stability of these systems under physiological conditions.

A first attempt to characterize this new class of phospholipid systems on a molecular level is the combined IR and NMR investigation presented in this work. The studies have been performed on fully hydrated bilayers containing either pure lipids or lipid/cholesterol mixtures, with a 40% molar fraction of

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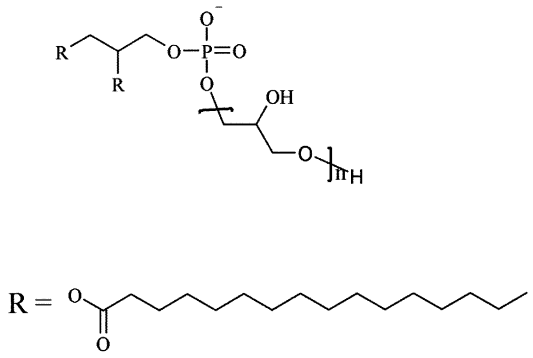

n = 1: 1,2-dipalmitoyl-*sn*-glycero-3-phosphatidylglycerol ($DPPG_1$)
n = 2: 1,2-dipalmitoyl-*sn*-glycero-3-phosphatidyldiglycerol ($DPPG_2$)
n = 3: 1,2-dipalmitoyl-*sn*-glycero-3-phosphatidyltriglycerol ($DPPG_3$)
n = 4: 1,2-dipalmitoyl-*sn*-glycero-3-phosphatidyltetraglycerol ($DPPG_4$)

Figure 1. Chemical structures of the phospholipid systems used in this work.

cholesterol. In the case of $DPPG_2$ a selectively deuterated sample (deuteration at carbon C-6) was available which was examined by both FT-IR and ^2H NMR spectroscopy. Apart from the analysis of the above-mentioned conformation sensitive IR bands and the ^2H NMR data, results from preliminary solid state ^{31}P NMR and ^{13}C MAS NMR studies will be presented. In the discussion we will compare the influence of the length of the glycerol unit as well as sample temperature and cholesterol content on the conformational and ordering behavior of 1,2-dipalmitoyl-*sn*-glycero-phosphatidyl-oligoglycerols. In addition, the data of the present systems will be contrasted with those from "classical" phospholipids, such as DPPC or DMPC.

Experimental Section

(a) Materials. The present spectroscopic investigations have been performed on nondeuterated ($DPPG_x$, DPPC) as well as on selectively deuterated phospholipids ($DPPG_2$, DPPC). Selectively deuterated lipids were obtained by the attachment of hexadecanoic acid-6,6-d_2 that also was synthesized during the present work. Likewise, for the synthesis of $DPPG_x$ the preparation of several oligoglycerols was required. In the final step the deuterated or nondeuterated hexadecanoic acid was coupled with choline or an oligoglycerol headgroup to give DPPC and $DPPG_x$, respectively. In the following, the various synthesis steps will be described briefly.

Synthesis of 6,6-d_2-Hexadecanoic Acid (Palmitic Acid).[22−24] The initial step of this synthesis is the preparation of the corresponding selectively deuterated 1-undecanol by reduction of the appropriate ester or carboxylic acid with $LiAlD_4$ in dry THF. The next step includes the bromination of the alcohol. After that, the Grignard agent of the resulting bromide was coupled with the magnesium bromide of the 5-bromo-valeric acid in the presence of stoichiometric amounts of $CuCl_2$/LiCl catalyst to give the desired selectively deuterated palmitic acid.

Synthesis of 1,2-Dipalmitoyl-sn-glycero-phosphatidylcholine.[25] In a first step the palmitic acid is transferred to the corresponding acid imidazolide using carbodiimidazolide (CDI; Fluka Chemie AG, Buchs, Germany). In the next step *sn*-glycero-3-phosphatidylcholine (GPC, Lukas Meyer GmbH, Hamburg, Germany) and 1,8-diazabicyclo-[5.4.0]-undec-7-ene (DBU, Fluka Chemie AG, Buchs, Germany) were added to the reaction mixture, which was stirred for 24 h, and the solvent was removed. The resulting lipid was purified via recrystallization in dry acetone.

Synthesis of Oligoglycerols and 1,2-Dipalmitoyl-sn-glycero-phosphatidyloligoglycerols.[26−29] The synthetic routes for the preparation of the different phosphatityloligoglycerols have been described earlier and follow the synthesis of phosphatidylglycerol.[26] To obtain the phosphatidyloligoglycerols, in the phosphorylation step isopropylidene glycerol was exchanged for oligoglycerols with the respective protecting groups. For instance 2-O-tetrahydropyranyl-glycero-1.3′-(1′.2′-isopropylidene)-glycerol was used for the preparation of phosphatidyl-diglycerol, (2-tetrahydropyranyl)-glycero-1.3′-O−(2′-O-tetrahydropyranyl)-1′.3″-O−(1″.2″-isopropylidene)-glycerol for the preparation of phosphatidyl-triglycerol and (2-tetrahydropyranyl)-glycero-1.3′-O-(2′-O-tetrahydropyranyl)-1′.3″-O−(2″-O-tetrahydropyranyl)-1″.3‴-O−(1‴.2‴-isopropylidene)-glycerol for the preparation of phosphatidyl-tetraglycerol. The synthesis of these protected oligoglycerols will be described in detail elsewhere.[27]

The purity of the described products is shown by thin layer chromatography. 1.2-Dipalmitoyl-*sn*-glycero-3-phospho-glycerol ($DPPG_1$) does not contain $DPPG_2$, $DPPG_3$ or $DPPG_4$. Correspondingly, 1.2-Dipalmitoyl-*sn*-glycero-3-phospho-diglycerol ($DPPG_2$) is free from $DPPG_1$, $DPPG_3$, or $DPPG_4$, etc. On the bases of HPTLC data, with Rf-values of 0.65 for $DPPG_1$, 0.55 for $DPPG_2$, 0.45 for $DPPG_3$, and 0.35 for $DPPG_4$ in the solvent system $CHCl_3$:CH_3OH:CH_3COOH:H_2O 100:60:20:5 (per volume), the purity of the four phosphatidyloligoglycerols $DPPG_1$, $DPPG_2$, $DPPG_3$, and $DPPG_4$ is better than 99%. This is much better than for the products described earlier by Maryama et al.[28] which have been obtained by transesterification of phosphatitylcholines with phospholipase D in the presence of impure, technical oligoglycerols.[29]

(b) Sample Preparation. The multilamellar, cholesterol free lipid dispersions were prepared by the addition of 15 mL deuterium depleted water to 10 mg of lipid to ensure a complete hydration of the glycerol headgroup. Homogenization was obtained by repeated freeze−thawing, centrifuging, vortexing, and incubation for about 3 h at temperatures well above the main transition of the membrane. The cholesterol containing dispersions were prepared by dissolving the appropriate amounts of lipid and cholesterol (40% molar ratio) in a small quantity of freshly distilled chloroform. The solvent was removed in a dry nitrogen stream, followed by evaporation in a vacuum for a few hours. The subsequent hydration and freeze−thawing steps were carried out as described above.

(c) Variable Temperature FT-IR Measurements. The nondeuterated phospholipid dispersions were measured using CaF_2 windows and a 25 μm zinc spacer, whereas for the detection of CD_2 rocking bands KRS5 windows and spacer with 6 μm thickness were used. All IR spectra were recorded on a Nicolet Nexus 470 FT-IR spectrometer (Nicolet, Madison, WI) using the OMNIC E. S. P. 5.1 software (Nicolet, Madison, WI) equipped with a DTGS detector. The phospholipid samples were thermostated in a variable temperature transmission cell (L. O. T.-Oriel GmbH, Langenberg, Germany), equipped with NaCl windows. The sample temperature was regulated with an automatic temperature control unit. The temperature accuracy was estimated to ±0.5 °C. Typically, 1024 interferograms, covering a spectral range from 4000 to 400 cm^{-1} at a resolution of 2 cm^{-1}, were collected in the temperature range between 223 and 353 K, apodized with a triangular function and Fourier transformed after one zero filling. Correction for background absorption was done by recording the background spectrum of the empty cell (measured with twice the number of interferograms as that used for the sample). The background spectrum was automatically subtracted from the subsequent spectra of

the phospholipid samples. Data from three independent samples were acquired at all temperatures for all samples studied. The whole series of variable temperature FT-IR spectra was measured twice for each sample. The data analysis is thus based on an average of six measurements.

(d) IR Data Analysis. The processing of the CH_2 and CD_2 stretching vibrations has been carried out using the OMNIC E. S. P. 5.1 software (Nicolet, Madison, WI). The analysis of the CH_2 wagging modes, CH_2 wagging progressions, and CD_2 rocking bands was performed on the Grams/32 software (Galactic, Salem, NH).

The frequencies of the CH_2 and CD_2 stretching bands were determined from the interpolated zero crossing of the first derivative spectra. For the analysis of the CH_2 wagging band progressions[10,11] it was necessary to subtract the underlying PO_2^- antisymmetric stretching band at around 1230 cm^{-1}. Therefore, an IR spectrum at the highest available temperature (here 353 K) was recorded and subtracted from the sample spectrum with progressions. Afterward, a flattened baseline was generated in the appropriate spectral region by selecting appropriate spectral minima. All progression intensities, the sum of the $k = 1$ to $k = 6$ components, were further normalized with respect to the progression band intensity obtained for the sample at 223 K.

For the analysis of the CH_2 wagging band region (1315−1400 cm^{-1}) a linear baseline correction was applied and it was made sure, that for each spectrum the same baseline points were taken. Afterward, the spectra were iterated using five to six vibration bands with Gaussian and Lorentzian contributions of variable amount. Their initial positions were 1378 cm^{-1} (symmetric methyl deformation mode), 1368 cm^{-1} (kink and gtg sequences), 1354 cm^{-1} (double gauche sequences), and 1342 cm^{-1} (end gauche sequences). In addition, we had to account for two other bands at 1328 cm^{-1} (a δ(O−H) mode from the glycerol moieties) and at 1385 cm^{-1} (band not further specified) in cholesterol rich samples. During the following least-squares fit analysis the band intensities and widths were varied independently, whereas the band positions were restricted to ±3 cm^{-1} of the values given above. The obtained integrated intensities of the CH_2 wagging bands were normalized with respect to the methyl deformation band at 1378 cm^{-1}. The amounts of the various gauche conformers were determined according to the procedure by Senak et al.,[13] which is based on reference measurements on *n*-alkanes and a theoretical approach using the rotational isomeric state (RIS) model.[30] It is important to point out that, unlike free alkanes, a phospholipid contains just one methyl group per fatty acid chain. The estimated uncertainty for the kink/*gtg* and double gauche sequence is 10−15%. Due to the partly overlap by the δ(O−H) mode, the uncertainty for the end gauche conformer is higher and estimated to 25−35%, depending on the length of the oligoglycerol chain.

For the processing of the CD_2 rocking bands we followed the same procedure as described for the CH_2 wagging bands. Again, care was taken that for each spectrum the same baseline correction has been applied. For the final iteration of the processed FT-IR spectra three bands were initially positioned, namely at 622 cm^{-1} (*tt* segments), at 646 cm^{-1} (ttgg or g′tgg) and at 651 cm^{-1} (g′tgt, ttgt). From the intensities of the two latter bands the percentage of gauche bonds at a specific CD_2 segment at a given temperature was calculated by using eq 1:[15]

$$\%\ \text{gauche} = \frac{I[651\ \text{cm}^{-1}] + I[646\ \text{cm}^{-1}]}{2I[622\ \text{cm}^{-1}] + I[651\ \text{cm}^{-1}] + I[646\ \text{cm}^{-1}]} \quad (1)$$

(e) NMR Measurements. ^2H NMR and ^{31}P NMR measurements were carried out on a CXP 300 NMR spectrometer (Bruker, Karlsruhe, Germany) at resonance frequencies of 46.01 and 121.5 MHz, respectively. The spectrometer was controlled by a MacSpect unit (Tecmag, Houston, TX) and was equipped with a 1 kW linear amplifier (LPI-10H, ENI, Rochester, NY). Measurements were done with a commercial double resonance probehead (Bruker, Karlsruhe, Germany). ^{31}P NMR measurements were performed with broadband proton decoupling during data acquisition (decoupling power: 20 W). The B_1 field strength was adjusted to give a $\pi/2$-pulse length of 2 μs for the ^2H and 3 μs for ^{31}P.

^{13}C MAS NMR measurements were carried out on a MSL 300 NMR spectrometer (Bruker, Karlsruhe, Germany), equipped with a 1 kW linear amplifier stage (LPPA-13010, Dressler, Stollberg/Germany), at a ^{13}C resonance frequency of 75.47 MHz. ^{13}C MAS NMR measurements were done under cross-polarization conditions using a commercial 4 mm MAS probehead (Bruker, Karlsruhe, Germany). The sample spinning frequency was 2.5 kHz. The B_1 field strengths were adjusted to give a $\pi/2$-pulse of 5.5 μs.

(f) Differential Scanning Calorimetry (DSC). The phase behavior of the various samples was examined by differential scanning calorimetry, using a Netzsch DSC 204 calorimeter (Selb, Germany). Typically, a temperature range between 240 and 330 K at heating rates of 2 K min^{-1} was covered.

Results and Discussion

In the following we present variable temperature FT-IR and NMR studies on model membranes based on the phospholipids $DPPG_1$, $DPPG_2$, $DPPG_3$, and $DPPG_4$, which are distinguished by the length of the glycerol moieties in the headgroup. It should be noted that, unlike the situation for phosphatidylcholines,[9−13] the number of FT-IR studies on phosphatidylglycerols is very limited.[19,31] In fact, a comprehensive FT-IR investigation of phosphatidylglycerols, such as DPPG (=$DPPG_1$), including several conformation sensitive bands and CD_2-rocking bands, has not been reported so far. The same holds for the present systems with oligoglycerol units in the headgroup $DPPG_2$ to $DPPG_4$, whose structures are given in Figure 1. The present work thus comprises experiments on pure $DPPG_x$/water dispersions (water content: 150 wt %) as well as $DPPG_x$/cholesterol mixtures (content of cholesterol: 40 mol %). For comparison, the same IR and NMR experiments were also performed on DPPC bilayers. In the case of DPPC and $DPPG_2$ selectively deuterated samples (deuteration at carbon C-6) were available which were examined by both FT-IR and ^2H NMR spectroscopy.

(a) Calorimetry and Phase Behavior. Representative DSC curves of the present phospholipids are shown in Figure 2. The derived phase transitions are summarized in Table 1. It can be seen that for pure $DPPG_1$ (Figure 2a) a pretransition ($L_\beta \rightarrow P_{\beta'}$) occurs at 309 K,[19,32] which also is known for example for DMPC and DPPC bilayers.[32] The main transition to the liquid crystalline phase (L_α phase) is registered at 315 K, which also shows up in the case of the pure $DPPG_2$/water dispersion (Figure 2b). For pure membranes consisting of $DPPG_3$ (Figure 2c) or $DPPG_4$ (Figure 2d), apart from a "high temperature" transition at about 315 K, a second transition at 278 K is found (indicated by arrows) that is pretty close to the melting point of water. As before, the transition at higher temperature is attributed to the main transition from the gellike to the liquid-crystalline phase.[19] The observed slight decrease of the main transition by 4 K for $DPPG_4$ membranes as compared with $DPPG_1$ bilayers might be related to a decrease in packing density of the acyl chains in the same direction.

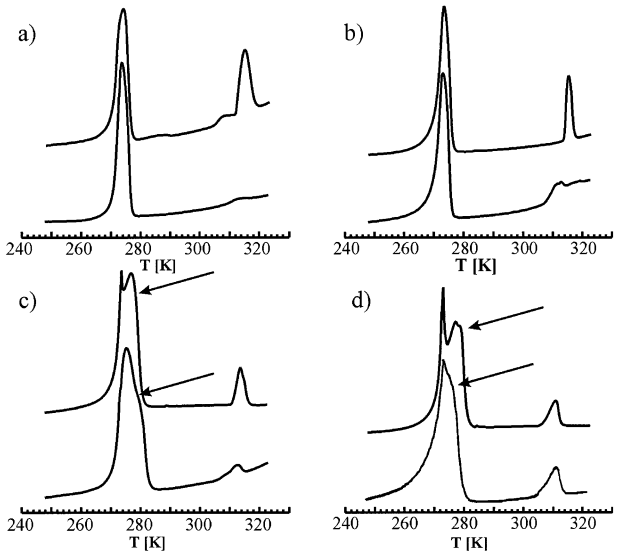

Figure 2. Experimental DSC curves between 240 and 330 K: (a) $DPPG_1$, (b) $DPPG_2$, (c) $DPPG_3$, and (d) $DPPG_4$ (top, pure lipid/water dispersion; bottom, sample with 40 mol % cholesterol). Arrows indicate additional phase transition (see text).

TABLE 1: Phase Transition Temperatures and Phase Assignments from Calorimetric and IR Studies on $DPPG_x$ (x = 1, 2, 3, and 4) Bilayers[a]

lipid	pure		40 mol % cholesterol	
$DPPG_1$	309 K	315 K (*313 K*)		
	L_β to $P_{\beta'}$	L_β to LC		
$DPPG_2$	315 K (*314 K*)			300−320 K (do.)
	L_β to LC			L_β to LC
$DPPG_3$	278 K	314K (*314 K*)	278 K	314 K (*310 K*)
	n. a.[b]	L_β to Q_α	n. a.	L_β to LC
$DPPG_4$	278 K	311 K (*310 K*)	280 K	311 K (*310 K*)
	n. a.	L_β to LC	n. a.	L_β to LC

[a] Data in brackets taken from the IR stretching data. [b] n.a.: not assigned.

For $DPPG_1$ membranes the addition of 40 mol % cholesterol, as in other phospholipids, such as DPPC or DMPC,[33] results in an almost complete suppression of the main transition, while for membranes from $DPPG_2$ a shift of the transition toward lower temperatures along with a broadening of the DSC transition can be observed. However, the addition of cholesterol does not alter the main transition in $DPPG_3$ and $DPPG_4$ membranes. For these latter systems the "low temperature" transition at 278 K is still visible, although being partially obscured by the transition due to the melting of the water.

At present, the origin of the low-temperature transition in the $DPPG_3$ and $DPPG_4$ samples is not understood. It might be speculated whether it is related to the melting of the oligoglycerol units or at least to some structural changes in the headgroup region, although further information on this topic is lacking. In this connection it is interesting to note that prolonged blood circulation times can be observed for lipid mixtures containing $DPPG_3$ and $DPPG_4$, while this effect is less pronounced for mixtures with $DPPG_2$ and completely absent for mixtures containing $DPPG_1$.[20]

(b) CH_2 Stretching Bands. The results from the analysis of the symmetric CH_2 stretching bands, referring to the pure lipid bilayers of $DPPG_x$ (upper graph) and the mixtures with cholesterol (lower graph), are summarized in Figure 3. It can be seen that, without exception, all $DPPG_x$/water dispersions exhibit a sudden band shift of the symmetric stretching absorption at the respective calorimetric main transitions. The

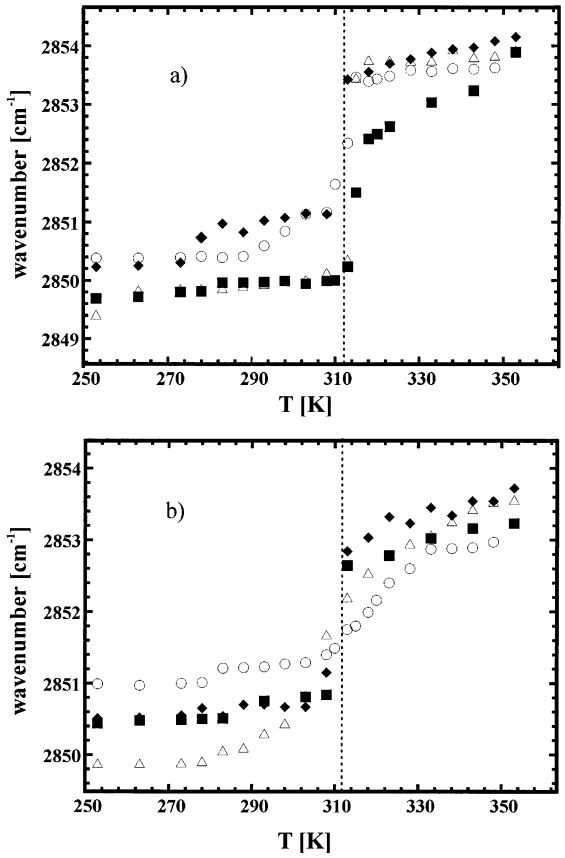

Figure 3. Position of symmetric CH_2 stretching bands of $DPPG_x$ samples (x = 1, 2, 3, and 4) as a function of temperature. Top: pure lipid/water dispersion. Bottom: samples containing 40 mol % cholesterol. (○) $DPPG_1$, (△) $DPPG_2$, (■) $DPPG_3$, and (◆) $DPPG_4$.

absorption wavenumbers fall in the range between 2850 cm^{-1} (gel phase) and 2854 cm^{-1} (liquid crystalline phase), which coincides with that from earlier work on related model membranes.[19,34] In the gel phase the acyl chains predominantly exist in the all-trans conformation which is responsible for the shift of the CH_2 stretching band toward low wavenumbers. The decrease of conformational order (i.e., increase of amount of gauche conformers) at the transition to the liquid crystalline phase is directly reflected by the pronounced shift of the CH_2 stretching band in the opposite direction, as shown earlier during investigations on pure hydrocarbons and other phospholipid membranes.[9,36] It should be emphasized that a similar shift is also observed for the antisymmetric stretching band (data not shown).

The absence of an additional shift or discontinuity at the calorimetric "low temperature" transition at 278 K for the $DPPG_3$ and $DPPG_4$ samples again supports the earlier assumption of a structural change that mainly involves the glycerol units in the headgroup. The hydrophobic part of the phospholipid molecules, i.e., the acyl chain region, therefore should remain unaffected, as indicated by the present FT-IR data. However, a final proof for this assumption has still to be found.

The data from the symmetric CH_2 stretching band analysis for the membranes containing 40 mol % cholesterol support the results from the aforementioned DSC measurements. Thus, for $DPPG_1$/cholesterol a gradual shift of the absorption band with increasing temperature can be found; a similar discontinuous change in the vicinity of the main transition of the pure phospholipid, as discussed above, now is completely missing. In addition, the overall shift of the absorption band is limited to only 2 cm^{-1} while in the pure $DPPG_1$/water dispersion a shift

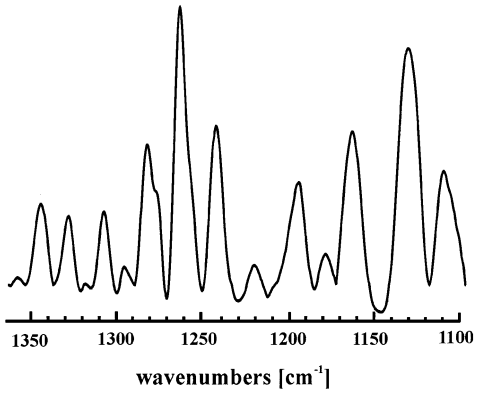

Figure 4. Experimental CH_2 wagging band progressions of pure $DPPG_1$ between 1360 and 1100 cm^{-1} at 298 K.

of 4 cm^{-1} has been found. Very similar results have been provided by former studies on DMPC or DPPC (own data from reference measurements on DPPC bilayers not shown), where after addition of cholesterol again a continuous shift for the CH_2 stretching band has been reported.[36,40]

For the $DPPG_2$/cholesterol sample the absorption frequency again changes gradually with temperature, however, exhibiting a larger overall shift than in the previous case. This is in line with the results from the calorimetric studies where the main transition is not completely suppressed. Rather, it is found to be smeared out over a temperature range of about 30 K. The graphs of the $DPPG_3$ and the $DPPG_4$/cholesterol mixtures are almost identical to those from the corresponding pure model membranes discussed above. Again, an abrupt band shift at the main transition is observed. This is in line with the calorimetric data where, unlike the situation for $DPPG_1$ and $DPPG_2$/cholesterol mixtures, the main transition remains unaffected by the addition of the steroid.

(c) CH_2 Wagging Band Progressions. It has been shown previously that a high conformational order in the hydrocarbon chain region causes a coupling of the methylene wagging modes.[10,11,35,37] The resulting progression of bands between 1150 and 1350 cm^{-1} can be described by the model of coupled harmonic oscillators. The intensity of the progression bands again can be used as a qualitative measure for the amount of all-trans chains in the sample. In Figure 4 representative progression bands for a pure $DPPG_1$/water dispersion at 298 K are shown. The existence of such progression bands clearly demonstrates that highly ordered fatty acid chains, being in an almost all-trans conformational state, prevail at temperatures well below the main transition. In Figure 5 these progression band intensities for the pure $DPPG_x$ membranes and the mixtures with cholesterol are plotted as a function of temperature.

As for the CH_2 stretching band data, the intensity of the progression bands can be used to monitor the change of the conformational properties in the vicinity of the main transition. In the present case it can be seen that the progression bands almost vanish at about 7 K above the main phase transition, reflecting the sudden increase of conformational disorder, along with a loss of the all-trans conformers, on going from gel to liquid crystalline phase. Again, the experimental curves clearly demonstrate that two kind of data sets can be distinguished, namely those from the samples with $DPPG_1$ and $DPPG_2$ on the one hand and from samples with $DPPG_3$ and $DPPG_4$ on the other hand. In the case of the pure lipid/water dispersions from $DPPG_3$ and $DPPG_4$, the conformational order starts to decrease at about 20 K lower than for the samples with $DPPG_1$ and $DPPG_2$. Thus, between 260 and 300 K for $DPPG_3$ and $DPPG_4$,

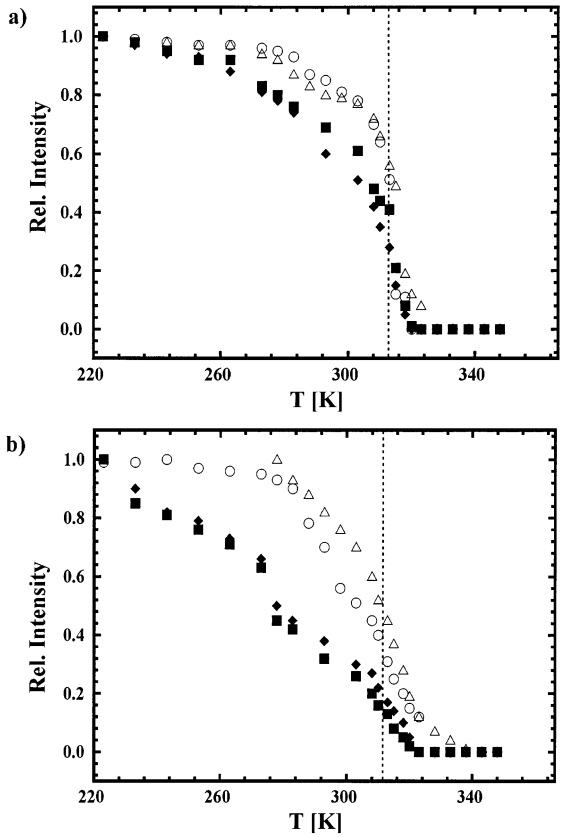

Figure 5. Experimental progression band intensities of $DPPG_x$ (x = 1, 2, 3, and 4) between 220 and 353 K. Top: pure lipid/water dispersion, bottom: samples containing 40 mol % cholesterol. (○) $DPPG_1$, (△) $DPPG_2$, (■) $DPPG_3$, and (◆) $DPPG_4$.

the intensity of the progression bands drops down by about 50%, whereas the lipids containing one or two glycerol groups show a decrease by only 20%. It should be recalled that a loss in progression band intensity of 50% corresponds to 5% gauche probability or 0.6 gauche conformers per chain.[10]

On a molecular level, the addition of cholesterol usually leads to an enhanced conformational order and to the formation of the so-called liquid ordered phase.[33] This also holds for the $DPPG_1$ and $DPPG_2$/cholesterol mixtures, as a finite progression band intensity can be found at even higher temperatures than for the corresponding lipid/cholesterol mixtures. In general, the presence of the steroid is accompanied by a more gradual change of the progression band intensities. Moreover, in the gel phase for the $DPPG_3$ and $DPPG_4$/cholesterol mixtures the experimental progression band intensities reflect a significantly lower amount of all-trans chains as compared with the samples without cholesterol. A reduction in intensity, although to a much smaller extent, also can be found for the $DPPG_1$ and $DPPG_2$/cholesterol mixtures, which again is very similar to the findings in DPPC or DMPC/cholesterol mixtures. Obviously, in the gel phase the impact on the amount of all-trans conformers by the addition of the steroid is much stronger for the higher oligoglycerols $DPPG_x$ (x = 3 and 4) than for their shorter counterparts with x = 1 and 2 or for other phospholipids bearing small headgroups.

(d) CH_2 Wagging Bands. Figure 6 presents FT-IR spectra for $DPPG_1$ (a), $DPPG_2$ (b), $DPPG_3$ (c), and $DPPG_4$ (d) above the main transition temperature in the liquid crystalline phase. For comparison a spectrum of DPPC (e) is also given. The spectral range from 1315 to 1395 cm^{-1} covers the conformation sensitive wagging mode region of the methylene groups. In the well-known case of DPPC[12] four absorption bands are observed.

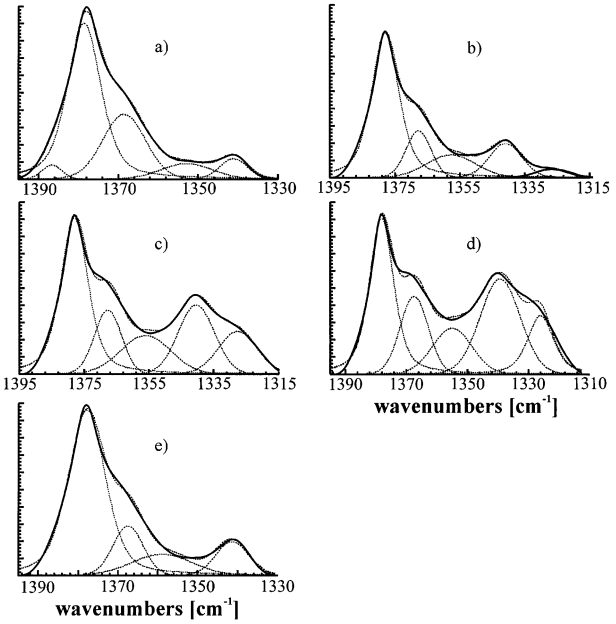

Figure 6. Experimental FT-IR spectra covering the CH_2 wagging band region (pure lipid/water dispersion): (a) $DPPG_1$, (b) $DPPG_2$, (c) $DPPG_3$, (d) $DPPG_4$, and (e) DPPC. Dotted lines: results from least-squares fit analysis.

The intense band near 1378 cm^{-1} arises from the methyl "umbrella" deformation mode and is used as an internal reference for the calibration of the other band intensities. At lower wavenumbers three CH_2 wagging bands, centered at 1368, 1354, and 1342 cm^{-1}, are visible that have been assigned to kink/gauche−trans-gauche (gtg), double gauche (gg), and end gauche (eg) sequences in the fatty acid chains. The relative amounts of these conformers (i.e., their integral values over the whole chain) are obtained via a curve fitting procedure, as described in the Experimental Section.

The corresponding analysis of the wagging band region of the oligoglycerols is complicated by an additional absorption band near 1330 cm^{-1}. It was assigned to the δ (O−H) mode,[38] which was verified by IR spectra of glycerol at different concentrations of water (data not shown). Moreover, the δ(O−H) band is shifted by the addition of 50 wt % water by five wavenumbers to 1335 cm^{-1}. It becomes a critical factor as the number of glycerol units, and therefore the amount of C−OH groups in the headgroup, increases from $DPPG_1$ to $DPPG_4$. Due to the overlap with the eg band at 1342 cm^{-1} it becomes increasingly difficult to separate these two bands and to derive the amount of eg conformers. The uncertainty for the amount of eg sequences in $DPPG_3$ and $DPPG_4$ therefore is estimated in this case to be between 25 and 35%.

To have a common basis for the comparison of the results from the oligoglycerols and DPPC, a complete analysis of the wagging band modes for the latter phospholipid was performed as well (data are given in Figure 7). In Figure 8 the amounts of eg, kink/gtg, and end gg conformers for $DPPG_x$ (left) and $DPPG_x$/cholesterol samples (right) are plotted as a function of temperature. In close analogy to the previous discussions of the calorimetric data, CH_2 stretching band and CH_2 wagging band progression data, the results from the wagging band analysis again imply a separation into two groups, namely, (i) $DPPG_1$ and $DPPG_2$ on one hand and (ii) $DPPG_3$ and $DPPG_4$ on the other hand.

In the case of the pure lipid bilayers, we get a similar amount of gg conformers for $DPPG_1$ and DPPC, ranging from 0.21 to 0.37 for the anionic lipid and 0.29 to 0.4 for the DPPC

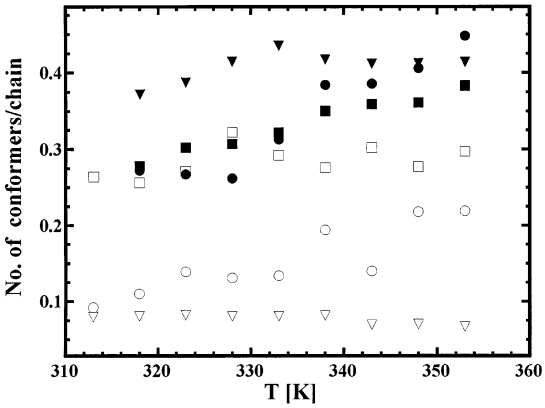

Figure 7. Number of gauche conformers per chain for DPPC bilayers (from CH_2 wagging band analysis). Filled symbols: pure lipid/water dispersion, open symbols: samples containing 40 mol % cholesterol. (○, ●) gg conformers, (□, ■) kink/gtg conformers, and (▽, ▼) eg conformers.

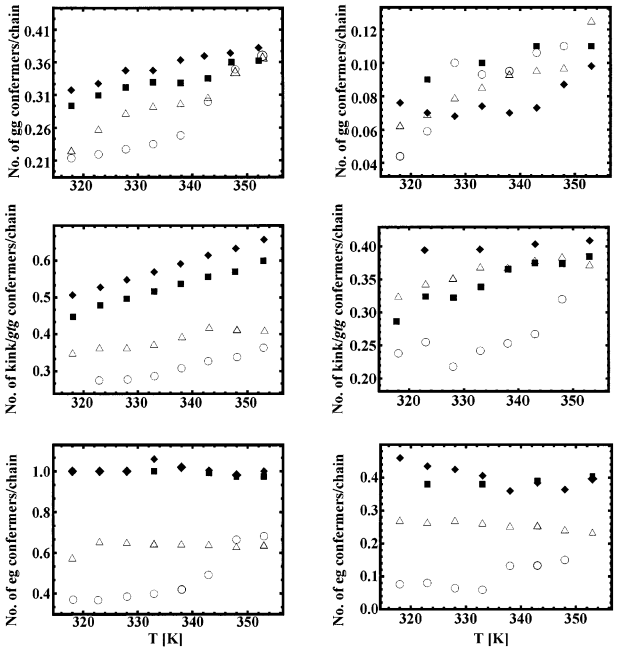

Figure 8. Number of gauche conformers per chain for $DPPG_x$ ($x = 1$, 2, 3, and 4) bilayers (from CH_2 wagging band analysis). Left column: pure lipid/water dispersion. Right column: samples containing 40 mol % cholesterol. (○) $DPPG_1$, (△) $DPPG_2$, (■) $DPPG_3$, and (◆) $DPPG_4$.

membrane, which is in good agreement with the data from other investigations.[12,31,39] For $DPPG_2$ the values are found to be about 20% higher than for $DPPG_1$. For the two lipids containing larger headgroups, the amount of gg conformers increases to values between 0.3 and 0.36 that are 30−50% higher than those of their shorter counterparts. In addition, the temperature dependence of the amount of gg sequences is less pronounced than for the low molecular weight oligoglycerols.

The data of the kink/gtg conformers exhibit quite similar trends. Again, we can distinguish between the values of the low molecular weight ($DPPG_1$, $DPPG_2$) and high molecular weight lipids ($DPPG_3$, $DPPG_4$). For the former ones values in the range from 0.3 to 0.4 are found, with $DPPG_2$ showing about 30% larger values. Again, the values for $DPPG_1$ are identical with those from DPPC, with values for kink/gtg conformers between 0.28 and 0.38. The higher amount of kink/gtg conformers in $DPPG_2$ can be understood by the presence of a bulkier headgroup (due to the hydration of the diglycerol headgroup) which creates free volume in the upper part of the fatty acid

chain region, where the kink sequences are expected to possess the highest probability.[34] A further support for this assumption can be obtained from the data of the high molecular weight systems, $DPPG_3$ and $DPPG_4$, which follow the trend toward an higher amount of kink/gtg conformers.

The derived values for the eg conformers behave very similar. Again, there is a clear tendency toward a higher amount of eg conformers with increasing number of glycerol units in the headgroup. However, it should be kept in mind that there is a higher uncertainty of these values for $DPPG_3$ and $DPPG_4$ samples due the aforementioned overlap with the δ(O−H) band. The higher amount of eg conformers points to a lower packing density now even at the lower end of the fatty acid chains.

The addition of 40 mol % cholesterol significantly lowers the amount of all gauche conformers in the systems studied here. Especially, the amount of gg conformers is reduced up to 70%, with values now ranging from 0.04 to 0.12. At the same time, the amount of kink/gtg and eg conformers also drop down, although the absolute reduction is less than for the gg conformers. Now, the values for the kink/gtg conformers increase from $DPPG_1$ (0.24 to 0.3) via $DPPG_2$ (0.32 to 0.34) and $DPPG_3$ (0.29 to 0.35) to $DPPG_4$ (0.4 to 0.41). Again, the values for the $DPPG_1$/cholesterol mixture are very close to those derived for the DPPC/cholesterol sample (0.26 to 0.32). Likewise, reduced values of the eg conformers are registered that again increase from $DPPG_1$ to $DPPG_4$, with values from 0.09 to 0.15 and values from 0.39 to 0.43, respectively. The above results and the minor temperature dependence of the gauche conformers in the cholesterol mixtures imply the presence of a higher molecular packing of the membranes which mainly arises from the incorporation of the cholesterol molecules in the acyl chain regions.

From the results presented so far the influence of the oligoglycerol chain length on both the conformational properties and the calorimetric data is quite obvious. The comparison of the pure lipid/water dispersions implies that the low mass system, $DPPG_1$, clearly can be distinguished from the higher oligomeric systems, $DPPG_3$ and $DPPG_4$, which behave very similar. The conformational behavior of $DPPG_2$ lies between both extremes, but nevertheless appears to be more close to that of $DPPG_1$. These differences can be explained by the assumption that highly hydrated oligoglycerol chains prevail in the $DPPG_3$ and $DPPG_4$/water dispersions. Owing to the rather bulky headgroup, these phospholipid bilayers should therefore possess a less dense packing in the acyl chain region along with a larger free volume. In fact, this assumption is supported by the distinct higher amount of gauche conformers in $DPPG_3$ and $DPPG_4$ bilayers, as revealed during the present FT-IR studies.

As for other phospholipid bilayers, the addition of 40 mol % cholesterol is accompanied by an ordering effect in the acyl chain region. In particular, the amount of gg sequences is reduced, since it is the sterically most demanding conformation. For $DPPG_3$ and $DPPG_4$ bilayers; however, the ordering effect by cholesterol is much less pronounced than for the low mass analogues $DPPG_1$ and $DPPG_2$ and other common lipid systems.[34,40] The most likely explanation is the aforementioned high hydration of the oligoglycerol units, resulting in very bulky headgroups along with a reduced packing of the acyl chain region. Unlike the situation in $DPPG_1$/cholesterol bilayers, for $DPPG_3$ and $DPPG_4$/cholesterol mixtures the steroid molecules can only partially compensate the free volume in the acyl chain region. This also would explain the observation that for the latter systems the main transition is not altered upon cholesterol addition.

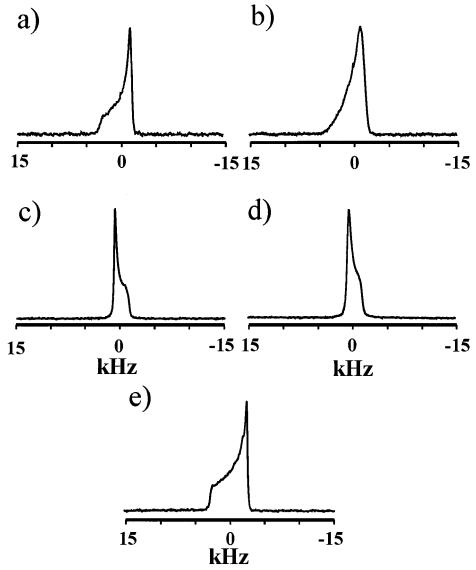

Figure 9. Representative solid state ^{31}P NMR line shapes pure lipid/water dispersions at 325 K. (a) $DPPG_1$, (b) $DPPG_2$, (c) $DPPG_3$, (d) $DPPG_4$, and (e) DPPC.

(e) ^{31}P and ^{13}C NMR Spectroscopy. During the present study also some orienting ^{31}P NMR experiments have been performed which can provide information about the mobility and ordering effects in the headgroup region.[41−43] In Figure 9 representative ^{31}P NMR line shapes are given which refer to the pure phospholipid/water dispersions from $DPPG_x$ and DPPC in the liquid crystalline phase at 325 K. In general, the overall width and shape of the ^{31}P NMR spectra prove the presence of motionally averaged systems. Moreover, the dependence of these NMR line shapes on the length of the oligoglycerol chains is quite obvious. Except for the $DPPG_2$ bilayers, characteristic axially symmetric ^{31}P NMR spectra are observed. Again, the spectra from $DPPG_3$ and $DPPG_4$ bilayers are different, as expressed by a lower and reversed chemical shift anisotropy ($DPPG_1$, 4.2 kHz; $DPPG_2$, 3.9 kHz; $DPPG_3$, −2.1 kHz; $DPPG_4$, −1.9 kHz; DPPC, 4.9 kHz).

The experimental ^{31}P NMR spectra of the latter samples are usually encountered for hexagonal and inverted hexagonal phases.[41,44] On the basis of the chemical structure (i.e., very bulky headgroup region) and with the known high amount of gauche conformers from the IR investigations (i.e., low packing density in the acyl chain region), the formation of both types of hexagonal phases for $DPPG_3$ or $DPPG_4$/water dispersions is very unlikely. In this connection, it should be recalled that the overall ^{31}P NMR line shapes also depend on the vesicle size and the chemical shift (CSA) tensor orientation with respect to the motional axis.[45] Owing to the large size of the oligoglycerol chains, it seems to be more appropriate to assume for $DPPG_3$ and $DPPG_4$ membranes a CSA tensor orientation that is different from that of the low mass analogues. A final proof of this assumption, however, is not possible on the basis of the present data. In this connection, X-ray investigations as well as variable temperature ^{31}P NMR investigations, including experiments on macroscopically oriented samples, would be very helpful. Again, we note that the overall appearance of the ^{31}P NMR spectrum of the $DPPG_2$/water dispersion appears to be between the limiting cases $DPPG_1$ on one hand and $DPPG_3$ or $DPPG_4$ on the other hand: a situation which was encountered before during the discussion of the FT-IR data.

As shown earlier by various investigations on *n*-alkanes and related systems, an increase in conformational disorder gives

TABLE 2: Temperature Dependent ^{13}C Chemical Shift Values Referring to Carbons C-4 to C-13 in the Acyl Chain Region of $DPPG_x$ and DPPC Bilayers

	chemical shift [ppm]									
	DPPC		DPPG		$DPPG_2$		$DPPG_3$		$DPPG_4$	
T [K]	pure	40 mol % cholesterol	pure	40 mol % cholesterol	pure	40 mol % cholesterol	pure	40 mol % cholesterol	pure	40 mol % cholesterol
288	33.3	33.0	33.1	33.3	33.0	33.2	32.9	33.0	32.9	33.0
318	31.1	32.3	31.0	31.8	30.7	31.5	30.5	31.1	30.6	31.1
333	30.9	31.5	30.6	31.3	30.5	31.0	30.2	30.7	30.0	30.7
343	30.7	31.3	30.5	31.2	30.3	30.9	30.0	30.2	29.9	30.2

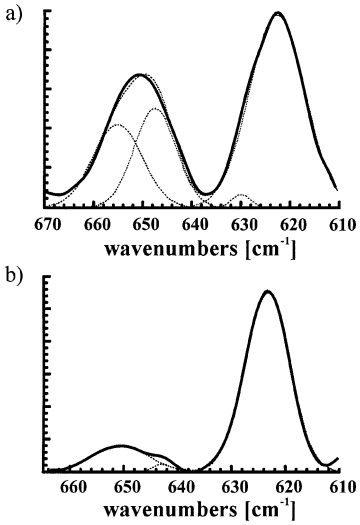

Figure 10. Representative FT-IR spectra (CD_2 rocking band region) lipid/water dispersions from $DPPG_2$ (top) and $DPPG_2$/cholesterol (bottom) at 323 K, labeled at position C-6 in acyl chains. Dotted lines: results from least-squares fit analysis.

rise to a high-field shift of the ^{13}C resonances.[46,47] Table 2 provides the experimental ^{13}C chemical shift values for the inner chain carbons, C-4 to C-13, obtained from ^{13}C MAS NMR investigations as a function of temperature and cholesterol content. In general, these chemical shift data display the expected high-field shift toward higher sample temperatures and with increasing length of the oligoglycerol chains due to the increase of the amount of gauche conformers which is in agreement with the findings of the FT-IR studies.

(f) CD_2 Rocking and CD_2 Stretching Bands. The CD_2 rocking bands between 620 and 670 cm^{-1}, in combination with selectively deuterated compounds, can be used to determine the amount of gauche sequences at a specific methylene unit.[14−17,48] The CD_2 rocking region contains three conformation sensitive bands, centered at around 622 cm^{-1} (tt), 645 cm^{-1} (ttgt, g′tgg), and 651 cm^{-1} (g′tgt, ttgt), reflecting the conformational states given in brackets. In Figure 10 representative FT-IR spectra covering the rocking band region are shown that were recorded for the $DPPG_2$/water dispersion and the $DPPG_2$/cholesterol mixture. In both cases the acyl chains were selectively deuterated at position C-6.

The results in terms of percentage of gauche conformers at position C-6, deduced from a band iteration process (see Experimental Section), are summarized in Figure 11. The graphs also contain data from the corresponding DPPC samples, that also have been deuterated at the same position. The inspection of these data reveals that the amount of gauche conformers at position C-6 of the fatty acyl chain in the $DPPG_2$/water dispersion is higher than in the corresponding DPPC bilayer. For the pure DPPC bilayer the gauche percentage at 323 K is about 20%, whereas $DPPG_2$ exhibits values of about 30%. Such a result was expected, since the wagging band data already pointed toward a higher gauche amount for $DPPG_2$ bilayers. Especially the kink/*gtg* sequences were found to be higher than in the DPPC membrane, most likely due to the bulkier diglycerol headgroup. In fact, the kink/gtg conformation generally is expected to have the highest probability in the upper part of the acyl chains.[34] For this reason the results from the rocking band analysis are consistent with the findings from the wagging band data, although the latter can only provide integral values over the whole acyl chain. Furthermore, the amount of gauche conformers derived from the CD_2 rocking bands also exhibit a characteristic discontinuity at the main transition for both the $DPPG_2$ and DPPC bilayers. Again, in a qualitative way this behavior has been seen earlier during the analysis of the variable temperature CH_2 stretching band and wagging band progressions.

The incorporation of 40 mol % cholesterol decreases the percentage of gauche conformers for the $DPPG_2$ and DPPC bilayer systems. The reduction, however, is more pronounced in the liquid crystalline phases from pure phospholipid/water dispersions which inherently contain a higher amount of gauche conformers. Moreover, the above-mentioned discontinuity at the main transition of the pure lipid/water dispersions does not show up in the curves of the cholesterol mixtures. This again is in agreement with the above results from the CH_2 stretching bands and wagging band progressions as well as calorimetric data from which a similar suppression of the main transition after addition of cholesterol has been deduced.

In a qualitative way the conformational behavior at a particular chain segment also can be followed by the position of the CD_2 stretching bands.[6] In close analogy with the CH_2 stretching bands (see above), a change of the conformational state gives rise to a shift of the symmetric and antisymmetric CD_2 stretching bands. This is demonstrated in Figure 11c, where for the $DPPG_2$ bilayers the positions of the antisymmetric CD_2 stretching band are plotted as a function of temperature. It can be seen that the overall appearance of these curves is very close to those given in Figure 11 for the percentage of gauche conformers. This holds for both the temperature dependence and the influence by the incorporation of cholesterol. The correlation of the these two data sets is further demonstrated by the straight lines given in Figure 11d, where the position of the antisymmetric CD_2 stretching band is plotted against the percentage of gauche conformers. Similar plots have been shown earlier for example during FT-IR investigations on hydrocarbon melts[6] and on chemically modified silica gels.[49]

(g) Combination of CD_2 Rocking and ^2H NMR Data. In Figure 12 solid state ^2H NMR spectra are depicted that refer to the pure $DPPG_2$/water dispersion and a $DPPG_2$/cholesterol mixture well above the main transition. As for other phospholipid membranes,[51−53] these ^2H NMR spectra are narrowed considerably when compared to the spectra of a completely rigid system. Detailed ^2H NMR studies on other phospholipid

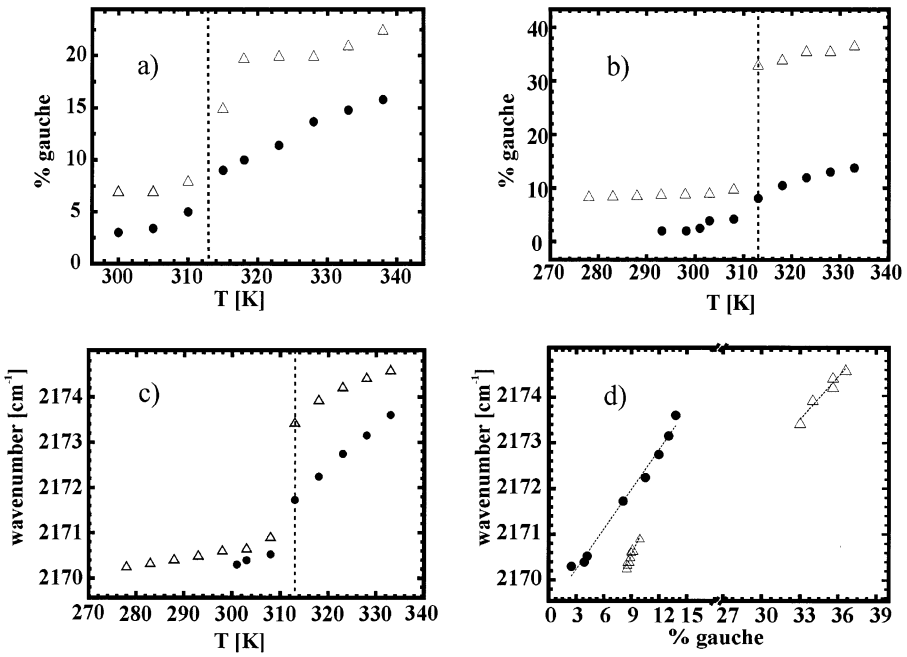

Figure 11. Results from analysis of the CD_2 rocking and antisysmmetric stretching band region for DPPC and $DPPG_2$, labeled at position C-6 in the acyl chains. Open and filled symbols refer to the pure lipid/water dispersions sample containing 40 mol % cholesterol, respectively. (a) Amount of gauche conformers in DPPC bilayers, (b) amount of gauche conformers in $DPPG_2$ bilayers, (c) position of antisymmetric CD_2 stretching band in $DPPG_2$ bilayers, and (d) position of antisymmetric CD_2 stretching band vs amount of gauche conformers in $DPPG_2$ bilayers.

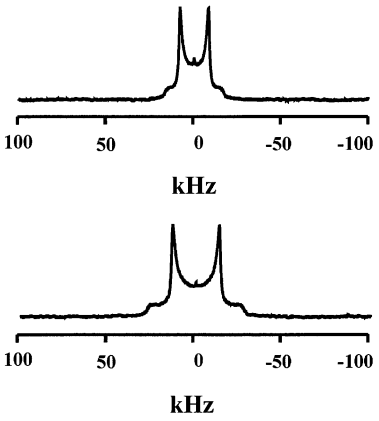

Figure 12. Representative ^2H NMR spectra of a $DPPG_1$ membrane at 323 K. Top: pure lipd water dispersion. Bottom: sample containing 40 mol % cholesterol.

membranes[51−53] have revealed the presence of various overall and internal motions of the lipid molecule, such as long-axis rotation, long-axis fluctuation, as well as trans−gauche isomerization.[53] If these motions are fast on the NMR time scale, then the quadrupolar splitting is directly connected with the overall order parameter S_{CD} of the lipid molecule by the following expression:

$$\Delta\nu_q = \frac{3}{4}\left(\frac{e^2qQ}{h}\right)S_{CD} \qquad (2)$$

Here, e^2qQ/h represents the static quadrupolar coupling constant and $\Delta\nu_q$ is the experimental quadrupolar splitting, i.e., the distance between the perpendicular singularities.[1] The order parameter S_{CD} can be further expressed by[53]

$$S_{CD} = S_\alpha S_\gamma \qquad (3)$$

S_α is the orientational order parameter which expresses the orientational order or degree of long-axis orientation of the lipid molecules with respect to a local preferential axis, the director.[53] S_γ represents the segmental order parameter at the corresponding CD_2 unit and thus reflects its conformational state. With the assumption that only kink conformers are contributing, the amount of gauche conformers, derived from the CD_2 rocking modes, can be related to the segmental order parameter S_γ by[16,53]

$$p_t = 1 - p_g$$
$$S_\gamma = {}^1/_2\, p_t \qquad (4)$$

Here, p_t and p_g are the trans and gauche populations, respectively. p_g can be directly taken from the analysis of the CD_2 rocking modes. With the known order parameters S_γ and S_{CD}, it is therefore possible to calculate the orientational order parameter S_α.

Figure 13 summarizes the order parameters S_{CD} from the ^2H NMR splittings and the derived values for S_α from the DPPC and $DPPG_2$ samples, comprising pure phospholipid bilayers and mixtures with cholesterol. For the pure lipid bilayers only the temperature range above the main transition is covered, since here at lower temperatures the overall motions are slowed and the above equations for the order parameters are not valid any more. However, for the cholesterol mixtures the liquid crystalline phase (with highly mobile lipid molecules) is stabilized and extended toward lower temperatures; a phenomenon which is well-known also from other lipid bilayers.[52]

In general, the derived order parameters S_{CD} and S_α show an almost linear decrease with increasing temperature which holds for both the pure lipid bilayers and the mixtures with cholesterol. Experimental S_{CD} data are also available from other ^2H NMR studies on pure DPPC bilayers, which are very close to the values reported here. For example, at 323 K our value is given to $S_{CD} = 0.26$, whereas values of 0.29[54], 0.22,[16] (position C-4), and 0.20[55] have been reported by other authors. Moreover, the order parameters S_{CD} of the samples with DPPC are found to be somewhat higher than those from $DPPG_2$. For the pure DPPC

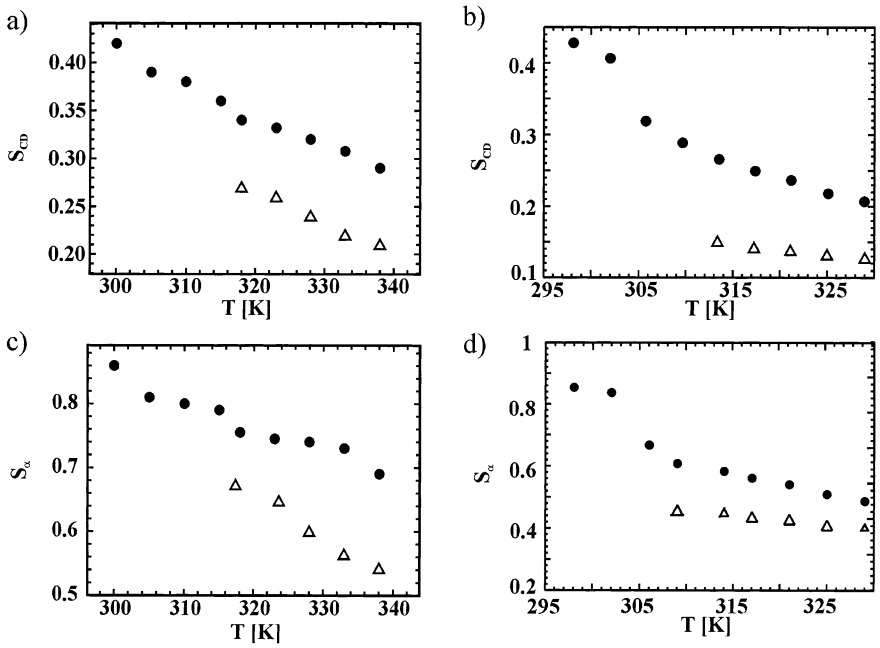

Figure 13. Experimental S_{CD} (top) and derived S_α (bottom) order parameters for DPPC (left column) and $DPPG_2$ bilayers (right column), labeled at position 6 in the acyl chains. Open and filled symbols refer to pure lipid/water dispersions and samples containing 40 mol % cholesterol, respectively.

bilayers values between 0.21 and 0.28 are obtained in the liquid crystalline phase, whereas for the $DPPG_2$/water dispersion values between 0.12 and 0.15 are observed. The addition of cholesterol is accompanied by an increase of the S_{CD} values. Now in the liquid crystalline phase the S_{CD} values range from 0.29 to 0.34 and 0.21 to 0.32 for DPPC and $DPPG_2$/cholesterol mixtures, respectively.

The orientational order, expressed by the order parameter S_α, exhibits similar trends as discussed for S_{CD}. That is, S_α values of the $DPPG_2$ samples are lower as compared to those from the DPPC systems. Likewise, the addition of cholesterol gives rise to an increase of the orientational order. $DPPG_2$ samples exhibit S_α values from 0.4 to 0.46 and from 0.5 to 0.82 for the pure bilayer and the cholesterol mixture, respectively. For the pure DPPC/water dispersion values between 0.52 and 0.86 are derived, which are in good agreement with earlier measurements on the same system,[16] where a value of 0.54 in the liquid crystalline phase at 324 K has been derived. Variable temperature ^{31}P NMR experiments also exist for[56] DMPC bilayers from which S_α values between 0.43 and 0.53 were reported.

These latter results clearly demonstrate that in general $DPPG_2$ samples are characterized by a lower degree of overall chain order than DPPC bilayers and most probably also $DPPG_1$ bilayers, although for the latter system, due to the lack of a deuterated sample, comparable data were not available. Again, the lower degree of orientational order of the $DPPG_2$ bilayers is attributed to the higher hydration of the headgroup region. The bulkier headgroup in $DPPG_2$ prevents a close molecular packing and therefore allows the system a greater conformational and motional freedom, as expressed by the low values for the order parameters S_{CD} and S_α. The rigid cholesterol increases the conformational order and minimizes the fluctuation amplitude of the lipid molecules, as reflected by the enhanced conformational and orientational order parameters. Nevertheless, due to the larger headgroup in $DPPG_2$, the addition of cholesterol is not sufficient in order to achieve a similar high packing density as for DPPC bilayers.

Conclusions

In the present work new synthetic phospholipids $DPPG_x$ ($x = 1-4$), bearing an oligoglycerol chain of variable length in the headgroup, were examined by variable temperature FT-IR and solid state NMR spectroscopy. Particular emphasis was given on the influence of the headgroup size and hydrophilicity on the conformational properties of the acyl chains, which was examined as a function of temperature and sample composition (i.e., presence or absence of cholesterol).

In the first step, the results from differential scanning calorimetry were contrasted with the data derived from the analysis of the symmetric CH_2 stretching modes and CH_2 wagging band progressions, both of which reflecting changes of the conformational state in the acyl chains. Furthermore, several conformational sensitive CH_2 wagging bands were analyzed from which the amount (i.e., integral value over the whole chain) of various types of gauche conformers could be obtained. It was found that the actual amounts of these conformers critically depend on the headgroup size, sample composition and sample temperature. The FT-IR investigations were completed by solid state ^{31}P and ^{13}C MAS NMR experiments. They could provide an independent support for the conclusions that were drawn on the basis of the FT-IR data. In addition, CD_2 rocking modes of selectively deuterated $DPPG_2$ have been analyzed in order to get information about the conformational properties at a particular acyl chain segment (in the present study at carbon C-6). These data were combined with those from complementary ^2H NMR investigations which allowed a distinction between the conformational order and the orientational order of the lipid molecules.

In general, the present membrane systems exhibit a similar temperature dependence as known from other phospholipid systems. That is, a higher sample temperature gives rise to a decrease in conformational and orientational order. Likewise, incorporation of cholesterol stabilizes the liquid crystalline phase, as expressed on the molecular level again by higher conformational and orientational order parameters. The actual length of the oligoglycerol units in the phospholipid headgroup

also has a significant effect on these order parameters. Here, the IR data showed a gradual decrease in conformational order with increasing oligoglycerol length. A similar trend is also discussed for the orientational order, although so far only data for $DPPG_2$ are available. These findings can be traced back to the higher hydrophilicity of the systems with longer oligoglycerol chains. Rather bulky lipid headgroups therefore prevail for these membrane systems that are responsible for a lower packing density in the acyl chain region which in turn can only partially be compensated by the incorporation of cholesterol.

It also should be emphasized that on the basis of the experimental IR and NMR data the low molecular mass systems $DPPG_1$ and $DPPG_2$ clearly can be distinguished from their higher mass analogues $DPPG_3$ and $DPPG_4$, which holds for both the conformational and overall ordering characteristics. In this connection it is interesting to note that the aforementioned prolonged in *vivo* blood circulation times are visible only for liposomes from $DPPG_3$ and $DPPG_4$. It nevertheless is still open whether both observations are correlated. Here, additional information is expected from forthcoming studies on related systems.

In summary, the present study has demonstrated that such FT-IR techniques are of general use for the evaluation of the conformational properties in phospholipid bilayers. The use of deuterated compounds was of particular advantage since FT-IR and ^2H NMR data could be combined in order to complete the picture about the conformational as well as the orientational order. Further work along this line is in progress.

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