

Chain Order in Lipid Bilayers: FTIR and Solid State NMR Studies on Bilayer Membranes from 1,2-Dimyristoyl-*sn*-glycero-3-phosphoglucose

Peter Wolfangel and Klaus Müller*

Institut für Physikalische Chemie, Universität Stuttgart, Pfaffenwaldring 55, D-70569 Stuttgart, Germany

Received: March 18, 2003; In Final Form: May 7, 2003

Multilamellar dispersions from a new synthetic phospholipid, 1,2-dimyristoyl-*sn*-glycero-3-phosphoglucose (DMPGE), bearing a glucose ring in the polar headgroup, are studied by means of FTIR, ^2H , and ^{31}P NMR spectroscopy. The investigations are focused on the evaluation of the molecular ordering of the lipid molecules as a function of temperature and cholesterol content. Information about the conformational order of the acyl chains is obtained from variable temperature FTIR investigations. Analysis of the CH_2 stretching and wagging modes of nondeuterated compounds provides the relative changes in conformational order in the vicinity of the main transition and integral values of distinct gauche conformers over the whole acyl chains, respectively. Likewise, CD_2 rocking bands are used to derive the amount of gauche conformers at a specific chain segment in samples containing selectively deuterated acyl chains. The present work represents the first report where the orientational order parameter simultaneously is obtained via two independent procedures. The first method is based on the combination of the CD_2 rocking band data with those from complementary ^2H NMR studies. The second procedure refers to a line shape analysis of variable temperature ^{31}P NMR spectra. A comparison of the available data for the present lipids with those from related systems, such as 1,2-dimyristoyl-*sn*-glycero-3-phosphocholine (DMPC), reveals a minor influence of the glucose headgroup on the (overall) orientational order while some effect is registered for the conformational order of the lipid molecules. In summary, the present work demonstrates the future potential of such combined FTIR and solid-state NMR studies in order to get detailed information of the lipid ordering in phospholipid bilayers. As shown here, such techniques are of general help in order to separate orientational and segmental (conformational) order in disordered alkyl chains, as there is no restriction to lipid systems as examined in the present work.

Introduction

During recent years, biological membranes were extensively studied by various spectroscopic techniques, such as NMR^{1–7} and IR spectroscopy.^{8–10} These studies were primarily addressed to the ordering characteristics as well as the lipid mobility of these systems, where the dependence on external parameters such as temperature or sample composition was also accounted for. NMR investigations, comprising ^2H , ^{31}P , and ^{13}C NMR line shape and relaxation experiments, have demonstrated their particular suitability to examine both molecular aspects,^{1–7,11–14} which usually requires a comprehensive and detailed computer-assisted data analysis. Likewise, FTIR spectroscopy can be used to sort out the conformational properties of the acyl chain region via analysis of several conformation sensitive vibrational bands.^{8–10,15,16}

For instance, the frequency shifts of the CH_2 stretching bands¹⁷ at about 2850 and 2925 cm^{-1} as well as the CH_2 wagging band progressions^{18,19} between 1150 and 1350 cm^{-1} can be used as a qualitative measure for the conformational changes in the vicinity of the main transition between the gel and liquid crystalline phases. Likewise, for the liquid crystalline lipid phases the CH_2 wagging band region^{20,21} between 1320 and 1390 cm^{-1} can be analyzed in order to get the (integral) amounts of various gauche conformers, that is, gauche–trans–gauche, double gauche, and end gauche conformers. If samples

with selectively deuterated acyl chains are available, then the analysis of the CD_2 rocking bands between 600 and 660 cm^{-1} can be used to obtain information about the percentage of gauche conformers at a specific methylene group.^{22–25} In principle, these latter data can be further combined with the order parameter S_{CD} , accessible via ^2H NMR investigations, from which the overall chain order in terms of the orientational order parameter S_{α} can be derived. In fact, so far only a few studies have been reported where this combination of NMR and FTIR data has been exploited.^{26,27}

In this contribution we present FTIR, ^2H , and ^{31}P NMR studies on a new model membrane that is based on 1,2-dimyristoyl-*sn*-glycero-3-phosphoglucose (DMPGE) (see Figure 1), a lipid bearing the monosaccharide glucose in the polar headgroup. The molecule thus possesses some characteristic features of natural phospholipids by the phosphate group as well as of natural glycerolipids, since a sugar unit is implemented in the headgroup. We have taken this compound as a model system for 1,2-dimyristoyl-*sn*-glycero-3-phosphoinositol (DMPI), which is of great physiological importance in natural membrane systems,^{28–30} since so far only little is known about the molecular behavior of pure inositol bilayers containing only a distinct chain length. This lack of information mainly is attributed to the fact that a total synthesis of phosphatidyl inositols turned out to be very complex and to the difficulty in isolating phosphatidyl inositols with a specific acyl chain length from natural sources, which typically are complex lipid mixtures.^{31,32} The present model lipid DMPGE is regarded to be a

* To whom correspondence should be addressed. Phone: ++ 49 (711) 685 4470. Fax: ++ 49 (711) 685 4467. E-mail: k.mueller@ipc.uni-stuttgart.de.

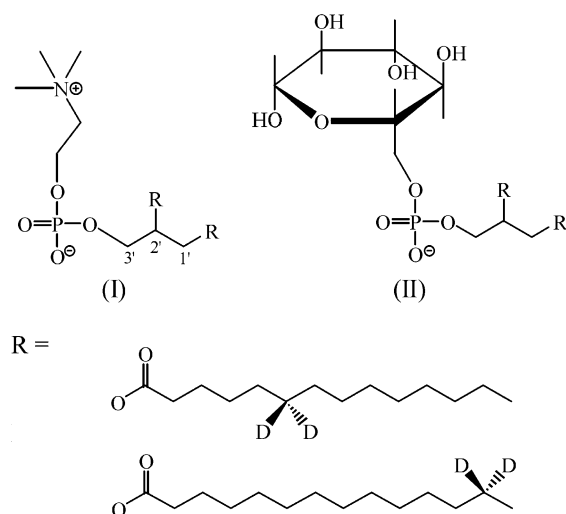


Figure 1. Chemical structures of the phospholipid DMPC (I) and DMPGE (II) used in the present study.

reasonable compromise which displays the main characteristics of DMPI: (i) the headgroup size, (ii) the overall electrical charge, and (iii) the large number of hydroxyl groups, which allows the formation of hydrogen bonds. The major difference between DMPGE and DMPI stems from the additional methylene segment between the phosphate group and the ring system, which certainly must be considered during the final interpretation of the experimental data.

The present contribution addresses the chain ordering of the new synthetic membrane DMPGE where FTIR and NMR investigations were performed on both pure lipid/water dispersions and lipid/cholesterol mixtures containing 20 mol % or 40 mol % cholesterol. Variable temperature FTIR investigations were performed on nondeuterated samples as well as on lipids bearing selectively deuterated acyl chains (position C-6). Explicitly, CH_2 stretching, CH_2 wagging, and CD_2 rocking band regions were analyzed from which the aforementioned information about the conformational properties of the lipid systems could be derived. Likewise, selectively deuterated lipids (deuterated acyl chain positions: C-6 and C-13) were examined by ^2H NMR spectroscopy. The results from the analysis of the ^2H NMR experiments and the FTIR data in the CD_2 rocking band region were further combined in order to separate the overall chain order (chain order parameter S_α) and the local conformational order (segmental order parameter S_γ). In addition, the overall chain order parameter S_α was determined independently on the basis of variable temperature ^{31}P NMR line shape studies. The derived data for the DMPGE bilayers are discussed and interpreted by considering available data on related compounds. In this connection, it appeared to be necessary to perform the same experiments on DMPC bilayers and DMPC mixtures, including deuterated and nondeuterated compounds. This ensures a common basis for the final discussion of the molecular properties of the present lipid systems.

Experimental Section

Materials. The starting materials for the synthesis were purchased either from Aldrich Chemicals (Steinheim, Germany) or from Fluka Chemie AG (Buchs, Germany). Cholesterol was obtained from Sigma Chemicals (Deisenhofen, Germany), and GPC (*sn*-glycero-3-phosphocholine), from Lukas Meyer GmbH (Hamburg, Germany). Myristic acid specifically deuterated at position C-6 or C-13 was prepared via standard multiple step

synthetic routes which, for example, can be found in refs 33 and 34.

For the preparation of DMPC a previously published preparation method was used.³⁵ Myristic acid (1 g, 4.38 mmol), dissolved in 40 mL of dry chloroform, was transformed into its imidazolide by addition of *N,N*-carbonyldiimidazole (810 mg, 5.0 mmol). The reaction mixture was then stirred at room temperature for about 45 min. Then, carefully dried *sn*-glycero-3-phosphocholine (488 mg, 1.9 mmol) and 1,8-diazabicyclo-[5.4.0]undec-7-ene (667 mg, 4.38 mmol) were coadded. The solvent was removed after additional stirring at room temperature for at least 24 h. DMPC of high purity was obtained from the residue by recrystallization in acetone.

The phospholipid DMPGE was obtained from DMPC by replacement of the choline headgroup in DMPC via transphosphatidyl transfer.^{36–39} This catalytic reaction required a biphasic system and the presence of the enzyme phospholipase D from *Streptomyces Species* (Asahi Chemicals Industry Co., Ltd., Tokyo, Japan). Therefore, phospholipase D and 14.4 g (80 mmol) of glucose were dissolved in 20 mL of a buffer solution consisting of sodium acetate (0.5 M), calcium chloride (0.5 M), and ethylenediaminetetraacetate (0.05 M) at a pH of 5.6. The organic phase contained 1.0 g (1.48 mmol) of DMPC in 35 mL of chloroform. The reaction mixture was heated to 45 °C and stirred vigorously for at least 10 h. Afterward, the phases were separated and the water layer was extracted with Folch solution (i.e., mixture of chloroform and methanol in the ratio 2:1). From the combined organic layers the solvent was evaporated and the crude product, consisting of DMPGE, DMPC, and DMPA, was purified by silica gel column chromatography.

FTIR Spectroscopy. Sample Preparation. Multilamellar lipid dispersions were prepared as described below for the NMR measurements, but the amount of water was increased to about 70–80 wt %. The construction of the IR cell (LOT/Oriel, Langenberg, Germany) excluded a loss of water; incomplete hydration thus can be excluded.

FTIR Measurements and Data Analysis. For the analysis of the CH_2 stretching and wagging mode regions, the infrared cell was equipped with CaF_2 windows separated by a spacer of 50 μm thickness. The CD_2 rocking band near 600 cm^{-1} was observed using KRS-5 windows without a spacer. In all experiments the IR cell was thermostated in a variable temperature transmission cell (LOT/Oriel, Langenberg/Germany). IR spectra were recorded with a Bruker IFS 66 FTIR spectrometer (Bruker, Rheinstetten/Germany). Further details about the experimental procedures can be found elsewhere.²⁷

Data Analysis. Details about the analysis of the CH_2 stretching bands, CH_2 wagging bands,²¹ and wagging band progressions^{18,19} as well as the CD_2 rocking band analysis^{10,25} are given elsewhere.²⁷

NMR Spectroscopy. Sample Preparation. Multilamellar dispersions (sample free of cholesterol) were prepared by hydration of 100 mg of dry phospholipid in the same amount of deuterium-depleted water. A homogeneous multilamellar dispersion was obtained by repeated freeze–thawing, centrifuging, and vortexing of the sample. The homogenized material was transferred into a 5 mm NMR tube and sealed under vacuum. DMPGE/cholesterol samples were prepared by dissolving appropriate amounts of phospholipid and steroid (20 mol % or 40 mol %) in freshly distilled chloroform. The solvent was partially removed by a stream of nitrogen gas, and the residual solvent was evaporated under vacuum for at least 5 h. After this procedure, the dry mixture was dispersed in deuterium-depleted water in the same way, as described above.

NMR Measurements. ^2H and ^{31}P NMR experiments were performed on a Bruker CXP 300 NMR spectrometer (Rheinstetten, Germany) at 46.07 MHz (^2H) and 121.5 MHz (^{31}P), interfaced to a MacSpect station (Tecmag, Houston, TX), using a broadband 5 mm double tuned probe. ^2H NMR spectra were obtained employing the quadrupole echo sequence $(\pi/2)_x - \tau - (\pi/2)_y - \tau - \text{acq.}$ ^{40,41} Typically, the $\pi/2$ pulse width was 2.0 μs and the delay τ between the pulses was fixed to 20 μs . The recycle delay varied between 0.3 s (liquid crystalline phase) and 10 s (gel phase). ^{31}P NMR experiments were performed in the presence of broadband proton decoupling (decoupling power: 20 W in the liquid crystalline phase and 55 W in the gel phase) using a single pulse sequence ($\pi/2$ pulse width: 3 μs). The recycle delays were between 3 s (liquid crystalline phase) and 300 s (gel phase). The number of scans varied between 512 and 2048, depending on the actual phase of the lipid bilayer. The sample temperature was controlled with a Bruker BVT 1000 temperature control unit. Generally, the temperature stability was found to be within $\pm 1\text{K}$.

Data Processing and Simulations. FORTRAN programs have been developed which describe the behavior of isolated $I = 1/2$ and $I = 1$ spin systems during the corresponding pulsed NMR experiments.^{42–44} Theoretical NMR line shapes, with particular emphasis on the slow motional region, are obtained by a numerical diagonalization of the corresponding relaxation matrixes using standard software packages.⁴⁵ In the present study these programs were used to analyze ^{31}P NMR spectra in the liquid crystalline and gel phases. Here, a rotational diffusion process^{13,14,46,47} was assumed which is described by a rotation about the long molecular axis and restricted fluctuation of the long molecular axis in an ordering potential,⁴⁸ with the main axis given by the liquid crystalline director axis. It should be noted that the rotational motion was further simplified by the assumption of a three-site jump process with equally populated jump sites. For the molecular fluctuations, next-neighbor jumps were assumed. The ordering potential is directly related to the orientational order parameter S_α , which gives rise to unequally populated fluctuation sites in the most general case.^{13,14,46,47} During the line shape simulations, several adjustable simulation parameters had to be accounted for: the magnitude and relative orientation of the ^{31}P chemical shift tensor with respect to the long molecular axis, the orientational order parameter S_α , a residual line width, and the correlation times τ_c for the two overall motions. The correlation times were set to the fast exchange limit ($\tau_c \leq 10^{-8}$ s) throughout the whole of the liquid crystalline (pure DMPGE bilayers) and liquid-ordered phases (DMPGE/cholesterol mixtures), and they were adjusted at lower temperatures for the pure DMPGE bilayers. The principle values for the chemical shift tensor were taken to be $\sigma_{11} = 85$ ppm, $\sigma_{22} = 16$ ppm, $\sigma_{33} = -110$ ppm and $\sigma_{11} = 85$ ppm, $\sigma_{22} = 18$ ppm, $\sigma_{33} = -105$ ppm for the pure lipid/water dispersions and lipid/cholesterol mixtures, respectively. The transformation from the principal axis system to the long molecular axis system involved two steps, from the principal axis system to the P–O bond axis and then to the lipid long molecular axis, as shown earlier during a study on DMPC bilayers.⁴⁹ We have taken the same transformation angles, namely $\phi_1, \theta_1, \psi_1 = 55^\circ, 105^\circ, 75^\circ$ and $\phi_2, \theta_2, \psi_2 = 0^\circ, 20^\circ, 0^\circ$, as also discussed in that earlier work. The fitting of the experimental spectra has been done by superimposing the experimental and theoretical spectra. Both the simulated and experimental spectra were processed on SUN workstations using the NMR1 and Sybyl/Triad software packages (Tripos, St. Louis, MO).

Differential Scanning Calorimetry (DSC). The phase behavior of the various samples was studied with a Netzsch DSC 204 calorimeter (Selb, Germany) in the temperature range between 200 and 350 K at the heating rate 5 K/min.

Results

In the present work multilamellar (nonoriented) dispersions of a new synthetic phospholipid, DMPGE (see Figure 1), were investigated by means of ^2H and ^{31}P NMR and FTIR spectroscopy between 240 and 350 K. The chemical structure differs from those of common model membrane phospholipids by the incorporation of glucose in the headgroup.

Phase Behavior. Three different membrane systems have been examined during the present spectroscopic studies: (i) pure hydrated lipids with a water content of 50–80 wt %, (ii) a hydrated phospholipid/cholesterol mixture with a steroid content of 20 mol %, and (iii) a mixture with a steroid content of 40 mol %. The two former model membranes exhibit a liquid crystalline phase and a gel phase, as established by differential scanning calorimetry. The transition temperature T_m of pure, hydrated DMPGE was found to be 309 K. Addition of 20 mol % cholesterol broadens the main transition at 309 K and decreases the transition enthalpy. However, the two phases can still be distinguished. The main transition is completely absent upon addition of 40 mol % cholesterol. In this latter case a liquid-ordered phase⁵⁰ is created, as well-known for other phospholipid systems.

FTIR Investigations. In Figure 2a the position of the CH_2 stretching bands in DMPGE bilayers is plotted as a function of temperature and sample composition. A highly ordered fatty acid chain in the all-trans conformation is known to display a stretching band near 2849 cm^{-1} . An increase of the number of gauche conformers, for example by heating the sample, should give rise to a distinct band shift toward higher wavenumbers.¹⁰ In fact, for the pure DMPGE/water dispersion, a pronounced and almost discontinuous shift can be observed in the vicinity of the phase transition. The wavenumber of the absorption maximum is found to change from about 2849 cm^{-1} in the gel phase to 2854.5 cm^{-1} in the conformationally disordered liquid crystalline phase. Addition of 20 mol % cholesterol lowers the overall shift of the CH_2 stretching band between the gel and liquid crystalline phases, although the discontinuity in the vicinity of the main transition remains still visible. As mentioned earlier, after incorporation of 40 mol % cholesterol, the calorimetric main transition vanishes completely. As a result, the band shift is less pronounced and is almost continuous at the main transition of the pure lipid/water dispersion. In addition, in the low temperature region ($T < 309\text{ K}$) the absolute band position reflects a somewhat lower conformational order than that for the pure lipid/water dispersion, while at higher temperatures the opposite effect is observed as a consequence of an ordering effect by the steroid molecules.

A second qualitative measure of the conformational order in membrane systems is given by the intensity of the CH_2 wagging band progressions between 1180 and 1380 cm^{-1} , as a result of the coupling of the methylene group wagging modes in all-trans alkyl chains.^{18,19} In Figure 2c CH_2 wagging progression bands are shown for the pure DMPGE/water dispersion in the gel phase and clearly prove the presence of highly ordered fatty acid chains. The overall temperature dependence of the progression band intensities of the various DMPGE bilayers in Figure 2b resembles the band shift effects found for the CH_2 stretching mode, including the discontinuity for the pure DMPGE/water dispersion at the calorimetric main transition. Addition of

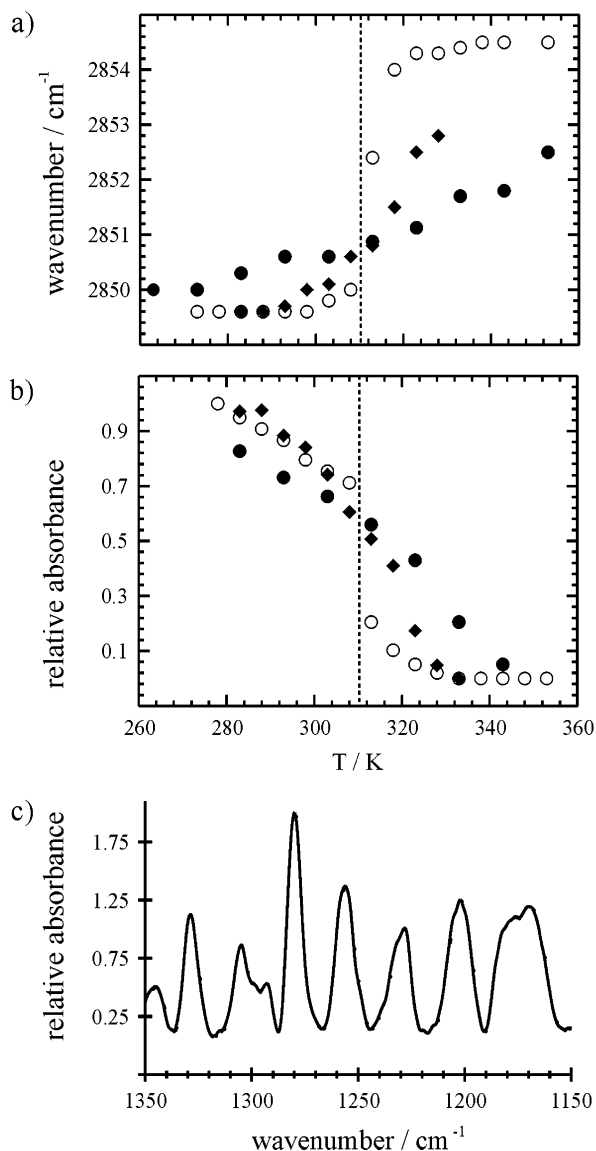


Figure 2. (a) CH₂ stretching band position and (b) wagging band progression intensities for pure DMPGE bilayers (○) and DMPGE with 20 mol % (◆) or 40 mol % cholesterol (●). The dashed line indicates the calorimetric phase transition of the pure DMPGE bilayer. (c) Wagging band progression for pure DMPGE bilayers at 285 K.

cholesterol again successively weakens this effect, reflecting a smaller overall temperature variation of the conformational order in such lipid/cholesterol mixtures.

In Figure 3 representative FTIR spectra covering the CH₂ wagging band region are given for pure DMPC (a) and DMPGE bilayers (b) in the liquid crystalline phase. The influence of the incorporation of 40 mol % cholesterol into the bilayer system is demonstrated by the corresponding FTIR spectra on the right-hand side (DMPC (c) and DMPGE (d)). In agreement with earlier studies, for DMPC bilayers three absorption bands are found at 1368, 1356, and 1342 cm⁻¹ that reflect kink(gtg')/gtg, double gauche (gg), and end gauche (eg) sequences in the fatty acid chains, respectively. A further intense band, centered at 1378 cm⁻¹, refers to the methyl group "umbrella" deformation mode. A decomposition of the wagging absorption band, employing a curve fitting procedure, eventually yields the integral amount of kink/gtg, gg, and eg sequences over the whole acyl chain. The temperature dependence of the amount of these conformers in DMPC bilayers is displayed in Figure 4. These plots reveal a pronounced increase with sample temperature for

the amount of gg sequences, while kink/gtg and eg sequences are less affected by the actual sample temperature.

The CH₂ wagging band region of DMPGE can be analyzed in quite the same way. Here, an additional band near 1390 cm⁻¹ has to be considered in the band shape analysis (see Figure 3b). However, we refrained from a further study of its origin, since this band can be clearly separated from the methyl "umbrella" deformation band. The final results for kink/gtg, gg, and eg sequences for the DMPGE bilayers are also summarized in Figure 4. A comparison of the experimental data reveals that an elevated number of kink/gtg sequences exists in bilayers from DMPGE as compared to pure DMPC bilayers. At the same time, the amount of gg sequences is similar in both membrane systems, while the amount of eg conformers in DMPGE bilayers is reduced by about 20–25%. Inspection of Figure 4 further demonstrates that addition of 40 mol % cholesterol reduces the number of gg and eg sequences considerably (about 40–50% for gg and almost 100% for eg), while the amount of kink/gtg sequence remains almost unaltered. In general, for the DMPGE model membranes, an almost continuous decrease of the gg sequence with increasing steroid content can be deduced. This is shown, for example, by the values of 0.38 (pure system), 0.33 (20 mol % cholesterol), and 0.22 (40 mol % cholesterol) at a reduced temperature of 15 K. At the same time, eg sequences are of minor importance in the DMPGE bilayers containing cholesterol. This is even true for the sample containing only 20 mol % steroid.

The total number of gauche conformers per alkyl chain in Figure 5 was calculated from the individual number of gauche conformers, given in Figure 4, by taking into consideration two gauche bonds for kink/gtg and gg conformers each and one gauche bond for eg conformers. The upper graph in Figure 5 refers to the results for the three DMPGE model membranes. For comparison the figure also contains the data for DMPC bilayers (bottom graph). Both systems reflect a substantially increased conformational order upon the addition of the steroid. This is shown for example by a total number of gauche conformers of 2.0, 1.5, and 1.1 at a reduced temperature of 25 K for pure DMPGE bilayers and mixtures with 20 and 40 mol % cholesterol, respectively. The ordering effect can be mainly traced back to a strong reduction of the gg and eg sequences, as shown before during the presentation of the individual contributions to the acyl chain conformational disorder (see Figure 4).

Unlike the case of CH₂ wagging bands, which only yield integral values for the amount of gauche conformers over the whole acyl chain, the absolute number of gauche conformers at a particular methylene segment can be obtained via the analysis of CD₂ rocking bands from selectively deuterated lipids. In this spectral region an intense band near 622 cm⁻¹ arises from the rocking vibrations of a CD₂ group adjoining two trans-bonds (tt), while ttgg'/g'tgg and g'tgt/ttgt conformers are responsible for the bands at 646 and 651 cm⁻¹, respectively.^{10,22–25} Figure 6 shows representative FTIR spectra of the CD₂ rocking mode regions of DMPC and DMPGE, selectively deuterated at position C-6 of both fatty acid chains. The left column refers to the pure phospholipid bilayers, and the right column, to samples with 40 mol % cholesterol. The spectra in Figure 6a and b were obtained for the DMPC/water dispersion in the gel and liquid crystalline phases. They clearly demonstrate the aforementioned increase of the amount of gauche conformers upon entering the liquid crystalline state. The spectra of DMPGE, shown in Figure 6c and f, turned out to be more complicated. Here, two additional bands near 637 and 642 cm⁻¹

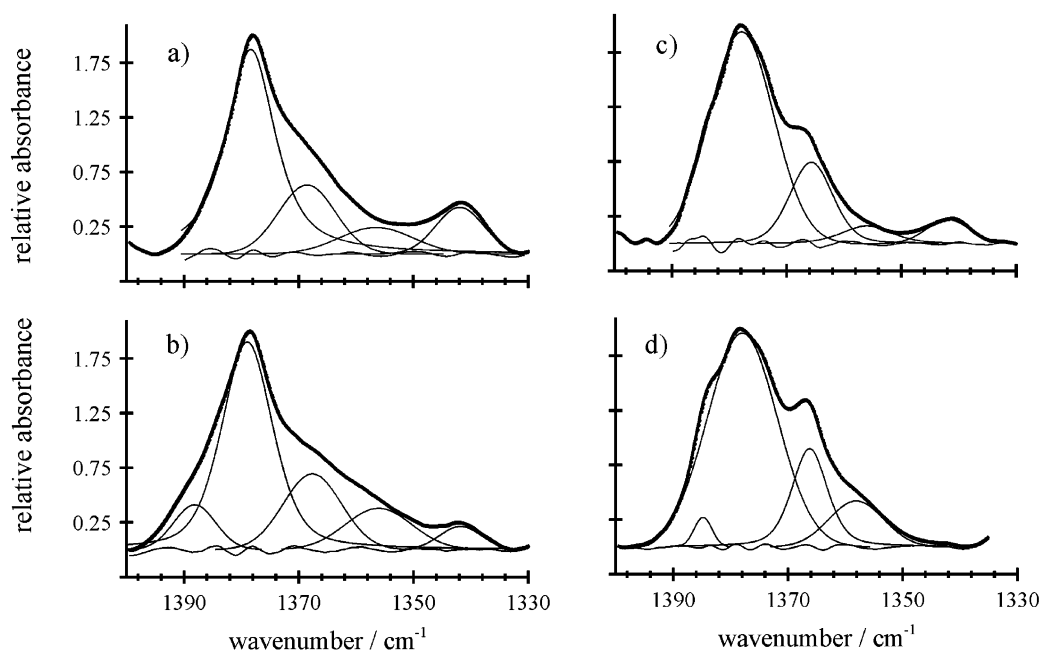


Figure 3. FTIR spectra (CH_2 wagging band region) for (a) pure DMPC bilayers, (b) pure DMPGE bilayers, (c) DMPC/cholesterol, and (d) DMPGE/cholesterol mixtures. The mixtures contain 40 mol % cholesterol. The spectra also contain the theoretical wagging band spectra (see Experimental Section) and the difference between experimental and simulated spectra.

arise in the spectral region of interest which were attributed to vibrations of the glucose unit of the headgroup. In a first attempt we tried to eliminate the interfering band via subtraction of the spectra of the deuterated and the nondeuterated samples, which due to the small signal intensities turned out to be not successful. We therefore performed the curve fitting process by including a total of five vibrational bands, that is, three conformation sensitive bands and two interfering bands, most probably from the monosaccharide. Nevertheless, as demonstrated below, reasonable data could be derived from the rocking band analysis of the DMPGE bilayers.

Figure 7 summarizes the results from the curve fitting analysis of the CD_2 rocking band region for the lipids DMPGE and DMPC, deuterated at carbon C-6 in the acyl chains. The pure lipid/water dispersions exhibit a distinct, almost discontinuous increase of the gauche amount in the vicinity of the phase transition, as also reflected by the aforementioned CH_2 stretching data. The gel phases are characterized by gauche populations of <5% which slightly increase to about 7% just below T_m . In the liquid crystalline state just above T_m the amount of gauche conformers is >20% which increases further upon sample heating. Addition of 40 mol % cholesterol raises the conformational order as compared to that of the pure lipid/water bilayers. This effect is clearly demonstrated at higher temperatures ($T > 309$ K) where the amount of gauche conformers is reduced by more than 60% for the DMPGE/cholesterol mixture. Within the limits of error, the differences between the two phospholipids DMPGE and DMPC are found to be negligible.

^2H and ^{31}P NMR Investigations. Variable temperature ^2H NMR studies were performed on lipid bilayers from DMPGE bearing selectively deuterated acyl chains. Here, three samples, namely the pure lipid/water dispersion and mixtures with 20 and 40 mol % cholesterol, were available with acyl chains deuterated at carbon C-6 ([6,6,6',6'- d_4]-DMPGE), and two samples, that is, the pure/lipid water dispersion and a mixture with 40 mol % cholesterol, were available with acyl chains deuterated at carbon C-13 ([13,13,13',13'- d_4]-DMPGE). In Figure 8 representative ^2H NMR spectra are given for multilamellar dispersions of pure DMPGE and a DMPGE/cholesterol

mixture (40 mol % steroid) in the liquid crystalline or liquid ordered phase, containing fatty acid chains selectively deuterated either at position C-6 or C-13. In the liquid crystalline phase, motionally narrowed ^2H NMR spectra are observed for the present DMPGE samples, as well-known for other phospholipid membranes.^{1–7} The spectral narrowing can be attributed to the presence of various overall and internal motions of the lipid molecule, such as long-axis rotation, long-axis fluctuation, and trans–gauche isomerization, as demonstrated earlier, for example, during extensive line shape and relaxation NMR studies on DMPC or DPPC bilayers.^{1–7,11–14} If these types of motion are fast on the NMR time scale, then the quadrupolar splitting $\Delta\nu_q$, that is, the distance between the perpendicular singularities, is directly connected with the overall order parameter S_{CD} of the lipid molecule by¹

$$\Delta\nu_q = -\frac{3}{4}\left(\frac{e^2qQ}{h}\right)S_{\text{CD}} \quad (1)$$

Here, e^2qQ/h represents the static quadrupolar coupling constant, which for aliphatic deuterons is about 169 kHz.

Figure 8 demonstrates that the actual quadrupolar splitting depends on both the sample temperature and sample composition. For the pure [6,6,6',6'- d_4]-DMPGE/water dispersions in the liquid crystalline phase, quadrupolar splittings between 28 kHz (310 K) and 20 kHz (350 K) are observed, whereas values of about 11 kHz (310 K) to 7 kHz (350 K) are derived for the corresponding bilayers from [13,13,13',13'- d_4]-DMPGE. In the latter case, two splittings are visible due to the inequivalence of the deuterons from *sn*-1 and *sn*-2 chains which also was reported for other systems.⁵¹ Typically, the difference of these splittings is on the order of 1–1.5 kHz. In some cases the second (larger) splitting only shows up as an additional shoulder, as seen in Figure 8a. The incorporation of 40 mol % cholesterol into the DMPGE membrane is manifested by a distinct increase of the experimental quadrupolar splitting, as can be taken from the ^2H NMR spectra in the lower row of Figure 8. Again, the ^2H NMR spectra of the DMPGE/cholesterol mixtures exhibit a

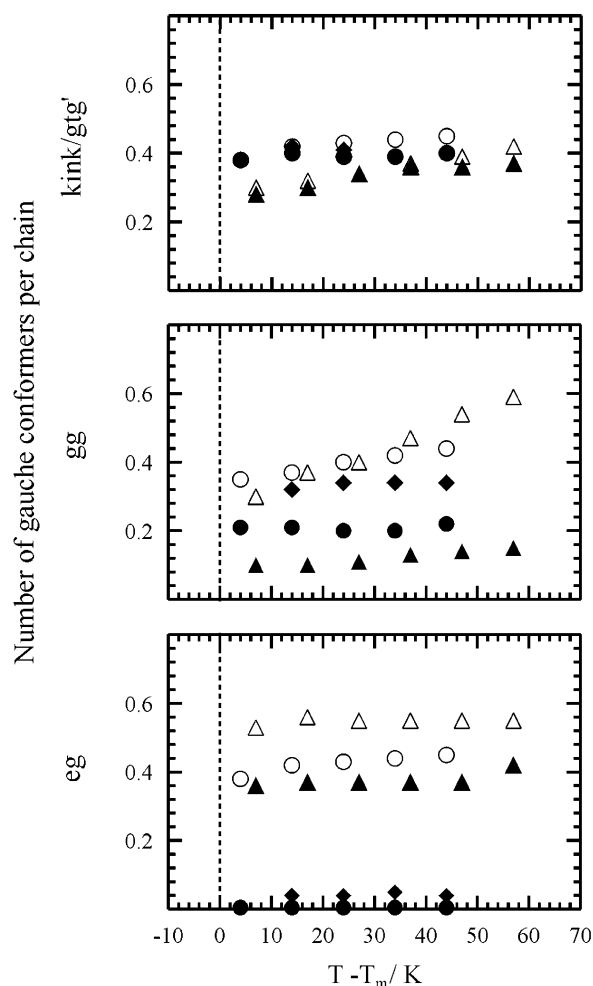


Figure 4. Number of kink/gtg, gg, and eg conformers per chain for pure DMPGE (○) and pure DMPC bilayers (△), DMPGE/cholesterol (◆, 20 mol % cholesterol; ●, 40 mol % cholesterol), and DMPC/cholesterol mixtures (▲, 40 mol % cholesterol).

characteristic temperature dependence, as the quadrupolar splitting increases upon lowering the sample temperature.

The order parameters S_{CD} , derived from the variable temperature ^2H NMR spectra by using eq 1, exhibit the same trends as those found for other lipid systems^{1-3,7,14,52} (see Figure 9). Here, the lower temperature limit for the S_{CD} curves is given by the appearance of slow motional effects in the experimental ^2H NMR spectra, where eq 1 no longer holds. For the present systems in the low temperature region, a temperature interval exists with two overlapping spectral components due to mobile molecules, for which eq 1 still holds, and lipid molecules in the slow motional regime.

In Figure 10 representative ^{31}P NMR line shapes of the liquid crystalline and gel phases are shown. The spectra refer to a pure DMPGE/water dispersion (Figure 10a) and to a sample with 40 mol % cholesterol (Figure 10b). Again, the liquid crystalline phase of the DMPGE/water dispersion as well as the liquid-ordered phase of the lipid/cholesterol mixture are characterized by the presence of axially symmetric powder spectra. The addition of steroid has only a minor influence on the widths of the experimental ^{31}P NMR spectra, as taken from the experimental chemical shift anisotropies $\Delta\sigma$ at higher temperatures. They vary from 41.2 to 43.8 ppm (pure DMPGE) and from 42.0 to 44.5 ppm (DMPGE/cholesterol), respectively. Such rather low values reflect the presence of fast motional processes that cause a substantial reduction of the chemical shift anisotropy

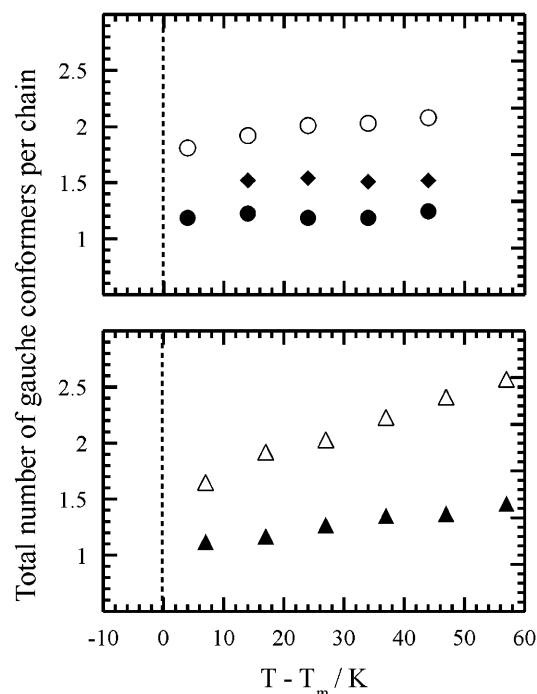


Figure 5. Total number of gauche conformers per chain for pure DMPGE (top) and DMPC bilayers (bottom): ○, △, pure samples; ◆, sample containing 20 mol % cholesterol; ●, ▲, samples containing 40 mol % cholesterol.

derived for static (immobile) systems.^{6,49} At sufficiently low temperatures in the gel phase, ^{31}P NMR spectra are observed that are typical for immobile phospholipids and that are used to derive the static ^{31}P chemical shift tensor components (see right column of Figure 10). For the present systems chemical shift tensor components of $\sigma_{11} = 85$ ppm, $\sigma_{22} = 16$ ppm, $\sigma_{33} = -110$ ppm and $\sigma_{11} = 85$ ppm, $\sigma_{22} = 18$ ppm, $\sigma_{33} = -105$ ppm were derived for the pure lipid/water dispersion and the lipid mixture with 40 mol % cholesterol, respectively. These values are very close to those reported for barium diethyl phosphate⁵³ ($\sigma_{11} = 80$ ppm, $\sigma_{22} = 19$ ppm, and $\sigma_{33} = -113$ ppm), which frequently is used as a reference system during ^{31}P NMR studies in phospholipids.^{6,49}

The variable temperature ^{31}P NMR spectra were further analyzed via line shape simulations in order to derive the (overall) orientational order parameter S_α of the phospholipid molecules. The line shape simulations are based on the accepted assumption that the lipid motion can be described by a rotational diffusion process in an ordering potential (see Experimental Section), that is, free rotation about the molecular long axis and restricted fluctuation of the long axis with respect to the local director axis (= z -axis). In the present analysis we have taken the chemical shift tensor values from the low temperature ^{31}P NMR spectra (see above) and the tensor orientation reported for barium diethyl phosphate, in analogy to the ^{31}P NMR data analysis in related phospholipid systems.⁴⁹ In the liquid crystalline L_α phase above 309 K, the rotational diffusion process could be considered to be in the fast exchange regime, while at lower temperatures also slow motional effects had to be taken into account. The derived order parameters S_α from the ^{31}P NMR data analysis are plotted in Figure 11. They show the typical decrease upon increasing the sample temperature.

The liquid crystalline phase of the pure DMPGE model membrane is characterized by order parameters between 0.75 (310 K) and 0.67 (350 K). In addition, a discontinuity can be observed at the main transition at 309 K, as S_α drops down

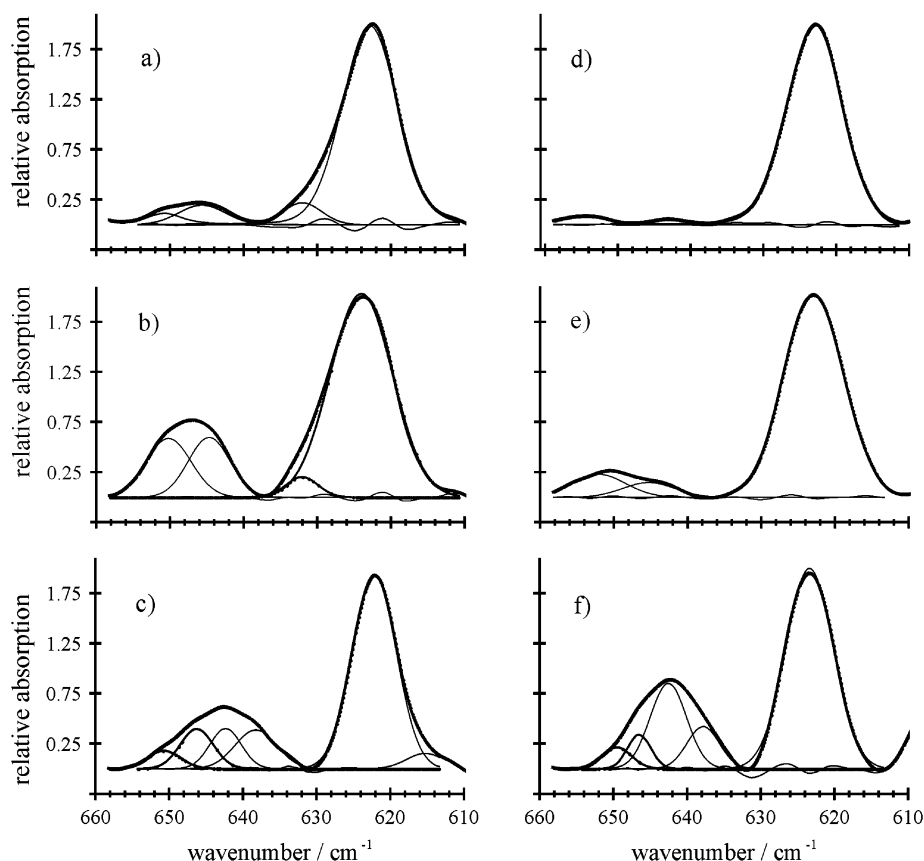


Figure 6. FTIR spectra (CD₂ rocking band region) for pure DMPC (293 K (a) and 313 K (b)) and pure DMPGE bilayers (313 K (c)), DMPC/cholesterol (283 K (d) and 313 K (e)), and DMPGE/cholesterol mixtures (313 K (f)). All phospholipids are selectively deuterated at position C-6 of both fatty acid chains. The mixtures with cholesterol contain 40 mol % cholesterol. The spectra also contain the theoretical spectra (see Experimental Section) and the difference between experimental and simulated spectra.

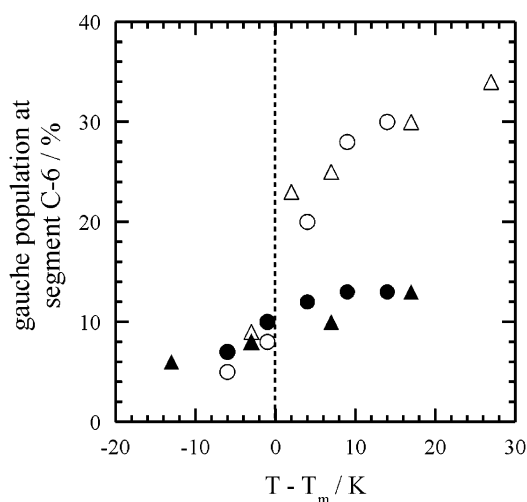


Figure 7. Population of the gauche conformation at chain position C-6 for pure DMPGE (○) and pure DMPC bilayers (△), DMPGE/cholesterol (●), and DMPC/cholesterol (▲) mixtures (40 mol % cholesterol).

from 0.9 in the gel phase to 0.75 in the liquid crystalline phase. In the gel phase the orientational order still increases up to a value of 0.95 at 248 K. The addition of 40 mol % cholesterol alters the molecular order throughout the whole temperature range studied here. At temperatures below 309 K, cholesterol reduces the orientational order by approximately 5–10% as compared to the case of the pure DMPGE bilayer. Since the discontinuity of the S_{α} curve at 309 K is completely missing, higher order parameters than those in pure DMPGE bilayers

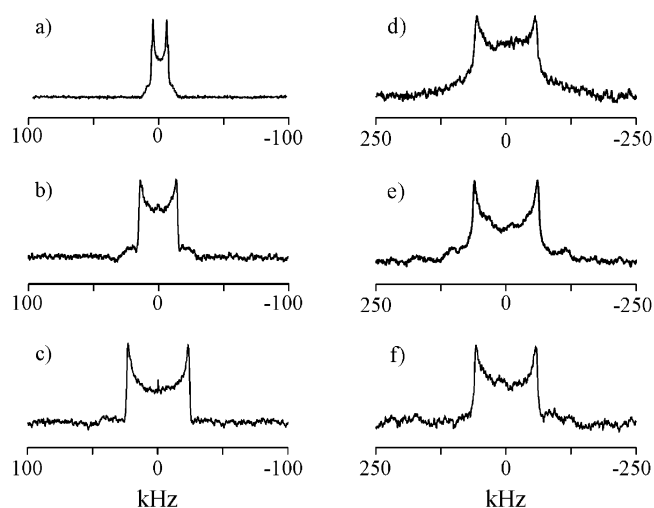


Figure 8. Experimental ²H NMR spectra of pure [13,13,13',13'-d₄]-DMPGE bilayers (315 K (a), 210 K (d)), [6,6,6',6'-d₄]-DMPGE bilayers (315 K (b), 225 K (e)), and [6,6,6',6'-d₄]-DMPGE/40 mol % cholesterol bilayers (315 K (c), 225 K (f)) at two different temperatures.

are obtained for temperatures above 309 K. The DMPGE sample containing only 20 mol % cholesterol exhibits an intermediate character. Here, the discontinuity at around 309 K is much less pronounced than that in pure DMPGE bilayers. The derived S_{α} values are between those found for pure DMPGE bilayers and the DMPGE/steroid mixture with 40 mol % cholesterol. It finally should be noted that neither the NMR nor the FTIR studies indicated a phase separation at higher temperatures, as published previously, for example, for DMPC/cholesterol mixtures.^{54,55}

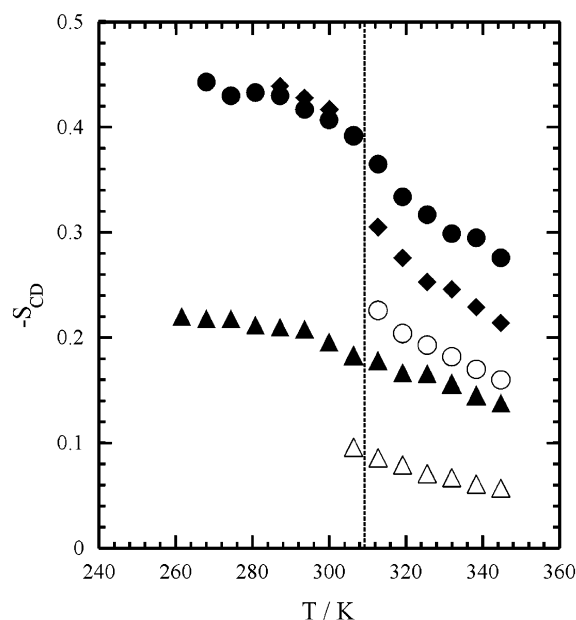


Figure 9. Molecular order parameter S_{CD} for DMPGE selectively deuterated at positions C-6 (○, ◆, ●) and C-13 (△, ▲). The data were calculated from the quadrupolar splittings in the NMR spectra. ○, △, pure DMPGE bilayers; ◆, sample containing 20 mol % cholesterol; ●, ▲, samples containing 40 mol % cholesterol.

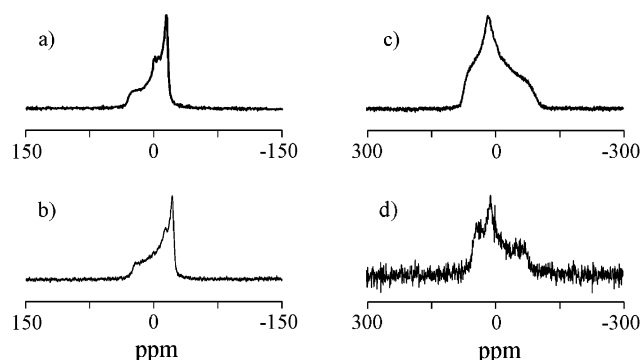


Figure 10. Experimental ^{31}P NMR spectra of pure DMPGE bilayers at 325 K (a) and 230 K (c), and DMPGE/cholesterol mixtures (40 mol % steroid) at 325 K (b) and 235 K (d).

Figure 11 also contains S_α values that were derived in a completely different way, namely by the combination of the ^2H NMR data (S_{CD} values from Figure 10) and FTIR data (amount of gauche conformers from the analysis of the CD_2 rocking data, see Figure 7) for [6,6,6',6'- d_4]-DMPGE samples with selectively deuterated acyl chains. This second method is based on ref 26 and has been used recently in another study on phospholipid bilayers.²⁷ 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^{26,56}

$$p_{\text{trans}} = 1 - p_{\text{gauche}} \quad (2)$$

$$S_\gamma = -\frac{1}{2}p_{\text{trans}} \quad (3)$$

S_α can then be calculated from the order parameters S_{CD} and S_γ using eq 4, which is based on the assumption that a single orientational order parameter S_α is sufficient to describe the overall ordering of the lipid molecules:⁵⁶

$$S_{CD} = S_\alpha S_\gamma \quad (4)$$

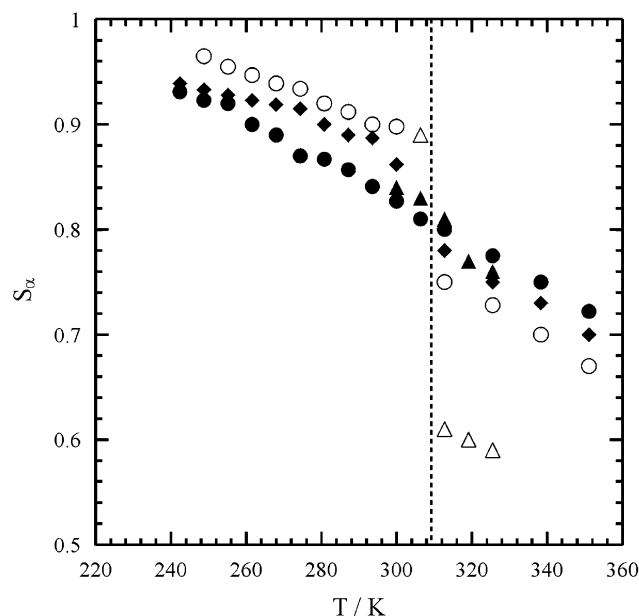


Figure 11. Orientational order parameter S_α from (a) the ^{31}P NMR data analysis for pure DMPGE bilayers (○) and DMPGE/cholesterol mixtures containing 20 mol % (◆) or 40 mol % cholesterol (●) and (b) a combined ^2H NMR and FTIR data analysis for pure [6,6,6',6'- d_4]-DMPGE bilayers (△) and a [6,6,6',6'- d_4]-DMPGE/cholesterol mixture (▲) with 40 mol % cholesterol. For further details, see text.

Orientational order parameters obtained via this second procedure for pure DMPGE bilayers and the DMPGE/cholesterol mixture (40 mol % cholesterol) are also given in Figure 11. For pure DMPGE bilayers values between 0.61 (313 K) and 0.59 (323 K) are observed in the liquid crystalline phase, which is about 15–20% lower than the values derived from the ^{31}P NMR line shape analysis. At the same time, consistent S_α values are found for the DMPGE/steroid mixture with 40 mol % cholesterol. Here, the S_α values from both methods (i.e., ^{31}P NMR analysis or combined ^2H NMR/FTIR analysis) are almost identical throughout the whole temperature range covered.

With the known order parameters S_α from the analysis of the ^{31}P NMR data (Figure 11), the molecular order parameters S_{CD} (Figure 9), and eq 4, we are able to calculate the segmental order parameter S_γ of the various DMPGE samples, selectively deuterated at positions C-6 and C-13 in the acyl chains. The results, summarized in Figure 12, show distinct dependences of S_γ on the cholesterol content, chain position, and sample temperature, in quite the same way as discussed for the S_{CD} curves. Thus, again, S_γ decreases with temperature and distance from the glycerol backbone and increases with the actual cholesterol content. Inspection of Figure 12 also reveals that the temperature dependence of S_γ is more pronounced for acyl position C-6 than for position C-13.

Discussion

To date, there exists a great number of studies dealing with phospholipids of quite different chemical constitution.^{1–27} Although investigations have been reported on lipids with various polar headgroups, like choline, glycerol, or serine, phospholipids bearing cyclic alcohols are only rarely considered. In the following we will compare the data from the present study on DMPGE with the those from the well-known phospholipid DMPC, which only differ in the polar headgroup. The main differences between both phospholipids therefore are the head-group size, the overall electrical charge in the upper part of the

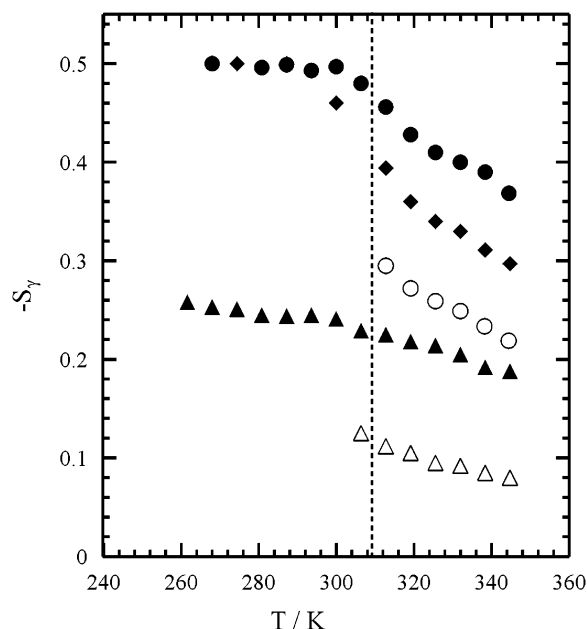


Figure 12. Segmental order parameter S_γ for DMPGE bilayers, selectively deuterated in the acyl chains at positions C-6 and C-13: ○, pure [6,6,6',6'-d₄]-DMPGE bilayers; ◆, [6,6,6',6'-d₄]-DMPGE bilayers with 20 mol % cholesterol; ●, [6,6,6',6'-d₄]-DMPGE bilayers with 40 mol % cholesterol; △, pure [13,13,13',13'-d₄]-DMPGE bilayers; ▲, [13,13,13',13'-d₄]-DMPGE bilayers with 40 mol % cholesterol. For further details, see text.

lipid bilayers, and the increased number of possible hydrogen bonds.

Orientational Order. In the present study for the first time two methods were employed for the determination of the orientational order parameter S_α . The first method is based on a ^{31}P NMR line shape analysis, where the motional averaging of the chemical shift tensor by the overall lipid reorientation (via anisotropic rotational diffusion) was explicitly considered. The second procedure refers to a combined ^2H NMR and FTIR data analysis of the measurements on lipids with selectively deuterated acyl chains ([6,6,6',6'-d₄]-DMPGE). The temperature dependence of the orientational order parameter S_α , shown in Figure 11, is found to be very typical for phospholipid membranes.^{13,14,49,52} The increase of the order parameter in the gel phase can be attributed to the presence of highly ordered lipid molecules in a dense molecular packing, which is well-known from other lipid/water dispersions. Likewise, in the liquid crystalline L_α phase thermally activated molecular processes give rise to a reduction of the alignment of the lipid molecules, as reflected by the continuous decrease of S_α with increasing temperature.

Addition of cholesterol (20 and 40 mol %) generally reduces the discontinuity of the orientational order parameter in the vicinity of the calorimetric main transition (from the liquid crystalline phase to the gel phase) of the pure phospholipid bilayers. The observation of a lower orientational order in the DMPGE/cholesterol sample below 309 K is well-known and reflects the property of the steroid to prevent a crystallization of the fatty acid chains.⁵⁷ At the same time, the DMPGE/cholesterol sample exhibits higher order parameters above 309 K if compared with the pure DMPGE bilayer. At these higher temperatures the incorporation of 40 mol % steroid increases the molecular packing density and thus prevents the distortion of the lamellar arrangement by extensive molecular fluctuations, as expected for pure DMPGE/water dispersions. Both results are consistent with the so-called “liquid ordered phase”, which

frequently is discussed for phospholipid/steroid mixtures.⁵⁰ It turns out that the present S_α values for the various DMPGE samples, as derived from the ^{31}P NMR data, are very similar to those reported earlier for DMPC or DPPC bilayers.^{27,49} Obviously, the influence of the present headgroup is of minor importance for the overall orientational order of the lipid molecules. The above results also demonstrate that the present assumptions for the ^{31}P NMR analysis (i.e., chemical shift tensor values and tensor orientation, rotational diffusion process) are correct.

The former discussion referred mainly to the data derived from the ^{31}P NMR data analysis. In the case of the pure DMPGE/water dispersion, inspection of Figure 11 reveals differences between the S_α data sets obtained from the ^{31}P NMR line shape analysis and the combined analysis of ^2H NMR and FTIR data. A possible explanation for these differences might be found in the assumption^{26,56} that only kink conformers contribute to the segmental order parameter S_γ , although the analysis of the CH_2 wagging data clearly proved that gg conformers also exist in the present lipid bilayers. In addition, isolated gauche defects might exist as well, which in the present work are not directly accessible. It is therefore very likely that the “true” segmental order parameter S_γ is lower and the present S_γ and S_α values represent the upper limit and lower limits, respectively. Another source for the discrepancies of the S_α curves of pure DMPGE bilayers could be found in the assumption of a single orientational order parameter for the whole lipid molecule. It might well be that for this system at higher temperatures a decoupling of the headgroup and the acyl chain region ordering is more appropriate; that is, the two molecular parts should be treated separately. In fact, previous ^2H and ^{31}P NMR data on DMPC also reported on differences for the orientational order of the acyl chain and the headgroup region.^{13,14,46,49} Likewise, recent relaxation studies⁵⁸ also pointed to a decoupling of the overall motions of the headgroup and acyl chain region which certainly should affect the ordering characteristics as well.

Surprisingly, for the DMPGE/cholesterol bilayers with 40 mol % cholesterol, the derived order parameters S_α are found to be almost identical and independent of the actual procedure used for the experimental data analysis. This sample, however, is characterized by a considerable lower amount of gauche defects; that is, a reduced contribution to S_γ from gg conformers and isolated gauche sequences is expected, as compared to that for the pure DMPGE/water dispersions. At the same time, the rigid cholesterol molecules should also reduce the aforementioned decoupling of the headgroup and the acyl region order, which might also explain the consistency of the S_α values in the DMPGE/cholesterol bilayers. A final proof for these assumptions as well as for their actual importance, however, cannot be given on the basis of the present experimental data.

Conformational Order. Information about the conformational order of the present lipid systems was obtained in various ways, including the analysis of different IR vibrational bands (CH_2 stretching bands, CH_2 wagging bands, and wagging band progressions, CD_2 rocking bands) as well as ^2H NMR data from DMPGE samples with selectively deuterated acyl chains.

On a more qualitative basis, the temperature-dependent CH_2 stretching bands and wagging band progressions clearly demonstrated that for pure DMPGE bilayers the conformational order of the acyl chains is significantly reduced at the transition to the liquid crystalline L_α phase, in close agreement with the observations for other lipids.^{8–10} Moreover, the observed discontinuity of the CH_2 stretching band positions and wagging

progression intensities at the calorimetric main transition of the pure DMPGE bilayers is largely reduced upon addition of cholesterol. Both observations can be explained by a stabilization effect of the steroid, which, in particular, holds for higher temperatures along with the formation of a liquid-ordered phase.⁵⁰ Here, due to thermal fluctuations, free volume is created in the acyl chain region which partially is compensated by the presence of the steroid.

Two general trends can be deduced from the derived amounts of gauche conformers from the CH₂ wagging data (see Figures 4 and 5), namely a relatively small temperature dependence for all DMPGE samples and a pronounced reduction of the amount of gauche conformers upon addition of cholesterol. This latter ordering effect can be mainly traced back to a strong reduction of the amount of gg and eg sequences (see Figure 4). The effect of cholesterol on the gg conformers can be rationalized by the large spatial requirement for this gauche sequence. A better packing caused by the addition of cholesterol therefore should destabilize, in particular, gg conformers. The gg amounts in DMPGE bilayers are found to be less affected by the cholesterol content. This observation can be attributed to the larger size of the DMPGE headgroup along with the larger free volume in the acyl chain region. Due to the smaller spatial constraints, the influence of the cholesterol content on the kink/gtg' sequences should be less pronounced, as also observed experimentally. The strong influence of the cholesterol content on the amount of eg sequences cannot only be caused by the presence of the steroid, since the rigid part of the cholesterol is located in the upper part of the acyl chain region. It might be speculated whether for the DMPGE/cholesterol mixtures the individual lipid layers partially interdigitate, resulting in the distinct reduction of eg sequences. This assumption is further supported by the fact that the amount of eg sequences in DMPGE samples is generally lower than that in DMPC samples, even in the absence of cholesterol. In addition, the comparison of the segmental order parameters S_γ (see below) at chain position C-13 in DMPC⁵⁹ and DMPGE is in qualitative agreement with these findings for the eg conformers. That is, at chain position C-13 the ordering effect of cholesterol in DMPGE (increase in chain order by about 55%) again is significantly higher than in DMPC (increase by about 40%).

A further comparison of the two phospholipids DMPGE and DMPC indicates that the actual headgroup has some influence on the overall acyl chain conformation and packing in the L _{α} phase. Thus, just above the main transition the averaged number of gauche conformers and thus the conformational disorder are higher in DMPGE bilayers, while DMPC bilayers exhibit a stronger temperature dependence. As outlined above, the higher gauche amount can be explained by the larger size of the DMPGE headgroup, which creates free volume in the acyl chain region even at low temperature. At the same time the choline group in DMPC seems to facilitate the formation of free volume at higher temperatures, as compared to the case of the glucose headgroup. This latter observation might point to the stronger intermolecular interactions between the sugar rings in the DMPGE headgroup due to the larger number of hydroxyl groups, which prevents a thermally activated formation of gauche defects at higher temperatures.

The gauche populations at a specific chain segment (C-6) were determined from the analysis of the CD₂ rocking vibration bands (see Figure 7). Here again, a strong influence by the addition of cholesterol could be registered for both the DMPGE and DMPC bilayers, as the gauche population drops down from about 30% (pure lipid bilayers) to about 12% (lipid/40mol %

cholesterol mixture) in the L _{α} phase. This influence of the cholesterol addition as well as the observed temperature variation are in qualitative agreement with aforementioned results from the CH₂ stretching and wagging data. These findings and the similar values reported for related lipids, such as DPPC,^{10,26,27} again reveal a minor influence of the glucose headgroup on the conformational order in the acyl chain region in DMPGE bilayers.

Another quantity that describes the conformational order at a particular methylene group is the segmental order parameter S_γ , as given in Figure 12 for the acyl positions C-6 and C-13 referring to various samples of different cholesterol content. This quantity was derived with the help of eq 4, that is, with the assumption that a single orientational order parameter S_α is sufficient to describe the overall ordering of the lipid molecules, which—as mentioned earlier—might not hold for all cases. Nevertheless, the observed dependences of S_γ on the sample temperature and cholesterol content are found to be in qualitative agreement with the results from the aforementioned FTIR data analysis. Thus, the rigid cholesterol molecules provide a stabilization of the lipid bilayers by increasing in addition the acyl chain conformational order. At the same time, thermally activated fluctuations give rise to a larger spatial freedom in the acyl chain region along with a larger conformational disorder upon temperature increase. The stronger temperature dependence of S_γ for carbon position C-6 might be related to the larger headgroup for the present DMPGE samples, which creates some spatial freedom in the upper part of the acyl chains. Nevertheless, the absolute S_γ values in all [6,6,6',6'-d₄]-DMPGE samples are still substantially higher than those found in [13,13,13',13'-d₄]-DMPGE bilayers.

With the assumption that only kink conformers are contributing,^{26,56} eqs 2 and 3 could be used to calculate from the segmental order parameter S_γ directly the amount of trans or gauche conformers at a specific deuterated chain segment. From this, however, unrealistically high amounts of gauche conformers show up, which holds in particular for chain position C-13 (i.e., amount of gauche conformers up to 80%). As already outlined before during the discussion of the orientational order parameter data, the simple consideration of only kink conformers as a contribution to the segmental order certainly is oversimplified. It is therefore very likely that the discrepancy can be attributed to the presence of double gauche conformers—as detected during the FTIR measurements—as well as single gauche sequences, the latter of which could not be quantified further by the present spectroscopic techniques.

Conclusions

In the present work a new synthetic phospholipid DMPGE, bearing the monosaccharide glucose as the polar part of the amphiphilic molecule, was investigated by FTIR and solid-state NMR spectroscopy. The present study addressed the evaluation of the ordering characteristics of the lipid molecules as a function of temperature and sample composition. Particular emphasis was given to the separation of the (overall) orientational order of the lipid molecules and the conformational order of the acyl chains.

The conformational order was accessible via the analysis of various conformation sensitive vibration bands. For the determination of the orientational chain order, two different procedures were employed simultaneously, which—to our knowledge—so far has never been done in this context. The first route was based on the line shape analysis of temperature dependent ³¹P NMR spectra, where, with the known molecular geometry of

the lipid molecules and magnetic shielding tensor orientation, the orientational order parameter S_α could be determined as a function of temperature and sample composition. These data exhibited a strong impact of the actual sample temperature and cholesterol content on the orientational order parameter. The influence of the actual headgroup structure on the orientational order, however, was found to be almost negligible. The second route to the orientational order parameter exploited the combination of the CD₂ rocking data and the results from the analysis of ²H NMR spectra from selectively deuterated phospholipids (for carbon position C-6). This latter analysis revealed a significant lower orientational order for the pure DMPGE lipid bilayer, as found during the aforementioned ³¹P NMR data analysis. It was speculated whether this latter observation can be attributed to a decoupling of the headgroup ordering and the acyl chain ordering. Another potential source was attributed to the presence of double gauche and isolated gauche sequences, which were not accounted for in the latter data analysis.

In summary, it could be demonstrated that the combination of variable temperature FTIR and solid-state NMR spectroscopy provides valuable information about the ordering characteristics of lipid bilayers. Further work along this line which addresses the specific molecular features of other unusual phospholipids is in progress.

Acknowledgment. Financial support for this project by the Deutsche Forschungsgemeinschaft, the Fonds der Chemischen Industrie (FCI), and the Bundesministerium für Forschung und Technologie (BMFT) is gratefully acknowledged. One of us (P.W.) wishes to acknowledge for a doctoral fellowship sponsored by the FCI and the BMFT. The authors would like to acknowledge valuable discussions with Dr. R. Lehnert.

References and Notes

- Seelig, J.; Seelig, A. *Q. Rev. Biophys.* **1980**, *13*, 19.
- Griffin, R. G. *Methods Enzymol.* **1981**, *72*, 108.
- Davis, J. H. *Biochim. Biophys. Acta* **1983**, *737*, 117.
- Davis, J. H. *Adv. Magn. Res.* **1989**, *13*, 195.
- Evans, J. N. *Biomolecular NMR Spectroscopy*; Oxford University Press: 1995.
- Gorenstein, D. *³¹P NMR, Principles and Applications*; Academic Press: New York, 1984.
- Seelig, J. *Biochim. Biophys. Acta* **1978**, *515*, 105.
- Lewis, R. N. A. H.; McElhaney, R. N. In *Infrared Spectroscopy of Biomolecules*; Mantsch, H. H., Chapman, B., Eds.; Wiley: New York, 1996; p 159.
- Mantsch, H. H.; Casal, H. L.; Jones, R. N. In *Spectroscopy in Biological Systems*; Clark, R. J. H., Hester, R. E., Eds.; Wiley: New York, 1986; p 1.
- Mendelsohn, R.; Snyder, R. G. In *Biological Membranes*; Merz, K. R., Roux, B., Eds.; Birkhäuser: Basel, 1996; p 145.
- Campbell, R. F.; Meirovitch, E.; Freed, J. H. *J. Phys. Chem.* **1979**, *83*, 525.
- Wittebort, J.; Blume, A.; Huang, T. H.; DasGupta, S. K.; Griffin, R. G. *Biochemistry* **1982**, *21*, 3487.
- Meier, P.; Ohmes, E.; Kothe, G.; Blume, A.; Weidner, J.; Eibl, H. *J. Phys. Chem.* **1983**, *87*, 4904.
- Mayer, C.; Müller, K.; Weisz, K.; Kothe, G. *Liq. Cryst.* **1988**, *3*, 797.
- Snyder, R. G. *J. Chem. Phys.* **1967**, *47*, 1316.
- Maroncelli, M.; Strauss, H. L.; Snyder, R. G. *J. Chem. Phys.* **1985**, *82*, 2811.
- Mantsch, H. H.; McElhaney, R. N. *Chem. Phys. Lipids* **1981**, *57*, 213.
- Senak, L.; Moore, D.; Mendelsohn, R. *J. Phys. Chem.* **1992**, *96*, 2749.
- Chia, N.; Mendelsohn, R. *J. Phys. Chem.* **1992**, *96*, 10543.
- Casal, H.; McElhaney, R. N. *Biochemistry* **1990**, *29*, 5423.
- Senak, L.; Davies, M. A.; Mendelsohn, R. *J. Phys. Chem.* **1991**, *95*, 2565.
- Snyder, R. G.; Poore, M. W. *Macromolecules* **1973**, *6*, 708.
- Maroncelli, M.; Strauss, H. L.; Snyder, R. G. *J. Phys. Chem.* **1985**, *89*, 4390.
- Davies, M. A.; Schuster, H.; Brauner, J. W.; Mendelsohn, R. *Biochemistry* **1990**, *29*, 4368.
- Mendelsohn, R.; Davies, M. A.; Brauner, J. W.; Schuster, H.; Dluhy, R. A. *Biochemistry* **1989**, *28*, 8934.
- Davies, M. A.; Hübner, W.; Blume, A.; Mendelsohn, R. *Biophys. J.* **1992**, *63*, 1059.
- Lehnert, R.; Eibl, H.-J.; Müller, K. *J. Phys. Chem.* **2003**, *B107*, 75.
- Beridge, M. J. *Nature* **1984**, *312*, 315.
- Low, M. G. *Biochim. Biophys. Acta* **1987**, *988*, 427.
- Mato, J. M.; Kelly, K. L.; Abler, A.; Jarrett, L. *J. Biol. Chem.* **1987**, *262*, 2131.
- Hansbro, P. M.; Stephen, J. B.; Bushby, R. J.; Turnbull, P. J. H.; Boden, N.; Saunders, M. R.; Novelli, R.; Reid, D. G. *Biochim. Biophys. Acta* **1992**, *1112*, 187.
- Bradshaw, J. P.; Bushby, R. J.; Giles, C. C. D.; Saunders, M. R.; Reid, D. G. *Nature structural biology*; 1996; Vol. 3, p 125.
- Norman, R. O. C.; Coxon, J. M. *Principles of Organic Synthesis*; Blackie Academic and Professional: London, 1993.
- Zimmermann, H. *Liq. Cryst.* **1989**, *4*, 591.
- Rürup, J.; Mannova, M.; Brezesinski, G.; Schmid, R. D. *Chem. Phys. Lipids* **1994**, *70*, 187.
- Pisch, S.; Bornscheuer, U. T.; Meyer, H. H.; Schmid, R. D. *Tetrahedron* **1997**, *53*, 14627.
- Shuto, S.; Ueda, S.; Imamura, S.; Fukukawa, K.; Matsuda, A.; Ueda, T. *Tetrahedron Lett.* **1987**, *28*, 199.
- Shuto, S.; Imamura, S.; Fukukawa, S.; Sakakibara, H.; Murase, J.-I. *Chem. Pharm. Bull.* **1987**, *35*, 447.
- Nakajima, J.; Nakashima, T.; Shima, Y.; Fukuda, H.; Yamane, T. *Biotechnol. Bioeng.* **1994**, *44*, 1193.
- Solomon, I. *Phys. Rev.* **1958**, *110*, 61.
- Davis, J. H.; Jeffrey, K. R.; Bloom, M.; Valic, M. I.; Higgs, T. P. *Chem. Phys. Lett.* **1976**, *42*, 390.
- Schmider, J.; Müller, K. *J. Phys. Chem.* **1998**, *A102*, 1181.
- Wolfangel, P.; Meyer, H. H.; Bornscheuer, U. T.; Müller, K. *Biochim. Biophys. Acta* **1999**, *1420*, 121.
- Liebelt, A.; Detken, A.; Müller, K. *J. Phys. Chem.* **2002**, *B106*, 7781.
- Smith, B. T.; Boyle, J. M.; Garbow, B. S.; Ikebe, Y.; Klema, V. C.; Moler, C. B. *Matrix Eigensystem Routines-EISPACK Guide*; Springer: Berlin, 1976.
- Meier, P.; Ohmes, E.; Kothe, G. *J. Phys. Chem.* **1986**, *85*, 3598.
- Müller, K.; Meier, P.; Kothe, G. *Prog. Nucl. Magn. Reson. Spectrosc.* **1985**, *17*, 211.
- Moro, G.; Segre, U.; Nordio, P. L. In *Nuclear Magnetic Resonance of Liquid Crystals*; D. Reidel Publ.: Dordrecht, 1985; p 207.
- Dufourc, E. J.; Mayer, C.; Stohrer, J.; Althoff, G.; Kothe, G. *Biophys. J.* **1992**, *61*, 42.
- Ipsen, J. H.; Mouritsen, O. G.; Zuckermann, M. J. *Biophys. J.* **1989**, *56*, 661.
- Seelig, A.; Seelig, J. *Biochemistry* **1974**, *13*, 4839.
- Blume, A. In *Phospholipids Handbook*; Cevc, G., Ed.; Marcel Dekker: New York, 1993; Chapter 13.
- Herzfeld, J.; Griffin, R. G.; Haberkorn, R. A. *Biochemistry* **1978**, *17*, 2711.
- Janiak, M. J.; Small, D. M.; Shipley, G. G. *J. Biol. Chem.* **1979**, *254*, 6068.
- Vist, M. R.; Davies, J. H. *Biochemistry* **1990**, *29*, 451.
- Petersen, N. O.; Chan, S. I. *Biochemistry* **1977**, *16*, 2657.
- Cevc, G., Ed. *Phospholipids Handbook*; Marcel Dekker: New York, 1993.
- Lehnert, R.; Wolfangel, P.; Müller, K. In preparation.
- Weisz, K.; Gröbner, G.; Mayer, C.; Stohrer, J.; Kothe, G. *Biochemistry* **1992**, *31*, 1100.