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FTIR studies of phospholipid membranes containing monoacetylenic acyl chains

Peter Wolfangel,[a] René Lehnert,[a] Hartmut H. Meyer[b] and Klaus Müller*[a]

[a] *Institut für Physikalische Chemie, Universität Stuttgart, Pfaffenwaldring 55, D-70569 Stuttgart, Germany. E-mail: k.mueller@ipc.uni-stuttgart.de*
[b] *Institut für Organische Chemie, Universität Hannover, Schneiderberg 1B, D-30167 Hannover, Germany*

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The conformational behaviour of phosphatidylcholine and phosphatidyl acid membranes derived from octadec-14-ynoic and octadec-9-ynoic acid is studied by FTIR spectroscopy comprising both the liquid crystalline and gel phases. In the vicinity of the transition from the gel to the liquid crystalline phase the conformational changes of the acyl chains are followed by the analysis of the CH_2 stretching bands and CH_2 wagging band progressions. In the liquid crystalline phase a quantitative determination of the amounts of *gauche-trans-gauche*, double *gauche*, and end *gauche* conformers is achieved by the analysis of the CH_2 wagging band region. In this connection, several model compounds (hex-2-yne, and hex-3-yne, oct-4-yne) have been examined to assist the assignment of a special vibration band at 1328 cm^{-1} due to the CH_2–C≡C unit. The results for the phospholipids studied here clearly demonstrate that the conformational properties critically depend on their actual lipid structure, sample composition and sample temperature. The derived data are discussed in conjunction with earlier investigations of more "conventional" lipid systems—mainly containing saturated acyl chains—to evaluate the specific influence of the incorporation of the C–C triple bond into the fatty acid chains.

Introduction

During recent years biological membranes have been studied and characterized by various spectroscopic techniques, such as NMR[1–3] and IR spectroscopy.[4–7] One major issue of the application of IR spectroscopy was to monitor the specific conformational features of the aliphatic chain region in phospholipid molecules.[6] Here, several vibration bands are accessible from which the desired information could be deduced. For instance, the frequency shift of the CH_2 stretching bands at about 2850 and 2925 cm^{-1} has been used as a qualitative measure of the changes in confomational order that sets in at the transition between the gel and liquid crystalline phase. The same holds for the intensity of the CH_2 wagging band progressions between 1150 and 1350 cm^{-1} that monitor the changes of the amount of all-*trans* chains at this phase transition.[8,9] Quantitative information about the presence and amounts of various *gauche* conformers (kink, double *gauche*, end *gauche* conformers) in the liquid crystalline phase is accessible by the analysis of the wagging band intensities[10–12] between 1330 and 1400 cm^{-1}. In the past, the wagging band intensities of a great number of different staturated and unsaturated phospholipids have been examined, comprising single and multicomponent lipid bilayers. It, however, should be kept in mind that the wagging band intensities can only provide integrated values for the various conformers over both acyl chains. Information about the conformation at a specific methylene segment on the other hand can be achieved from the analysis of CD_2 rocking bands[13,14] between 610 and 660 cm^{-1}. In that case, lipids with specifically deuterated acyl chains are required. Since, in addition, CD_2 rocking bands are very weak and are sometimes obscured by other strong absorption bands, the number of studies of CD_2 rocking bands in lipid membranes is rather limited.

In this contribution we present variable temperature FTIR studies of new model membranes that are based on 1,2-dioctadec-(14-ynoyl)-*sn*-glycero-3-phosphatidylcholine [DO(14-yne)PC] and 1,2-dioctadec-(9-ynoyl)-*sn*-glycero-3-phosphatidylcholine [DO(9-yne)PC], *i.e.* phospholipids bearing an acetylenic group at carbon 14 or at carbon 9 of the acyl chains.[15–17] These phospholipids have gained some interest during an ongoing program on the application of phospholipids in the stabilization of membrane proteins. DO(14-yne)PC is of particular interest due to the main transition being close to physiological temperatures.[15,16] It should be noted that the molecular behaviour of these lipid systems has also been studied independently by dynamic ^2H, ^{31}P and ^{13}C NMR spectroscopy. Those studies focus on the impact of the C≡C unit on the specific conformational and overall (reorientational, lateral) lipid dynamics.[17,18] The phospholipids studied here also should be distinguished from the well-known lipids with diacetylenic groups that are of potential interest in the field of drug delivery.[19]

The present FTIR studies have been performed on fully hydrated bilayers containing either the pure lipids or lipid/cholesterol mixtures. Particular emphasis was given to the analysis of the CH_2 stretching and wagging bands from which the conformational features in the acyl chain region could be evaluated. The comparison with the data derived from other studies on saturated phospholipids—such as DMPC—revealed the specific conformational behaviour of the present model membranes that is related to the incorporation of an isolated C–C triple bond in the fatty acid chains.

Experimental section

Materials

The starting materials for the synthesis were purchased either

from Aldrich Chemicals (Steinheim, Germany) or from Fluka Chemie AG (Buchs, Switzerland). Cholesterol and myristic acid were obtained from Sigma Chemicals (Deisenhofen, Germany) and GPC, *sn*-glycero-3-phosphatidylcholine, from Lukas Meyer (Hamburg, Germany). Octadec-14-ynoic acid, octadec-9-ynoic acid and octadec-14-ynoic acid, deuterated at position C-13, have been synthesized, as described earlier.[17] The methyl ester of octadec-14-ynoic acid was obtained by the reaction of the corresponding acid with CH_2N_2, according to standard procedures.

For the preparation of the phospholipids 1,2-dioctadec-(14-ynoyl)-*sn*-glycero-3-phosphatidylcholine [DO(14-yne)PC] and 1,2-dioctadec-(9-ynoyl)-*sn*-glycero-3-phosphatidylcholine [DO(9-yne)PC] a previously published preparation method was used.[15,16] Thus, the desired octadecynoic acid (1 g, 3.57 mmol), dissolved in 40 ml of dry chloroform, was first transformed into its imidazolide by addition of *N*,*N*-carbonyldiimidazole (630 mg, 3.89 mmol) and stirring at room temperature for about 45 min. Afterwards, carefully dried *sn*-glycero-3-phosphatidylcholine (335 mg, 1.3 mmol) and 1,8-diaza-bicyclo[5.4.0]undec-7-ene (543 mg, 3.57 mmol) were co-added. After further stirring at room temperature for at least 24 h, the solvent was removed. DO(14-yne)PC and DO(9-yne)PC of high purity were obtained from the residue by recrystallization in acetone. 1,2-Dimyristoyl-*sn*-glycero-3-phosphatidylcholine (DMPC) was obtained in an analogous way by using myristic acid instead of octadecynoic acid.

To obtain the corresponding phosphatidyl acid, 1,2-dioctadec-(14-ynoyl)-*sn*-glycero-3-phosphatidyl acid [DO(14-yne)PA], the choline head group was split off by the enzyme phospholipase D (PLDP), employing the following procedure: 300 mg (0.38 mmol) DO(14-yne)PC were dissolved in 10 ml chloroform and heated to 45 °C. Subsequently, 5 ml of a buffered solution (0.1 M NaAc, 0.1 M $CaCl_2$, 0.01 M EDTA at pH 5.6) containing the enzyme were co-added. The biphasic reaction mixture was stirred vigorously for about 2–3 h. By addition of 50 ml of Folch solution ($CHCl_3$/MeOH = 2 : 1) the reaction was stopped. The product was separated from the organic phase and further recrystallized in acetone.

Sample preparation

Multilamellar dispersions (steroid-free samples) were prepared by the hydration of 10 mg of dry lipid in about 15 ml deuterium-depleted water. Homogenization was obtained by repeated freeze–thawing, centrifuging and vortexing of the sample. The construction of the IR cell excluded a loss of water and thus incomplete hydration. The cholesterol containing samples were prepared by dissolving the appropriate phospholipid and cholesterol (3 : 2 molar ratio) in a small amount of freshly distilled chloroform. The solvent was partially removed by a stream of nitrogen gas, and the residual solvent was evaporated under vacuum for a few hours. After this procedure the dry mixture was dispersed in deuterium-depleted water in the same way, as described above. Liquid alkynes were used without any further treatment and octadec-14-ynoic acid was dissolved in a small amount of tetrachloromethane.

IR measurements

Both phospholipid dispersions and reference alkynes were placed in 25 or 50 μm thick infrared cells with CaF_2 windows. The IR cell was thermostatted with a variable temperature unit from LOT/Oriel (Langenberg, Germany). All IR spectra were recorded with a Bruker IFS 66 FT IR spectrometer (Karlsruhe, Germany). Typically 32 to 128 interferograms were collected, apodized with a triangular function and Fourier transformed with one level of zero-filling. The spectral resolution was 2 cm^{-1}. For each system three samples of the same composition were prepared. The whole series of variable temperature spectra was measured twice for each sample. The data analysis is thus based on an average of six measurements.

IR data analysis

For the analysis of the CH_2 wagging band region the Bruker IFS 66 spectrometer software (Opus 3.0) was used. In the case of the phospholipid model membranes the spectral region of interest (1315 to 1400 cm^{-1}) was first flattened by a linear baseline. Afterwards, the spectra were iterated using five 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), 1342 cm^{-1} (end *gauche* sequences) and 1330 cm^{-1}. During the least-squares fit analysis the band intensities and widths were varied independently. Variation of the band positions on the other hand was restricted to ± 2 cm^{-1} of the values given above. The integrated intensities of the CH_2 wagging bands were furthermore normalized with respect to the methyl deformation band. The amount of specific *gauche* sequences were calculated according to the procedure given by Senak *et al.*[11] that is based on reference measurements on *n*-alkanes and a theoretical approach using the rotational isomeric state (RIS) model.[20] Care was taken over the fact that the phospholipids contain only one methyl group per fatty acid chain in contrast to two methyl groups in *n*-alkanes. The total number of *gauche* conformers per chain is obtained by taking into account that two *gauche* bonds are necessary to build up one kink/gtg or double *gauche* sequence. The estimated uncertainty for the various conformers is 10 to 15%.

The experimental data from the measurements on octadec-14-ynoic acid, dissolved in a small amount of tetrachloromethane, were treated slightly different. At first, the vibrational bands arising from the solvent were eliminated by the subtraction of a pure CCl_4 spectrum recorded under the same experimental conditions. This was followed by a further linear baseline correction. The final determination of the band intensities was done in the same way as described above.

For the analysis of the CH_2 wagging progressions,[8,9] subtraction of the underlying PO_2^- antisymmetric stretching band near 1230 cm^{-1} was required. For this reason, an IR spectrum at the highest available temperature in the liquid crystalline phase (typically at 353 K) was recorded and subtracted from the spectrum acquired at the other temperatures. Afterwards, a flattened baseline was generated in the spectral region of interest by selecting appropriate spectral minima. All progression intensities—a sum of the $k = 1$ to $k = 4$ components—were further normalized with respect to the progression band intensity obtained for the pure phospholipid/water dispersions at 278 K. Differences in the PO_2^- antisymmetric stretching band region caused by the addition of cholesterol have been taken into account by comparison with the IR spectrum at 353 K. Further details about the actual procedures can be found in refs. 8 and 9.

The frequencies of the CH_2 symmetric stretching vibrations were determined from the interpolated zero crossings in the first derivative spectra.

Differential scanning calorimetry (DSC)

The phase behaviour of the various samples was established by differential scanning calorimetry. Typically a temperature range of 200 to 350 K at heating rates of 2 K min^{-1} or 5 K min^{-1} was examined using a Netzsch DSC 204 calorimeter (Selb, Germany).

Results and discussion

In the following we present the results from a variable temperature FTIR study of model membranes based on the phos-

pholipids DO(14-yne)PC, DO(14-yne)PA and DO(9-yne)PC, whose structures are given in Fig. 1. These compounds differ from common phospholipids by the incorporation of a C–C triple bond at carbon C-14 or C-9 of the acyl chains. In the present work the following membrane systems have been examined between 270 and 350 K: (i) pure hydrated lipids with a water content of 75 wt.% and (ii) hydrated lipid/cholesterol mixtures with a steroid content of 40 mol%. The hydrated lipids exhibit a liquid crystalline and a gel phase, as established by differential scanning calorimetry.[16,17] The transition temperatures were found to be 296, 325 and 271 K for DO(14-yne)PC, DO(14-yne)PA and DO(9-yne)PC, respectively. Addition of 40 mol% cholesterol to DO(14-yne)PC sample is accompanied by a lowering of the transition temperature to 273 K that is superimposed by a further water peak. Differential scanning calorimetry measurements of the samples with DO(14-yne)PA/cholesterol and DO(9-yne)PC/cholesterol showed gel to liquid crystalline phase transitions at 319 and 268 K, respectively.

The present FTIR study is focussed on the evaluation of the conformational properties in the acyl chain of such unusual phospholipids. Former FTIR studies on pure hydrocarbons and hydrocarbon solutions[21,22] have shown that the chain conformation can be monitored by analysing CH_2 and CD_2 stretching bands, CH_2 wagging and CD_2 rocking bands. In a similar way, such techniques have been employed to the study of the acyl chain conformations in phospholipid bilayers. In this connection, studies have been reported on membrane systems comprising single component and multicomponent bilayers (lipid mixtures, mixtures with cholesterol or proteins, *etc.*) as well as lipids with saturated and unsaturated acyl chains. In the latter case, so far only lipids containing olefinic groups have been considered. In this contribution, FTIR investigations are reported on phospholipids bearing monoacetylenic acyl chains. The study primarily addresses the evaluation of the specific conformational features of such unusual membrane systems that might arise from their unique molecular structure. For this reason CH_2 stretching, and CH_2 wagging bands, as well as CH_2 wagging band progressions, have been analysed.

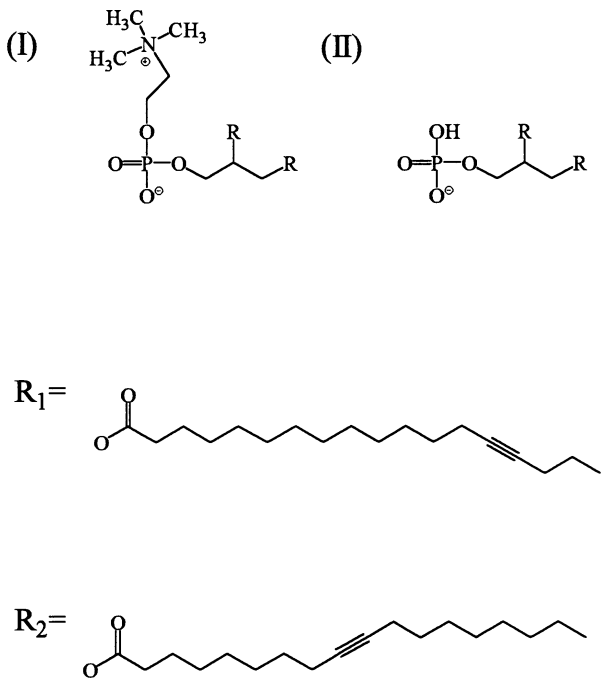

Fig. 1 Chemical structure of the phospholipids used in the present study (I) phosphatidylcholine, (II) phosphatidyl acid; R_1 = octadec-14-ynoic acid, R_2 = octadec-9-ynoic acid.

(i) CH_2 stretching bands

The results from the analysis of the CH_2 stretching bands are summarized in Fig. 2. In the corresponding graphs the temperature dependent frequency shifts of the symmetric CH_2 stretching bands are given that are observed for DO(14-yne)PC, DO(14-yne)PA and DO(9-yne)PC. Open and filled symbols refer to the pure phospholipid/water dispersions and to the samples containing 40 mol% cholesterol, respectively. It can be seen that the pure model membranes exhibit a pronounced band shift of the symmetric CH_2 stretching absorption at the respective main calorimetric transitions. For the present samples the wavenumbers of the band maxima change from about 2848 cm^{-1} in the gel phase to 2854 cm^{-1} in the liquid crystalline phase. Similar frequency shifts due to changes of the acyl chain conformation in the vicinity of the gel to liquid crystalline phase transition have been reported for other phospholipid membranes.[4,6] This can be understood by the fact that in the gel phase the acyl chains prevail in the all-*trans* conformation along with an absorption at lower wavenumbers. The increase in conformational disorder upon transition to the liquid crystalline phase is then reflected by a distinct frequency shift of the CH_2 stretching band towards higher wavenumbers.

A quite similar trend of the CH_2 stretching band frequencies can be observed for the mixtures with cholesterol. The DO(14-yne)PA and DO(9-yne)PC membranes exhibit almost identical curves for both the pure phospholipid

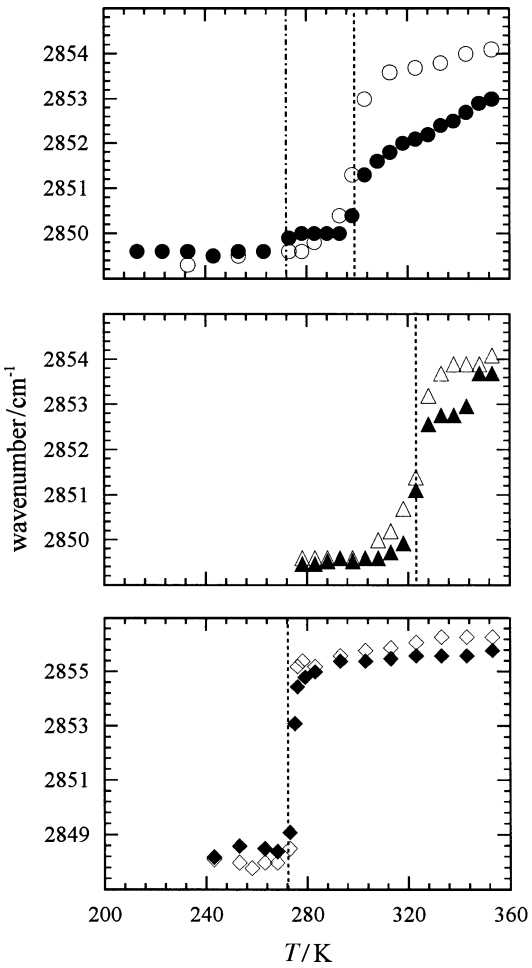

Fig. 2 CH_2 stretching wavenumbers for DO(14-yne)PC (top), DO(14-yne)PA (center) and DO(9-yne)PC samples (bottom) as function of temperature. Dashed lines indicate the calorimetric phase transitions. For clarity the phase transition of DO(14-yne)PA/cholesterol has been omitted. The open and filled symbols refer to the samples containing the pure lipids and the lipid/cholesterol mixtures, respectively.

systems and the mixtures with cholesterol while the experimental curves from the samples with DO(14-yne)PC display larger differences. In the latter case only a slight shift at about 273 K—the calorimetric phase transition—is registered for the mixture with cholesterol. In fact, the major frequency shift of the CH_2 stretching band occurs at higher temperatures, exhibiting a more gradual change than for the pure lipid. Overall, the experimental temperature dependences of the CH_2 stretching band data behave very similarly to the CH_2 wagging band progressions. Therefore they will be discussed below in conjunction with the latter data.

(ii) CH_2 wagging bands in the liquid crystalline phase

Fig. 3 presents FTIR spectra for DO(14-yne)PC (a), DO(9-yne)PC (b) and DO(14-yne)PA (c) bilayers in their liquid crystalline phases. For comparison, an IR spectrum referring to DMPC is also given. The spectral range from 1315 to 1395 cm^{-1} covers the conformation sensitive wagging mode region of the CH_2 groups. In the case of DMPC, which bears saturated acyl chains, four absorption bands are observed. The intense band near 1378 cm^{-1} refers to the methyl group "umbrella" deformation state which serves as an internal reference band 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, respectively, in the fatty acid chains. As demonstrated previously, such a spectrum can be decomposed by using a curve fitting procedure from which the amount of the three conformational sequences can be deduced.[11]

It was anticipated that a similar analysis is also possible for the present IR spectra of DO(14-yne)PC, DO(14-yne)PA and DO(9-yne)PC bilayers. Inspection of the spectra given in Fig. 3, however, reveals two features that are not observed for DMPC and similar lipids with saturated acyl chains and that should be related to the incorporation of the C–C triple bond: (i) an additional band centered at about 1328 cm^{-1} and (ii) an unusual signal enhancement of the band near 1340 cm^{-1} (eg conformer) for DO(14-yne)PC and DO(14-yne)PA bilayers.

This latter observation is in agreement with earlier IR data of small model compounds bearing an isolated acetylenic group.[23] In that study the authors reported on a single, unusual strong absorption band between 1325 and 1336 cm^{-1} that was assigned to a special wagging band of the CH_2 group in the CH_2–C≡C segment. The increased spectral intensity was attributed to polarization effects by the adjacent C≡C bond. Further information—such as the behaviour of the other CH_2 wagging bands—however, was not provided. During the present work, therefore, IR spectra of several model compounds have been recorded to get a better insight into the special features within the wagging band region of such phospholipids. The model compounds examined here were hex-2-yne, hex-3-yne and oct-4-yne, whose IR spectra are given in Fig. 4. The IR spectrum of hex-3-yne contains only two absorption bands. They can be assigned to the "umbrella" deformation band at 1378 cm^{-1} and to a strong CH_2 wagging band at 1325 cm^{-1} due to the CH_2–C≡C segment. It should be mentioned that the latter signal occurs outside the normal CH_2 wagging band region. The IR spectra of hex-2-yne and oct-4-yne exhibit three bands that are of interest within the spectral range given here: (i) the umbrella deformation band mentioned previously, (ii) an absorption band of relatively high intensity at 1340 cm^{-1} and (iii) the former CH_2 wagging band of the CH_2–C≡C segment being shifted to 1331 cm^{-1} and of lower intensity than for hex-3-yne. The reduced intensity of the latter band can only partially be explained by the presence of a single CH_2–C≡C segment. Rather, these data imply that the polarization effect by the C–C triple bond also differs with the actual chemical structure, *i.e.* whether the CH_2–C≡C segment is attached to a methyl, ethyl group or an even larger alkyl chain segment. The strong IR absorption band for hex-2-yne and oct-4-yne at 1340 cm^{-1} has been assigned to the eg wagging band from the C≡C–CH_2–CH_2–CH_3 segment. The intensity of this IR band is found to be much stronger than for saturated alkanes, most probably due to a polarization effect from the adjacent C≡C group (see also below). In addition, a weak shoulder of unknown source is visible at about 1350 cm^{-1} in the IR spectra of hex-2-yne and oct-4-yne.

The above interpretation has been verified by additional IR measurements on a bilayer membrane from selectively deuterated [13,13,13′,13′-d_4]-DO(14-yne)PC. Here, one CH_2 group next to the triple bond has been replaced by CD_2. Compari-

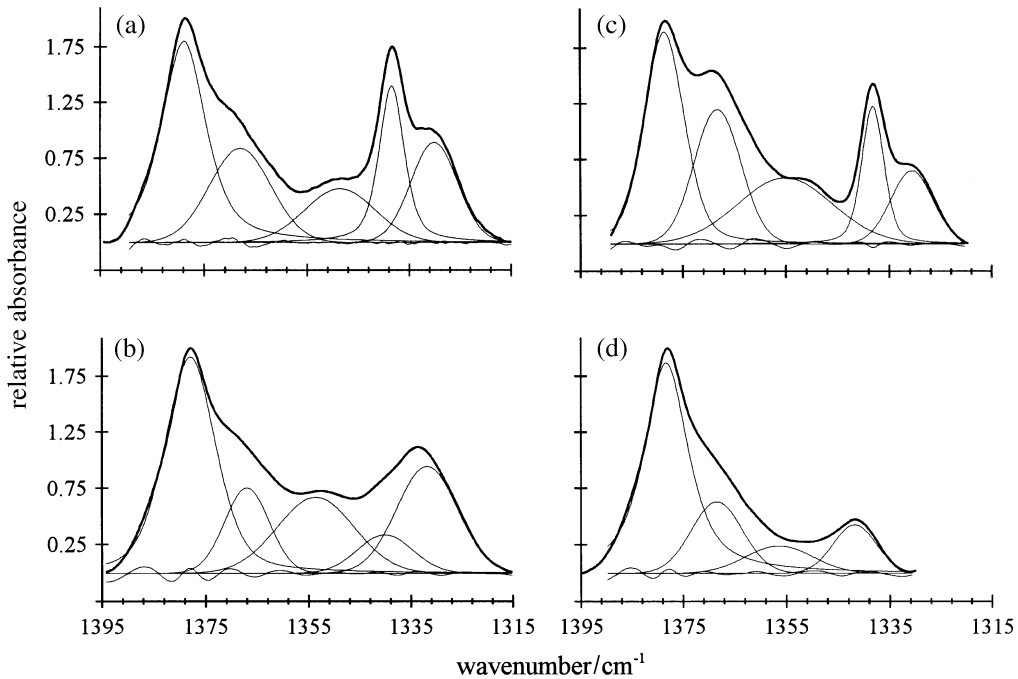

Fig. 3 FTIR spectra (wagging band region) recorded in the liquid crystalline phases of various phospholipid membranes: (a) pure DO(14-yne)PC, (b) DO(9-yne)PC, (c) DO(14-yne)PA and (d) DMPC. The spectra also contain the theoretical wagging bands due to the various conformers (see text) and the difference spectra between experiment and simulation.

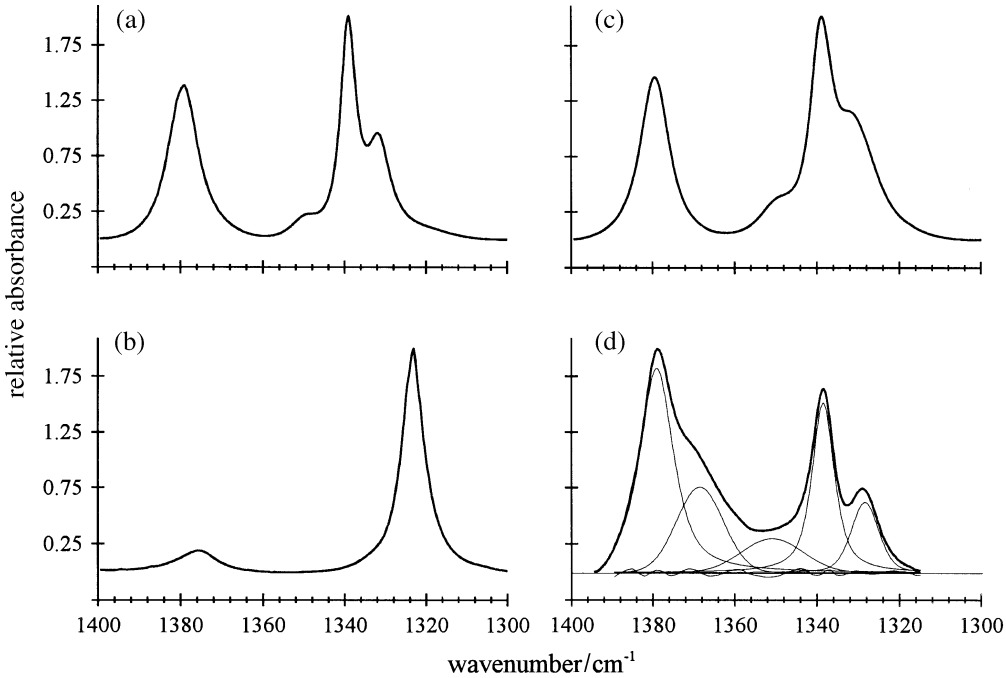

Fig. 4 FTIR spectra (wagging band region) of various model alkynes, recorded at room temperature: (a) hex-2-yne, (b) hex-3-yne and (c) oct-4-yne. In addition, an FTIR spectrum of a DO(14-yne)PC membranes is given in (d) that was selectively deuterated at C-13 in the acyl chains. The latter spectrum also contains the theoretical wagging bands due to the various conformers (see text) and the difference spectrum between experiment and simulation.

son of the IR spectrum, given in Fig. 4, with that for the non-deuterated DO(14-yne)PC sample (see Fig. 3 (a)) reveals that the intensity of the 1328 cm^{-1} wagging band in fact is reduced by almost a factor of two, while all other band intensities remain almost unaffected. This result can be understood by the fact that for the CD_2 group (with a larger molecular mass) the corresponding 1328 cm^{-1} band is shifted towards lower wavenumbers, *i.e.* outside the given spectral window. Consequently, the experimental band intensity should be halved, as in fact is observed. It can therefore be concluded that the CH_2 segments next to the C≡C unit only contribute to the special absorption band at 1328 cm^{-1} and play no role in the conformation sensitive CH_2 wagging bands at wavenumbers beyond 1335 cm^{-1}.

In principle, the CH_2 wagging band region of the lipids with monoacetylenic acyl chains can be analysed in a quite similar way, as done during previous successful studies on lipids with saturated acyl chains.[4–6] However, some special features should be kept in mind: (i) apart from the three conformation sensitive bands, a further intense wagging band of the CH_2-C≡C segment at about 1328 cm^{-1} (see previous paragraph) had to be considered. (ii) For the lipids with octadec-14-ynoic acid [DO(14-yne)PC, DO(14-yne)PA] an intensity increase of the eg wagging band is seen (see also below) which did not occur for DO(9-yne)PC bilayers. (iii) For the DO(9-yne)PC samples the eg wagging band was obscured almost completely by the strong vibration band of the CH_2–C≡C unit. An accurate determination of the amount of eg conformers for this bilayer system therefore was not possible.

The enhanced intensity of the conformation sensitive eg wagging bands (see part (ii) of previous paragraph) for DO(14-yne)PC and DO(14-yne)PA bilayers was taken into account by a new scaling factor that was derived from complementary FTIR measurements on octadec-14-ynoic acid, dissolved in tetrachloromethane. The experimental IR spectra of this sample (see spectrum in Fig. 5) were subject to the same fitting procedure described in the experimental part in order to derive the content of the various *gauche* conformers. On the basis of the RIS model[20] it could be shown that the amounts of gg and kink/gtg conformers for octadec-14-ynoic acid are almost identical with those reported for tetradecane at the same temperature and are unaffected by the C–C triple bond. A quite similar conclusion was drawn during a former IR study[11] on DOPE (1,2-dioleoyl-*sn*-glycero-3-phosphatidylethanolamine) membranes bearing a C–C double bond. It therefore appeared to be justified to employ the RIS model for the determination of the amount of eg confomers in the iso-

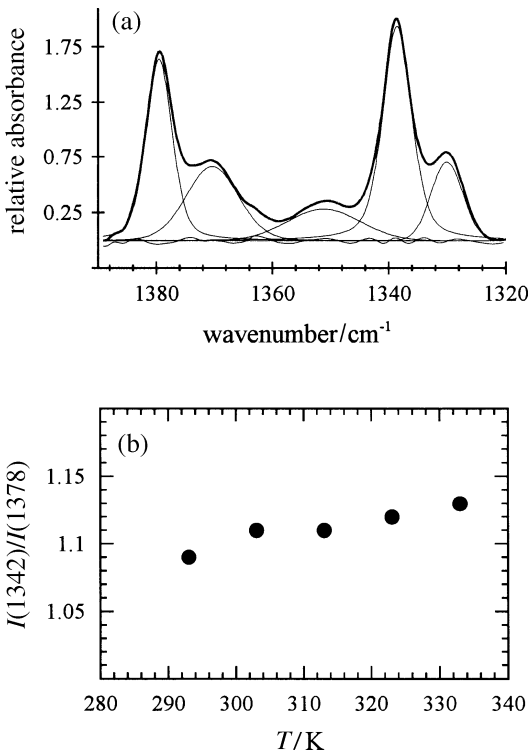

Fig. 5 (a) Experimental FTIR spectrum of octadec-14-ynoic acid, dissolved in tetrachloromethane, recorded at room temperature. (b) Scaling factors for the eg wagging band derived from the variable temperature FTIR studies for octadec-14-ynoic acid.

tropic solution of octadec-14-ynoic acid, resulting in a new scaling factor of 1.11 for this vibration band at 313 K. This value is considerably larger than the 0.18 obtained for *n*-octadecane at the same temperature, which is attributed to the electronic influence from the C–C triple bond on the dipole moment of the eg vibrational mode. The derived scaling factors between 290 and 350 K, summarized in Fig. 5, are found to exhibit a very small temperature dependence.

On this basis it was possible to analyse the CH_2 wagging band region of the FTIR spectra from DO(14-yne)PC, DO(14-yne)PA and DO(9-yne)PC bilayers in quite the same way as for other saturated phospholipids. The derived amounts of *gauche* conformers are summarized in Fig. 6. In general, the increase in sample temperature is accompanied by a lowering of the conformational order in all model membranes. The temperature dependence, however, differs with the actual sample and specific *gauche* conformer.

Comparison between the samples containing pure DO(14-yne)PC and DMPC (data not shown) reveals an increased amount of gg sequences for the former system (0.4 for DO(14-yne)PC *vs.* 0.2 for DMPC) a few degrees above the respective main transition temperature, being almost constant within the temperature range covered here. At the same time, an increase is registered for the amount of kink/gtg sequences (0.5 *vs.* 0.27) along with a strong temperature dependence, while the amount of eg conformers is lowered with respect to DMPC (0.35 *vs.* 0.55).

Overall, the total number of *gauche* conformers per chain is higher for DO(14-yne)PC than for DMPC, as can be seen from the values 3.0 and 2.4 at $T = 333$ K, respectively. The

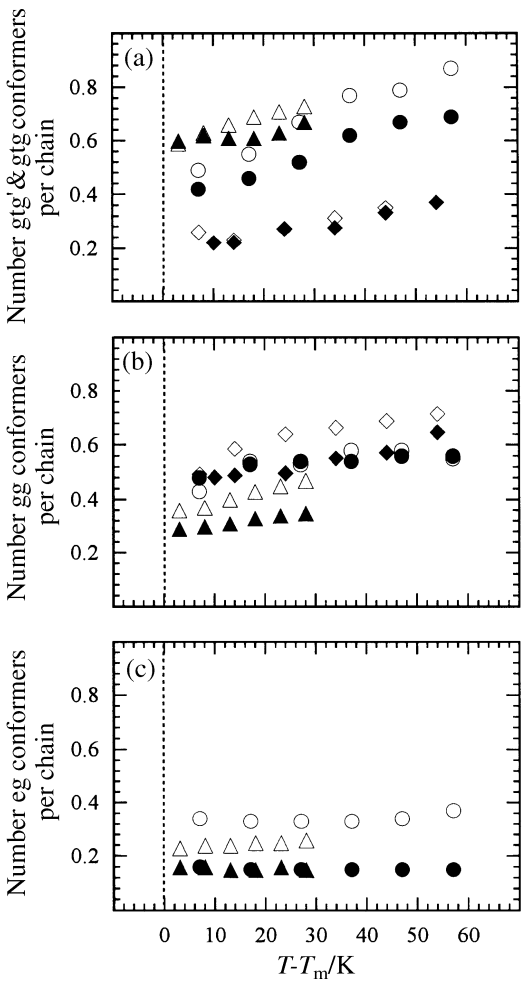

Fig. 6 Number of kink/gtg (a), gg (b), and eg conformers (c) per chain for bilayers from DO(14-yne)PC (○, ●), DO(14-yne)PA (▲, △) and DO(9-yne)PC (◆, ◇). Open and filled symbols refer to the data from the pure lipids and lipid/cholesterol mixtures, respectively.

increase of conformational disorder in DO(14-yne)PC bilayers can be understood by a less dense chain packing due to the sterically more hindered terminal pentyne group. The larger amount of free volume in the fatty acid chain region thus facilitates the formation of chain disorder. The kink sequences—which are expected to dominate the kink/gtg wagging band—give rise to a parallel displacement of the whole hydrocarbon chain which requires only a small amount of free volume. They are assumed to occur over the whole fatty acid chains and therefore exhibit a distinct temperature dependence. The higher amount of gg sequences for DO(14-yne)PC cannot be explained satisfactorily. Here, the further bend of the acetylenic unit might cause some stabilization of the usually sterically demanding *gauche* sequence. On the other hand, it should be noted that the determination of the gg conformers is accompanied by the largest error since the corresponding absorption band is rather broad and to some extent is obscured by the kink/gtg and eg wagging bands. Finally, the observed lower amount of eg conformers for DO(14-yne)PC is attributed to an ordering effect by the adjacent acetylenic group in such lipid bilayers or to a mutual penetration of the hydrophobic region from adjacent layers.

The addition of cholesterol to DO(14-yne)PC is accompanied by a reduction of the kink/gtg and eg confomers while the amount of gg sequences is almost unaffected. The overall chain ordering effect of the steroid on DO(14-yne)PC membranes is much less pronounced than for DMPC, as can be seen from the comparison of the calculated total number of *gauche* conformers per chain. For instance, at 333 K for DO(14-yne)PC values of 3.0 (pure lipid) and 2.5 (lipid/cholesterol mixture) were found, while for DMPC values of 2.4 (pure lipid) and 1.3 (lipid/cholesterol mixture) have been derived. These results can be rationalized by the fact that the rigid ring system of cholesterol is located in the upper part of the aliphatic chain region between positions C-2 and C-8 or C-9 and thus primarily influences the amount of kink/gtg conformers. On the contrary, gg sequences are expected to be located more towards the C–C triple bond and thus should experience only a minor influence by the steroid. The reduction of the amount of eg conformers upon addition of cholesterol might originate from stronger interactions between the two phospholipid layers that constitute a bilayer, *i.e.* a mutual penetration of the hydrophobic regions of adjacent layers along with a better bilayer packing. Finally, it is interesting to note that even for DO(14-yne)PC with cholesterol a relatively high amount of kink/gtg sequences is observed, supporting the assumption of a large free volume due to the incorporation of the acetylenic unit.

The influence of the polar head group on the conformational disorder in the fatty acid chain region is reflected by the data for the DO(14-yne)PA bilayers, given in Fig. 6. A few degrees above the phase transition of the DO(14-yne)PA membrane the amounts of gg and eg conformers are reduced by about 15 and 25%, respectively, as compared with DO(14-yne)PC. At the same time the amount of kink/gtg conformers are found to increase by about 20% for DO(14-yne)PA. During former investigations on the saturated chain analogues DMPA and DMPC[24,25] a general decrease of all *gauche* conformers has been observed on going from PC to PA phospholipids. This behaviour has been traced back to the larger diameter of the phosphocholine head group. The glycerol moiety in DMPC is thus arranged parallel to the bilayer surface, resulting in a bend of the *sn*-2 chain at position C-2. The two fatty acid chains cannot be extended equally deep into the bilayer interior and free volume is created. On the contrary, in the DMPA membrane the smaller head group leads to a perpendicular arrangement of the glycerol backbone with respect to the bilayer normal. The almost identical arrangement of both fatty acid chains within the lipid bilayer is accompanied by a smaller amount of free volume in the

lower part of the acyl chain region which prevents the creation of gg and eg conformers. It is anticipated that these assumptions also hold for the present PC and PA membranes with monoacetylenic hydrocarbon chains. The alterations observed for the amounts of gg and eg conformers upon variation of the head group are thus almost independent of the particular chemical structure in the acyl chain region. The most likely explanation for the observed increase of the kink/gtg conformers in DO(14-yne)PA bilayers can again be found in the general less dense packing, particularly in the upper part of the fatty acid chain region, where the kink sequences are expected to possess the highest probability. Finally, it should be noted that direct comparison of DO(14-yne)PA and DMPA also reveals an increase of the conformational disorder for the phospholipids bearing unsaturated acyl chains. This is reflected by the total number of *gauche* conformers per chain that are found to 2.3 for DO(14-yne)PA and 1.4 for DMPA at 333 K.

As for other membrane systems,[4,6] the addition of 40 mol% cholesterol has an ordering effect in the fatty acid chain region of the DO(14-yne)PA/water dispersion. The amount of gg sequences is reduced by about 20% whereas kink/gtg sequences remain less affected by the incorporation of the steroid. This again supports the assumption of a different arrangement of the glycerol backbone in the two phospholipids. For DO(14-yne)PA/cholesterol bilayers the overall increase in conformational order is less pronounced, as can be deduced from the total number of *gauche* conformers per chain. At 333 K they are 2.3 and 2.0 for DO(14-yne)PA and DO(14-yne)PA/cholesterol, respectively.

For DO(9-yne)PC and DO(9-yne)PC/cholesterol bilayers, only the kink/gtg and gg wagging bands could be analysed. As demonstrated by the graphs in Fig. 6, both membrane systems are characterized by a relatively low amount of kink/gtg conformers, while the amount of gg conformers is even higher than found for DO(14-yne)PC and DO(14-yne)PA bilayers. Furthermore, the kink/gtg and gg conformers exhibit a pronounced temperature dependence. The addition of cholesterol is reflected by a reduction of the amount of gg sequences, while kink/gtg conformers are found to be almost unaffected. These findings can be rationalized by the specific molecular structure of DO(9-yne)PC, where the rigid and bulky acetylenic group is located closer to the lipid head group. In addition, it is known that in the liquid crystalline phase the phospholipid molecules are highly mobile and undergo fast chain rotational and wobble motions, as shown by recent ^{13}C and ^{31}P NMR investigations.[26] The particular molecular structure of DO(9-yne)PC implies that a good chain packing is not possible with the acyl chains in the all-*trans* conformation. Rather, a substantial amount of gg conformers has been derived from the present IR investigations. They are, most likely, located in the upper part of the acyl chains and should give rise to a better membrane packing. Thus, the addition of cholesterol has an impact on the amount of gg conformers, while the amount of kink/gtg conformers—which are sterically less demanding—is not altered, being in line with a general low chain packing in DO(9-yne)PC membranes.

(iii) CH_2 wagging band progressions

It has been shown previously that a high conformational order in hydrocarbon chains causes a coupling of the methylene wagging modes.[8,9,27] The resulting progression of bands between 1150 and 1350 cm^{-1} can be described by the model of coupled harmonic oscillators for which the eigenvalues of the vibrational secular equation are given by[27]

$$4\pi^2\nu_k^2 = H_0 + 2\sum_m H_m \cos(m\phi_k) \quad (1)$$

H_0 and H_m are matrix elements of the secular determinant. The ϕs are the phase differences between adjacent oscillators. They are given by

$$\phi_k = k\pi/(n+1) \quad (k = 1, 2, 3, \ldots, n) \quad (2)$$

where n is the number of oscillators in the chain. If the integers k are correctly identified for an observed band progression, a smooth curve results from a plot of the progression band frequency ν_k against ϕ_k.

As an example, the wagging progression bands of the methyl ester from octadec-14-ynoic acid, recorded in the solid phase at 273 K, are shown in Fig. 7(a). The CH_2 wagging mode progressions are located near 1225, 1250, 1276, 1301, 1324 and 1345 cm^{-1}. Former studies on unsaturated methyl alkenoates have demonstrated that the double bond acts as a barrier that decouples the methylene vibrational modes that exist in the alkyl segments at both sides of the C=C unit. The same effect can be found for the C–C triple bond in the methyl ester of octadec-14-ynoic acid. The phase angles of the oscillators thus have been calculated according to eqn. (2) with the assumption that only 12 methylene groups between the carbonyl group and the triple bond contribute to the progression of bands. In Fig. 7(c) the progression band frequencies of the methyl ester of octadec-14-ynoic acid are plotted as a function of the derived phase angles. For comparison, the data of Chia *et al.*[9] for the methyl ester of nervonic acid (13 methylene groups between the carbonyl and the double bond at C-15)

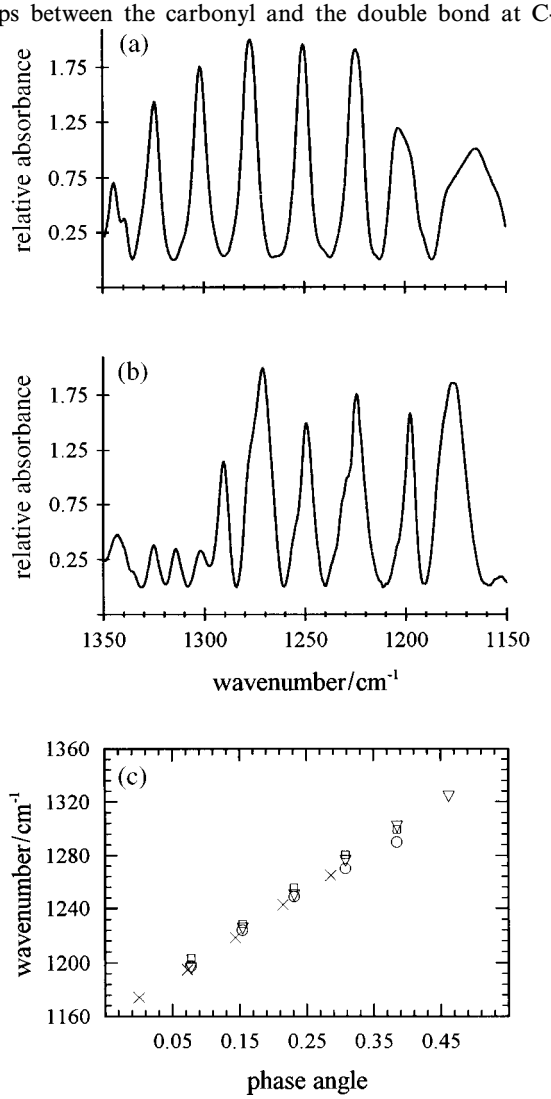

Fig. 7 Wagging band progressions for (a) methyl ester of octadec-14-ynoic acid and (b) pure DO(14-yne)PC. In (c) the progression band wavenumbers are plotted as a function of the derived phase angles: DO(14-yne)PC (○), DMPC (□), methyl ester of octadec-14-ynoic acid (▽) and methyl ester of nervonic acid (×).

are also given. The observed linear relationship between the progression band frequencies and the phase angles clearly proves the correct assignment of the progression bands and the use of the correct number of coupled methylene groups for the derivation of the phase angles.

In a similar way, we have studied the progression bands in the lipid bilayers. In Fig. 7(b) a representative series of progression bands for the DO(14-yne)PC/water dispersion is given that refers to the gel phase at 278 K. In this case, the IR spectrum recorded in the liquid crystalline phase at 353 K has been subtracted from the original gel phase spectrum at 278 K to eliminate the broad PO_2^- band. The observed wagging band progression in Fig. 7(b) clearly demonstrates the presence of highly ordered fatty acid chains being in an almost all-*trans* conformational state. The variation of the amount of all-*trans* conformers with temperature can be followed by the intensities of the experimental progression bands. In Fig. 8 this is shown for the pure DO(14-yne)PC and DO(14-yne)PA membranes and the corresponding lipid/cholesterol mixtures, where the experimental data points refer to the averaged sum of the $k = 1$ to $k = 4$ progression band intensities.

Inspection of the curves in Fig. 8 reveals that for the phospholipids studied here the progression band intensities have vanished almost completely at 5 to 10 K above the respective main transitions of the pure lipids, which reflects an increase of conformational disorder on going from the gel to the liquid crystalline phase. For instance, in the case of the pure DO(14-yne)PC membrane a strong intensity decay occurs directly at the corresponding main transition. Likewise, the DO(14-yne)PC/cholesterol mixture exhibits a drop down of the progression band intensity by 50% at about 273 K—the calorimetric phase transition of this sample—which is followed by a gradual decay to zero at higher temperatures. It should be noted that such an intensity loss of 50% corresponds to 5% *gauche* probability at each position or 0.6 *gauche* conformations per chain.[8] Finally, the progression band intensity curves of both DO(14-yne)PA samples are found to be nearly identical. They, however, are characterized by a more gradual decay of the curves with temperature.

The above findings are in qualitative agreement with the behaviour of the previously mentioned CH_2 stretching bands.

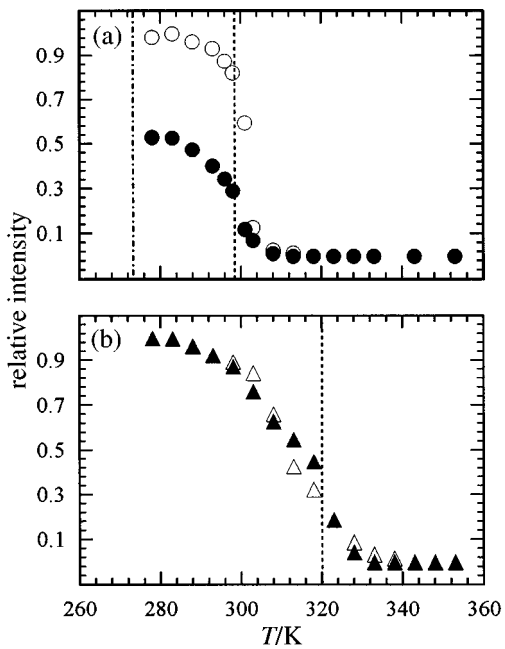

Fig. 8 Wagging band progression intensities for DO(14-yne)PC (top) and DO(14-yne)PA (bottom). Open and filled symbols refer to the data from the pure lipids and lipid/cholesterol mixtures, respectively. Dashed lines indicate the calorimetric phase transitions.

That is, a shift in the CH_2 stretching band frequency usually is accompanied by a change of the progression band intensity. For example, for the DO(14-yne)PC/cholesterol sample the intensity loss, observed at about 273 K, is reflected by a discontinuity of the CH_2 stretching band frequency shift (see Fig. 2). In this particular case the finite progression band intensity persists for about 30 K in the liquid crystalline phase and drops down further at about 296 K. At the same time, a pronounced frequency shift of the CH_2 stretching band position is observed. Obviously, the addition of cholesterol gives rise to a stabilization of the all-*trans* conformers in DO(14-yne)PC membranes well above the respective main transition of the lipid/cholesterol mixture. Such a behaviour has also been reported previously for DPPC/cholesterol bilayers.[9]

Similar plots for the DO(9-yne)PC membranes are missing, since here the progression band region was obscured by other strong vibration bands. An analysis of the CH_2 wagging band progression therefore was not possible. All in all, the present studies have demonstrated clearly that, for the phospholipids bearing monoacetylenic chains, the progression band intensities as well as the CH_2 stretching frequencies can again be used as a qualitative measure for acyl chain conformational order and its variation with temperature and phospholipid phase.

Conclusion

In the present work new synthetic phospholipids DO(14-yne)PC, DO(14-yne)PA and DO(9-yne)PC, bearing monoacetylenic fatty acid chains, have been investigated by FTIR spectroscopy. The study primarily was addressed to the evaluation of the conformational ordering in the fatty acid chain region as a function of temperature and sample composition, *i.e.* presence or absence of cholesterol. To do so, the CH_2 stretching and wagging band regions as well as the CH_2 wagging band progressions have been analysed. It could be demonstrated that for the present systems the same procedures are applicable as used previously for lipids with saturated or olefinic acyl chains. From the analysis of the CH_2 wagging bands several *gauche* conformers could be identified. Their actual amounts varied with sample composition and sample temperature and could be related to the specific chemical structure of the phospholipids examined here. In summary, the present study has demonstrated that such FTIR techniques are of general use for the evaluation of the conformational properties in phospholipid bilayers and independent of the particular acyl chain structure. Further work along this line is in progress.

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