Hydration-Induced Gel States of the Dienic Lipid 1,2-Bis(2,4-octadecadienoyl)-*sn*-glycero-3-phosphorylcholine and Their Characterization Using Infrared Spectroscopy

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The lyotropic phase behavior of the dienic lipid 1,2-bis(2,4-octadecadienoyl)-*sn*-glycero-3-phosphorylcholine (DODPC) has been investigated by means of IR spectroscopy at 25 °C. Gradual hydration has been realized exposing the lipid to an atmosphere of variable relative humidity (RH). Upon scans of decreasing RH, the liquid crystalline lipid undergoes the chain-freezing transition to a metastable gel state. By storage of the sample at low RH, the gel transforms to a crystalline subgel designated as SG_I. Subsequent hydration induces the conversion to a second subgel (SG_{II}). The subgel phases are characterized by the dense packing of the acyl chains as indicated by the correlation field splitting of the CH_2 rocking and bending modes. Band shifts of phosphate group vibrations as well as the splitting of the carbonyl stretching mode are correlated with the hydration of the polar region of the bilayer given in terms of the molar ratio of water to lipid. The $\nu_{1,3}$(OH) absorption band of water yields qualitative information about the water−lipid interaction. The drastic sharpening of this band in the SG_I phase was attributed to the reduction of water binding sites on the lipid, leading to a more uniform population of water molecules adsorbed onto the lipid headgroup. The external conditions of phase transformation of DODPC were compared with corresponding data of dimyristoylphosphatidylcholine (DMPC) having the same number of subsequent methylene segments in the acyl chains. Apparent differences can be attributed to the influence of the diene groups representing a rigid spacer inserted between the methylene chains and the ester groups of the lipid, i.e., in a position near the polar/apolar interface of the bilayer.

1. Introduction

In phospholipids the nature of the chemical linkage between the glycerol backbone and the hydrocarbon chains, and the structure of the fatty acid moieties are known to modify the conformation of the molecules and consequently affect the physical properties of the aggregates that they form in an aqueous environment. Only a few approaches for studying the interrelation between the interfacial structure and the phase behavior of lipids have involved ester- and ether-linked hydrocarbon chains,[1−4] conformationally restricted backbones,[5,6] and 1,3- and 1,2-linked analogues.[7,8] For example, the replacement of the ester-linked hydrocarbon chains with the corresponding ether-linked analogues results in only modest increases of the gel liquid crystalline phase transition temperature and in the enhanced capacity to form interdigitated gel phases.[1,2,4] Apparently, structural effects of these chemical modifications are strongly intensified in gel-phase systems. At present, the molecular basis for the marked sensitivity of lipid physical properties to the chemical structure of the polar/apolar interface of lipid aggregates is not yet clarified completely.

Recent investigations on long chain phosphatidylcholines[9,10] have identified a new fully hydrated gel phase. It was noted[11] that the discovery of a new phase that can form even in an extensively investigated lipid pointedly highlights the limitations in our knowledge of even simple lipid systems. Obviously, the understanding of factors governing acyl chain packing is weak. This can be attributed, at least partially, to the deficiency of experimental data that directly address the influence of lipid interfacial structure on lipid phase behavior and in particular on the formation of a variety of gel states.

In the synthetic phospholipid 1,2-bis(2,4-octadecadienoyl)-*sn*-glycero-3-phosphorylcholine (DODPC) in each of the fatty acid chains, a polymethylene unit is linked to the glycerol residue via a 1,4-disubstituted *trans*,*trans*-butadiene (diene) group. That is, the anchoring of the hydrophobic moiety to the more polar part of the amphiphile is modified by a rigid, extended spacer inserted between the ester groups and the flexible $-(CH_2)_{12}-CH_3$ chains. The experimental investigation of the consequences exerting this chemical modification on the molecular structure and dynamics within the lipid aggregates in combination with their phase behavior is expected to yield further insight into the role of the polar/apolar interface in amphiphilic assemblies.

Phosphatidylcholines with diene groups in the fatty acid chains, and in particular DODPC, have been investigated mainly in order to stabilize bilayer structures by means of polymerization.[12−14] Hence, the physicochemical properties of aqueous vesicle systems of DODPC have been studied before and after polymerization to optimize the polymerization conversion, to modify the architecture of the polymerized membranes, and finally, to control the stability and the leakage rate of vesicles.[15−20] Despite the intensive and imaginative work on polymerizable amphiphiles, the mesomorphism and consequently the phase behavior of most of the synthesized monomers were only little characterized with the exception of diacetylene lipids, which form tubular structures in the gel state.[21−23]

The most easily controlled and most often used perturbing variable in physical studies of lipid systems under ambient pressure conditions is that of temperature. High-pressure studies also have been applied to modify model membrane systems indicating a very rich and complex barotropic phase behavior of diacyl phospholipids.[24]

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On the other hand, phospholipids show lyotropic mesomorphism, i.e., they potentially undergo phase transformations upon changing the degree of hydration by means of water adsorption or desorption. The interface between the polar headgroup and the apolar hydrocarbon chains of the lipid is located just in the region of the linkage between the acyl chains and the glycerol moiety. Thus, the sensitivity of lipid structures to chemical alterations in this region can be attributed to a substantial degree to a modification of the strength of lipid/lipid and lipid/water interactions. In particular the degree of hydration and the hydrogen bonding of water to the region of the modified polar/apolar interface of DODPC is expected to vary its phase properties with respect to other phosphatidylcholines.

The direct approach for modifying the polar interface in a definite way represents the investigation of the dependence of lipids on the degree of hydration. The continuous variation of lipid hydration can be realized by adjusting the relative humidity of the atmosphere surrounding the lipid.[25,26] We used equipment that allows continuous variation of the relative humidity in a wide range and thus allows the precise detection of hydration-induced phase transformations.

In the present study we have investigated the lyotropic phase behavior of DODPC at room temperature. Our attention is focused on lipid properties as well as on the characterization of the adsorbed water. DODPC forms lamellar gel and subgel states in wide ranges of incomplete hydration. As in the case of the ester/ether substitution mentioned above, we expect that crystalline structures would more readily manifest thermal, structural, and conformational effects caused by the presence of the diene groups.

Vibrational spectroscopy provides macroscopic as well as microscopic information on the structural and dynamic properties of membranes by means of semiempirical parameters. Discrete frequency ranges in the spectra can be assigned to different parts of the DODPC molecule (headgroup, acyl chains, and carbonyl and diene groups), and therefore, detailed information about local molecular properties in the hydrophobic core of the bilayer as well as at the polar/apolar interface is available.

2. Experimental Section

Materials. 1,2-Bis(2,4-octadecadienoyl)-*sn*-glycero-3-phosphorylcholine (DODPC) was purchased from Nippon Oil & Fats Co., Ltd. The purity was confirmed by thin layer chromatography (eluant: chloroform/methanol/water in 65/25/4 v/v proportion) showing a single spot on the TLC plates. Ethanolic stock solution (5 mg/mL, spectroscopic grade ethanol) of the monomeric DODPC was used for sample preparation.

Sample Preparation and Hydration. Lipid samples were prepared by spreading homogeneously 200 μL of the stock solution on one side of a ZnSe attenuated total reflection (ATR) crystal (50 mm $\times$ 5 mm; face angle of 45°) and by evaporating the solvent under a stream of nitrogen. The lipid arranges spontaneously into layers oriented preferentially parallel to the crystal surface (see below). The amount of lipid corresponds to about 10^3 bilayers, yielding an average lipid film thickness of about 4 μm. Equally spaced Bragg peaks in the small-angle X-ray diffraction pattern of samples prepared analogously on glass slides give evidence of the lamellar structure under the external conditions used (data not shown).

The ATR crystal was mounted on a commercial horizontal Benchmark unit (Specac, U.K.) modified in order to realize relative humidity (RH) and temperature control at the crystal surface coated with the lipid. The unit was covered with a thermostated (flowing water) copper block forming a closed atmosphere above the crystal. This block was supplemented with gas influx and outflux lines and with sensors measuring the RH (capacitive RH sensor) and temperature (Pt-100 thermocouple) within the sample compartment. During the measurement nitrogen gas of definite humidity and temperature flows through the cell (10^{-3} m^3/h), thus realizing the hydration of the lipid. The relative humidity of the N_2 inflow was adjusted in a home-built moister cell by means of subtle water evaporation regulated with Peltier elements. The moister cell was integrated into a regulating circuit that additionally consists of a computer-controlled electronic device and of the humidity sensor measuring the RH within the sample compartment directly above the ATR crystal. This equipment allows us to adjust the RH continuously between 10% and 95% with an accuracy of $\pm$1%.

An analogous moisture-regulating device was combined with a microbalance system (Sartorius) in order to determine gravimetrically the amount of water adsorbed by the lipid ($\sim$3 mg) as a function of RH.

The samples were investigated by means of increasing (hydration scan) and decreasing (dehydration scan) RH at constant temperature, $T = (25 \pm 0.2)$ °C. The RH was varied in steps of 2%. Before measurement the sample was allowed to equilibrate for at least 10 min after reaching the prescribed RH in each step.

Infrared Measurements. Infrared spectra with a resolution of 2 cm^{-1} were measured using a BioRad FTS-60a Fourier transform infrared spectrometer equipped with a deuterated triglycine sulfate detector. Typically, 256 scans were coadded. Absorbance spectra of the sample were calculated using the respective single-channel spectra of the empty ATR crystal (without lipid) as background.

Selected absorption bands are characterized in terms of the wavenumber at maximum absorbance and the center of gravity (COG). This parameter is sensitive to shifts of the band maximum as well as to changes of the band shape, and consequently, it is well suited for detecting subtle spectral changes induced by conversions of the physical state of the sample. The COG representation gives rise to a well-defined characterization of spectral details in terms of a single parameter, the behavior of which is dominated by the selected absorption band and usually can be correlated to local molecular properties.

In some cases the band shapes are fitted by the superposition of two Gaussian lines yielding the maximum wavenumbers, bandwidths, and integral absorbances of the component bands.

3. Results

Spectral Assignments. The spectra of monomeric DODPC resemble the general features of diacyl phosphatidylcholines (PCs)[27,29−32] showing characteristic absorption bands from the hydrophobic acyl chains as well as from the carbonyl ester and the headgroup of the lipid. Assignments and designations of selected absorption bands common in diacyl PCs are taken from ref 27 and listed in Table 1. In contrast to the FTIR spectra of phosphocholines with saturated fatty acid residues, new bands appear in the spectra of DODPC originating from the diene group. The conjugated double bonds give rise to coupled C═C (cf. ν_{as}(C═C) and ν_s(C═C)) and C−C (ν(C−C)) vibrations as well as to the well-resolved C−H wagging mode (γ_w(C−H)).[28] In 1,4-trans dienes the ν_s(C═C) band is usually observed at wavenumbers higher than that of the ν_{as}(C═C) mode.[28] Furthermore, the diene group is conjugated with the carbonyl group, thus causing a drastic downward shift of the C═O stretching vibration by about 20 cm^{-1}, i.e., from about 1735 cm^{-1}, measured in fully hydrated disaturated PCs, down to about 1711 cm^{-1} in DODPC. The modifications induced by the diene

TABLE 1: Assignments of Selected Absorption Bands of DODPC and the Corresponding Wavenumbers at Maximum Absorbance in the L_α, Gel, SG_I, and SG_{II} Phases[b]

			wavenumber/cm^{-1}			
group vibrations	assignment	symbol	L_α	gel	SG_I	SG_{II}
methylene	antisymmetric stretching	$\nu_{as}(CH_2)$	2922	2919	2918	2918
	symmetric stretching	$\nu_s(CH_2)$	2853	2851	2851	2851
	scissoring/bending	$\delta(CH_2)$	1467	1467	1472 sh, 1466	1472 sh, 1467
	rocking	$\gamma_r(CH_2)$	720	721	726 sh, 720	726 sh, 720
1,4-*trans*-butadiene	C═C symmetric stretching	$\nu_s(C{=}C)$	1643	1643	1637	1637
(diene)	C═C antisymmetric stretching	$\nu_{as}(C{=}C)$	1616	1616	1609	1609
	C−C stretching	$\nu(C{-}C)$	1137	1140	1141	1141
	C−H wagging	$\gamma_w(CH)$	998	995	1000	1000
carbonyl	C═O stretching	$\nu(C{=}O)$	1711	1714	1716 sh, 1700	1716 sh, 1704
phosphate	PO_2^- antisymmetric stretching	$\nu_{as}(PO_2^-)$	1239[a]	1252−1240[a]	1255−1249[a]	1247[a]
	P−$(OC)_2$ antisymmetric stretching	$\nu_{as}(P{-}(OC)_2)$	826	822, 802 sh	806, 800 sh	817, 800 sh
choline	N^+−$(CH_3)_3$ antisymmetric stretching	$\nu_{as}(N{-}CH_3)$	970	969	970	967
(trimethylammonium)	C−N^+−$(CH_3)_3$ symmetric stretching	$\nu_s(N{-}CH_3)$	927	925	927	923

[a] Maximum shifts with progressive dehydration. [b] The appearance of a shoulder is designated by "sh". The assignments of methylene, phosphate, and choline group vibrations are taken from ref 27 and that of the diene group vibrations from refs 28 and 34. Peaks are resolved to whole wavenumbers.

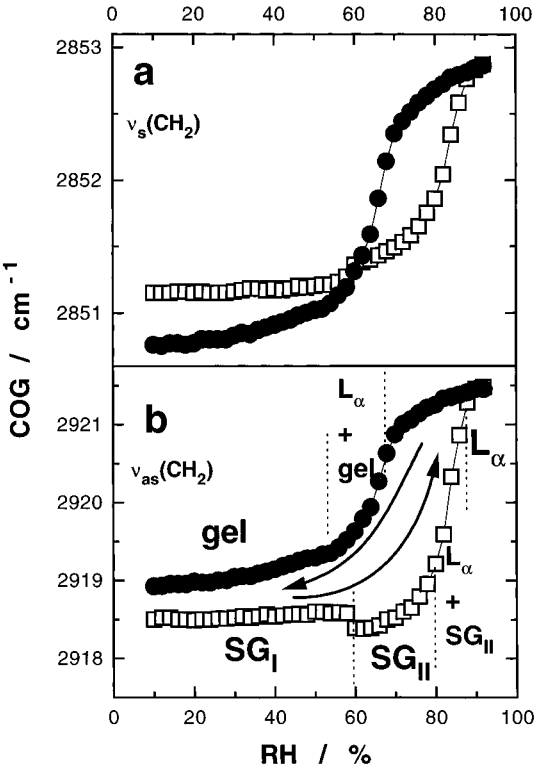

Figure 1. Center of gravity (COG) of the symmetric (a) and antisymmetric (b) CH_2 stretching bands, $\nu_s(CH_2)$ and $\nu_{as}(CH_2)$, of DODPC as a function of relative humidity (RH) obtained from hydration (□) and dehydration (●) scans. The corresponding phase sequences are indicated in part b.

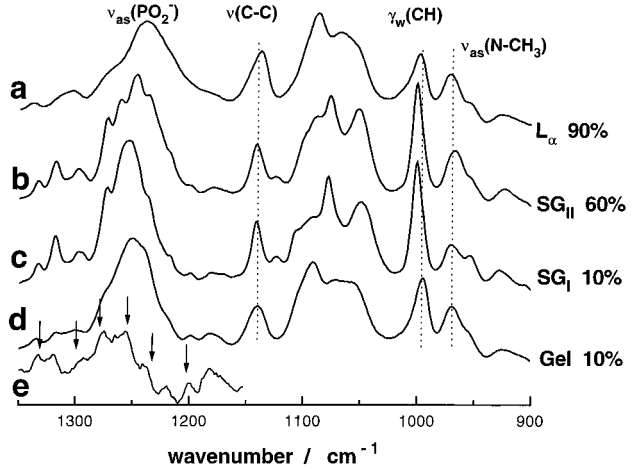

Figure 2. Absorbance spectra of DODPC in the spectral range 1350−900 cm^{-1}. The vertical dotted lines emphasize spectral shifts of the $\nu(C{-}C)$, $\gamma_w(CH)$, and $\nu_{as}(N{-}CH_3)$ bands (from left to right). The spectra correspond to the different phases of DODPC: (a) L_α (RH = 90%), (b) SG_{II} (RH = 60%, hydration scan), (c) SG_I (RH = 10%, hydration scan), and (d) gel (RH = 10%, dehydration scan). The difference of two spectra recorded in the L_α and gel states upon dehydration (RH = 68% and 55%, respectively) is shown in spectrum e. The arrows indicate the positions of the CH_2 wagging progression bands, $\gamma_w(CH_2)$.

group are also evident in the FTIR spectra of 1,4-disubstituted *trans,trans*-butadienes given in refs 33 and 34. Preliminary polymerization studies on DODPC (unpublished results) have shown that the absorption bands assigned to the diene group disappear during the UV induced polyreaction.

Dehydration Scan. The centers of gravity of the $\nu_s(CH_2)$ and $\nu_{as}(CH_2)$ absorption bands of DODPC, COG($\nu_s(CH_2)$), and COG($\nu_{as}(CH_2)$) each decrease monotonically upon dehydration, showing a sigmoidal course in the humidity range between 67 and 55% (Figure 1). In general, the liquid crystalline (L_α) to gel phase transition of lipids is marked by an abrupt decrease of the frequency of the symmetric and antisymmetric CH_2 stretching vibrations correlating with the freezing of the acyl chains.[29] A linear correlation between the degree of conformational ordering of the hydrocarbon chains given in terms of the ^2H NMR order parameter on one hand and in terms of the COG of the $\nu_s(CH_2)$ band on the other was revealed empirically in several lipid systems investigated in the liquid crystalline phase.[35] Thus, the decrease of the COGs of the $\nu_s(CH_2)$ and $\nu_{as}(CH_2)$ bands of DODPC upon dehydration can be attributed to the increasing ordering, i.e., rigidization, of the hydrocarbon chains due to the osmotic stress induced in the sample by the removal of water (see below).

A more detailed inspection of the FTIR spectra recorded at humidities below 67% reveals the existence of a sequence of nearly equally spaced bands between 1198 and 1340 cm^{-1} (cf. spectrum e in Figure 2). This frequency pattern is typical for the methylene wagging band progression resulting from a number of coupled CH_2 oscillators in the all-trans conformation[36] and thus indicates all-trans polymethylene fragments in the octadecadienoyl chains of DODPC. Note that additional bands in this spectral range originate from the strong $\nu_{as}(PO_2^-)$ vibration as well as from the CH bending modes of the diene group complicating the unequivocal identification of the position of all wagging bands. Typically, the wagging band progression present in lipid systems at temperatures below the main phase

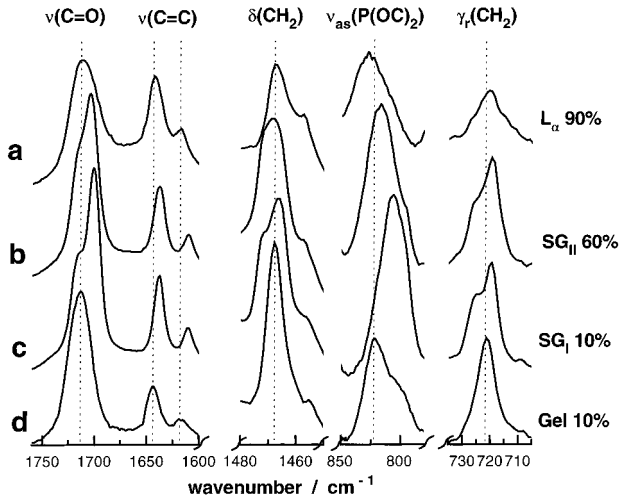

Figure 3. Absorbance spectra of DODPC in the region of the ν(C═O), ν_s(C═C), ν_{as}(C═C), $\delta(CH_2)$, ν_{as}(P−(OC)$_2$), and $\gamma_r(CH_2)$ absorption bands (from left to right). Vertical dotted lines are drawn at the wavenumbers of maximum absorbances of selected bands of the gel phase spectra in order to emphasize spectral shifts. Spectra a−d correspond to the same conditions as those given in Figure 2 .

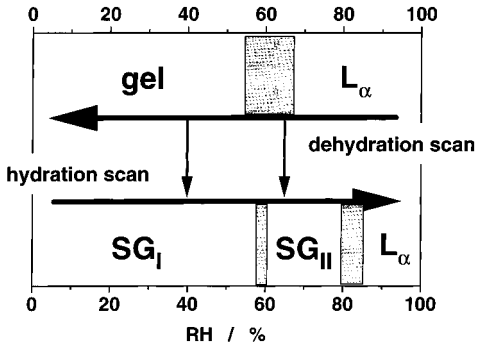

Figure 4. Schematic illustration of the phase sequences passed upon gradual dehydration and hydration of DODPC. Phase coexistence regions are shown by gray areas. The smaller arrows indicate transitions to the subgels.

transition temperature decreases considerably in intensity upon heating, and ultimately, it disappears at the gel-to-liquid crystalline phase transition.[32,37] Hence, the identification of the wagging band progression in the DODPC spectra below RH = 67% gives strong evidence of frozen hydrocarbon chains and consequently of a dehydration-induced liquid crystalline-to-gel phase transition.

This behavior is completely reproducible for the slower average reduction rate of RH (3%/h) and is also independent of the sample history (freshly prepared, repeated scan, previously heated and annealed in the L_α phase). In the phase transition range X-ray diffraction patterns show the superposition of two series of equally spaced Bragg peaks that refer to the L_α and gel states and thus indicate L_α/gel coexistence (data not shown).

Lipid Crystallization. When the sample is stored for more than 4 h after completing the dehydration scan at a constant RH chosen in the intermediate range between 30% and 55%, the lipid starts to convert from the gel state into another rigid state characterized spectroscopically by a sharpening and shift of most of the absorption bands (see below). A similar tendency was typically found upon crystallization of lipid systems because of restricted conformations of the molecules in comparison with that of the liquid crystalline or gel states.[27] Consequently, we will designate this crystalline state arbitrarily as subgel I (SG_I).

The most characteristic spectral changes observed during the conversion of the lipid from the gel into the SG_I state are the following (compare spectra d and c in Figures 2 and 3 and columns gel and SG_I in Table 1): (i) the appearance of shoulders on the high-frequency side of the CH_2 scissoring and rocking modes (Figure 3); (ii) the distinct right-hand shift of the C═O, C═C, and P−(OC)$_2$ stretching bands (Figure 3); (iii) the left-hand shift of the CH wagging, of the symmetric N−CH_3 stretching vibrations, and of the intense band around 1140 cm^{-1}, assigned to the C−C stretching vibration (Figure 2); (iv) the appearance and increase of the intensity of the CH bending modes in the frequency range 1330−1290 cm^{-1}; (v) the splitting of the broad shoulder between 1080 and 1020 cm^{-1} into two intense, well-resolved components (Figure 2).

It was found that DODPC can be transformed into the SG_I state, independent of the history of the sample, by incubation at the conditions given above. We conclude that the gel state of DODPC in the dehydration scans is metastable whereas the crystalline SG_I state corresponds to thermodynamic equilibrium. The gel/SG_I transition is inhibited or slowed, if one stores the lipid at RH < 30%, probably because of the almost complete immobilization of molecular motions and the dense packing of the lipid.

Hydration Scan. Now we consider the lipid phase behavior in the case of the hydration scan started in the SG_I phase (sample stored at RH = 40% for 7−15 h and subsequently dried and stored for several hours at RH = 10%).

The FTIR spectrum of DODPC in the SG_I state remains almost unchanged upon subsequent hydration. At RH = 58−60% subtle spectral changes indicate a further conversion to another rigid state designated arbitrarily as SG_{II} (compare spectra c and b in Figures 2 and 3 as well as columns SG_I and SG_{II} in Table 1). They are indicated by the following observations: (i) only a slight broadening of the $\delta(CH_2)$ absorption band and the relative decrease of the intensity of the high-frequency shoulder of the $\gamma_r(CH_2)$ band (Figure 3); (ii) a distinct left-hand shift of the C═O and P−(OC)$_2$ stretching bands (Figure 3); (iii) a right-hand shift of the antisymmetric N−CH_3 vibrations (Figure 2).

Some of these spectral modifications (e.g., (i)) seem to compensate partially the changes observed at the gel/SG_I conversion, thus giving rise to absorption bands looking like the superposition of the bands obtained in the gel and SG_I states. On the other hand, the bands originating from the diene and carbonyl groups do not change significantly at the SG_I/SG_{II} conversion in contrast to their large shifts at the gel/SG_I transformation. Moreover, the choline group bands shift from their wavenumber of maximum absorbance determined in the gel state (see Table 1). Consequently, these spectral changes cannot be understood as a superposition of the respective band contours recorded in the gel and SG_I phases, but they characterize a separate phase.

Upon a further rise of RH, DODPC converts to the liquid crystalline phase as indicated by identical FTIR spectra from dehydration and hydration scans. It should be stressed that the lipid with increasing RH is completely melted at about 85% whereas the acyl chains start to freeze only at about 67% upon dehydration. This freezing−melting hysteresis was found unchanged even at slower dehydration/hydration scan rates, and therefore, it should be interpreted as an intrinsic property of the different solid states of DODPC. Diacetylene lipids with rather rigid moieties in the acyl chains show a similar freezing−melting hysteresis in contrast to naturally occurring phospholipids.[13] When a dehydration scan at RH = 60−65% is stopped, the sample starts to convert into the SG_{II} phase after a storage for 4−8 h at these RHs. We conclude that up to RH = 65% the subgels correspond to thermodynamic equilibrium, which

TABLE 2: Center Positions and Intensity Ratios of Gaussian Functions Fitted to the CH_2 Rocking and Bending Modes in Different Phases of DODPC (cf. Figure 5)[a]

vibration	property	L_α	gel	SG_I	SG_{II}
CH_2 rocking $\gamma_r(CH_2)$	max/cm^{-1}	720.0	721.0	725.5, 719.5	725.5, 719.5
	$A_{\gamma'}/A_\gamma$			0.68	0.41
	σ/deg			39	33
CH_2 bending $\delta(CH_2)$	max/cm^{-1}	1467.1	1467.1	1472.2, 1466.3	1471.7, 1466.9
	$A_{\gamma'}/A_\gamma$			0.61	0.43
	σ/deg			38	33

[a] The setting angle σ is defined in the text. The resolution of the center positions is ± 0.1 cm^{-1}.

is, however, reached very slowly from the gel or gel/L_α coexistence range. At RH = 65−80% no conversions of L_α to SG_{II} or vice versa are observed. On the other hand, the SG_I/SG_{II} phase transition is reversible without significant hysteresis using identical scan rates in both directions.

These results give rise to a differentiation of the solid DODPC into three states denoted as gel (RH < 55%, dehydration scan, metastable), SG_I (RH < 60%), and SG_{II} (60% < RH < 80%). The phase sequences found in the experiments are illustrated schematically in Figure 4.

Acyl Chain Packing. Most of the spectral changes observed upon transformation of DODPC into the subgel states refer to vibrations of the phosphocholine, carbonyl, and diene groups, suggesting structural changes predominantly in the polar region of the bilayers. The band positions of the CH_2 stretching vibrations, $\nu_s(CH_2)$ and $\nu_{as}(CH_2)$, are affected only slightly. These bands are almost sensitive to the conformation of the CH_2 chains, and consequently, no drastic modification of the intrachain structure seems to take place except for an increase of the fraction of chains in the all-trans conformation as indicated by the moderate enhancement of the CH_2 wagging progression bands, $\gamma_w(CH_2)$ (Figure 3). An alternative interpretation of this effect can be given on the basis of the fact that the molar extinction coefficient of the $\gamma_w(CH_2)$ modes of the acyl chains is influenced strongly by the nature and/or conformation of the polar endgroup.[27] Hence, the intensity increase of the $\gamma_w(CH_2)$ bands in the subgel states can be induced also by conformational changes at the linkage between the methylene and diene groups during the gel/SG_I transformation, for example, by changes of the dihedral angle of the CH_2−CH_2−CH=CH fragments.

On the other hand, the CH_2 scissoring and rocking vibrations, $\delta(CH_2)$ and $\gamma_r(CH_2)$, are sensitive to interchain interactions and consequently are highly diagnostic for the structure of lipid assemblies in the frozen state.[9,38,39] Owing to the coupling of the $\delta(CH_2)$ or $\gamma_r(CH_2)$ modes of neighboring all-trans methylene chains, the respective bands can split into two or more components depending on the symmetry of the acyl chain packing. Such correlation field splitting was observed in case of a number of hydrated saturated phosphatidylcholines in the crystalline state. On the basis of the IR spectroscopic analysis of crystalline *n*-alkanes,[40] they are assigned to several acyl chain packing modes.[24,38]

The appearance of shoulders on the high-frequency side of the CH_2 scissoring and rocking modes of DODPC during the gel/SG_I transformation (Figures 3 and 4) indicates the splitting of these bands and consequently gives evidence of an almost complete damping of reorientational fluctuations among the octadecadienoyl chains. The correlation field splitting of the CH_2 rocking and bending modes provides the possibility of evaluating the interchain configuration of DODPC by means of a ratio of the integrated absorbances of the high- and low-wavenumber components of the respective absorption band,[40] $A_{\gamma'}/A_\gamma$ (cf. Figure 5). In orthorhombically packed *n*-alkanes the setting angle between the polymethylene planes and the axis of the unit cell, σ, is related to $A_{\gamma'}/A_\gamma$ by $\tan^2 \sigma = A_{\gamma'}/A_\gamma$.[40] This

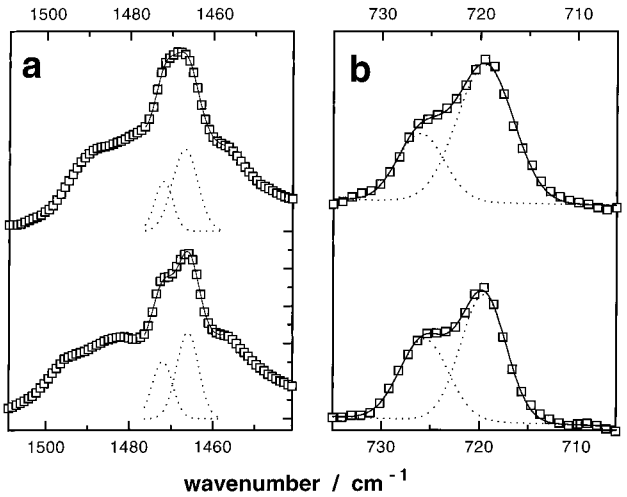

Figure 5. Absorbance spectra in the region of the CH_2 bending (a) and rocking (b) modes of DODPC in the SG_I (bottom) and SG_{II} (top) states. The conditions are the same as given in the legend of Figure 2. The Gaussian functions fitted to the bands are shown by dotted lines and their superposition by solid lines.

ratio represents an accepted parameter for comparing the packing properties of crystalline lipid systems,[4,39] although its interpretation in lipid systems is not as simple as in *n*-alkanes not the least due to the two acyl chains per molecule.

In the SG_I phase the intensity ratio yields 0.68−0.61, thus indicating a nonparallel arrangement of the polymethylene planes of neighboring chains on the average (Table 2). Similar $A_{\gamma'}/A_\gamma$ ratios are determined in completely hydrated crystalline DPPC at high pressure (G_{III} phase).[39] In this phase the correlation field splitting was attributed mainly to an almost fixed orientation between the polymethylene planes of the two acyl chains within each lipid molecule, which are assumed to arrange almost nonparallel to each other, while the correlation between the chains of neighboring DPPC molecules is insignificant because of reorientational fluctuations of the whole lipid molecules.

In the SG_{II} phase the integrated areas of the splitting components indicate an increased deviation of the packing of the acyl chains from an orthorhombic perpendicular lattice possibly due to their more parallel alignment on the average. However, it seems more likely that this tendency is caused by loosening the packing density of the chains, which should be accompanied by the reduction of intermolecular vibrational coupling. This hypothesis is confirmed by the slight upward shift of the right-hand $\delta(CH_2)$ band to a position similar to that observed in the gel state (see Table 2) where no splitting and consequently no favoring of any interchain alignment were detected. The possible misinterpretation of band splitting data in crystalline, phase-separated lipid bilayers (L_c/gel) is discussed in ref 41.

Adsorbed Water. Upon a decrease of the RH, the number of water molecules per lipid, R_{WL}, reduces continuously without drastic changes at the L_α/gel transition (cf. Figure 6a). The

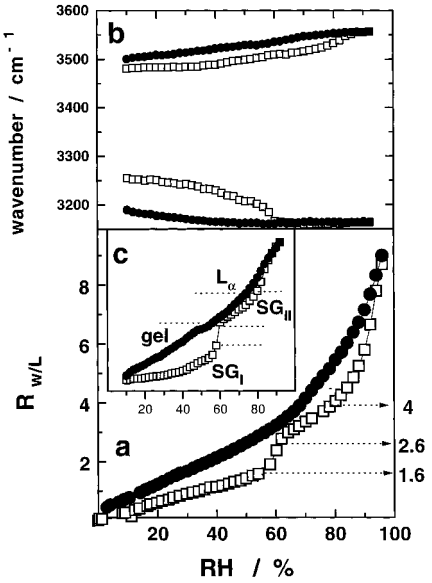

Figure 6. Number of water molecules per lipid, R_{WL} (a), wavenumbers at half-maximum absorbance (cf. Figure 7c) (b), and the integrated absorbance (c) of the $\nu_{1,3}$(OH) absorption band of water adsorbed onto DODPC as a function of relative humidity RH obtained from hydration (□) and dehydration (●) scans. R_{WL} was measured gravimetrically with an accuracy of ±0.2. It is given at the right axis for the subgel phase boundaries (a). The absorbance of the $\nu_{1,3}$(OH) band was integrated over the spectral range 3700−2700 cm^{-1} after separation from the CH stretching bands below 3100 cm^{-1} and drawn in arbitrary units (c).

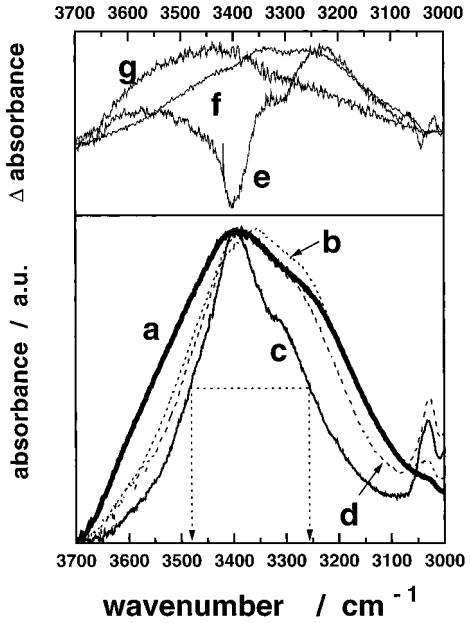

Figure 7. Normalized absorbance (below) and difference (above) spectra of water adsorbed onto DODPC in the wavenumber range of the $\nu_{1,3}$(OH) band. The assignment of spectra a−d given in the figure corresponds to the conditions in the legend of Figure 2, i.e., to the L_α, SG_{II}, SG_I, and gel states. The difference spectra are calculated by subtraction of the absorbance spectra recorded at RH = 10% (dehydration scan) and 10% (hydration scan) (e), 60% and 58% (both hydration) (f), and 84% and 82% (g). Thus, curves e, f, and g represent the difference spectra of gel − SG_I, SG_{II} − SG_I, and L_α − SG_{II}, respectively. The wavenumbers at half-maximum absorbance at the right and left flanks are illustrated by vertical dotted arrows in spectrum c (see also Figure 6).

hydration capacity of the SG_I phase is lower compared with that of the gel. It is enhanced remarkably at the SG_I/SG_{II} and, to a lesser extent, the SG_{II}/L_α conversions. The course of the integrated absorbance of the O−H stretching region of water ($\nu_{1,3}$(OH)) versus RH behaves almost like that of R_{WL} (cf. parts a and c of Figure 6). It represents an approximate measure of the amount of water bound to the lipid[31,46,47] regardless of slight variations of the extinction coefficients of the water fractions differing in their H-bond characteristics.[43]

Typical $\nu_{1,3}$(OH) bands of gradually hydrated DODPC are displayed in Figure 7. The band shapes obtained in the L_α and gel states are very similar and resemble the typical structure of the $\nu_{1,3}$(OH) band of fully or partially hydrated PC lipids in the fluid state.[42−44] The broad feature consists of subbands assigned to the symmetric and antisymmetric stretching vibration of free, i.e., non-hydrogen-bound, OH groups (3615 cm^{-1}) and of hydrogen-bound OH-groups (<3615 cm^{-1}) and to the Fermi resonance (3280 cm^{-1}) at $2\nu_2$(OH), where ν_2(OH) ≈ 1650 cm^{-1} is the H_2O bending vibration.[45] In general, the $\nu_{1,3}$(OH) bandwidth reflects the distribution of hydrogen bonds of variable strength among a variety of binding sites. In the frame of this interpretation the band maximum centered around 3400 cm^{-1} is related to the major fraction of H bonds and the shoulders on its low- and high-frequency sides can be assigned to populations of stronger and weaker bound hydroxyl groups, respectively.

The gel/SG_I transition is characterized by the relative increase of the absorbance in the center of the $\nu_{1,3}$(OH) band at the expense of the left-hand and, to a much greater extent, of the right-hand shoulder (see difference spectrum in Figure 7). This effect can be explained by the reduction of the amount of more tightly bound water at the gel/SG_I transition. The sharp contour of the $\nu_{1,3}$(OH) band in the SG_I state indicates the relative uniform H-bond characteristic possibly due to the interaction of the water molecules (R_{WL} < 1.6) with a unique binding site. The narrow shape resembles the $\nu_{1,3}$(OH) band of the well-ordered lauroylpropanediolphosphorylcholine (LPPC) monohydrate where the water was assumed to form only bridges between the phosphate groups.[42]

The SG_I/SG_{II} conversion at RH = 60% is characterized by a considerable enhancement of the subbands in the 3350−3200 cm^{-1} range, indicating the increased strength of interaction of the water molecule (ΔR_{WL} = 1) adsorbed additionally by each lipid on the average. The RH dependence of the wavenumbers at half maximum intensity shown in Figure 6b (see also Figure 7c for illustrating this quantity) reflects this broadening of the $\nu_{1,3}$(OH) band in terms of the downward shift of the right flank to a value found also in the gel phase.

On the other hand, during the subsequent SG_{II}/L_α transformation at about RH = 80% the left shoulder of the $\nu_{1,3}$(OH) band is enhanced considerably and finally coincides with that measured in the L_α phase (cf. Figures 6b and 7). Possibly a substantial fraction of the adsorbed water molecules (R_{WL} > 4) does not interact or only weakly interacts with the lipid via H bonds. Obviously, hydration swells the size of the lipid headgroup and thus creates the free volume necessary for acyl chain melting. A similar modification of the $\nu_{1,3}$(OH) band shape occurs, if one compares the spectra of DODPC in the gel and L_α states. It was found also in the Raman spectra of multilamellar dispersions of DMPC.[46]

Phosphate Group. The courses of the COGs of the $\nu_{as}(PO_2^-)$ and the $\nu_{as}(P-(OC)_2)$ band change similarly as the molar ratio R_{WL} (compare Figures 6a and 8d). It is justified to conclude that the spectral shifts of the two phosphate bands are caused mainly by the hydration of the phosphate group representing the primary hydration site of PC lipids.[48] Consequently, the transformations among the gel, SG_I, and SG_{II} states are correlated with subtle changes of water/phosphate interactions.

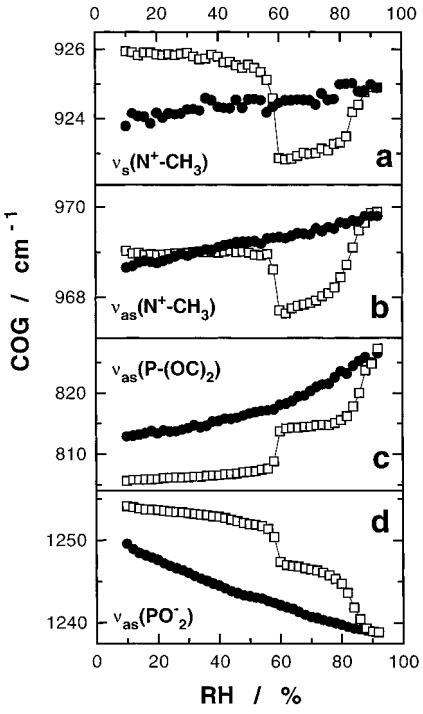

Figure 8. Center of gravity (COG) of absorption bands of the choline and phosphate groups of DODPC as a function of relative humidity (RH) obtained from hydration (□) and dehydration (●) scans: (a) ν_s-(N−CH_3); (b) ν_{as}(N−CH_3); (c) ν_{as}(P−$(OC)_2$); (d) $\nu_{as}(PO_2^-)$.

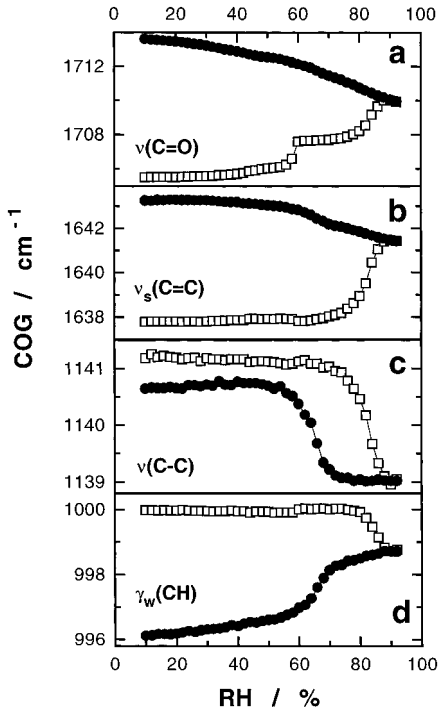

Figure 9. Center of gravity (COG) of absorption bands of the carbonyl and diene groups of DODPC as a function of relative humidity (RH) obtained from hydration (□) and dehydration (●) scans: (a) ν(C═O); (b) ν_s(C═C); (c) ν(C−C); (d) γ_w(CH).

It is widely accepted that the frequency of the $\nu_{as}(PO_2^-)$ vibration is very sensitive to lipid hydration mainly because of direct H binding to the charged phosphate oxygens.[27,44,48,49] But also other effects may influence the $\nu_{as}(PO_2^-)$ band position. Theory predicts frequency shifts caused by direct interaction of the trimethylammonium group with the phosphate as well as by changes of the torsion angles of the C−O−P−O−C fragment.[48] The course of COG(ν_{as}(P−$(OC)_2$)) increases upon hydration, whereas that of COG($\nu_{as}(PO_2^-)$) decreases, a difference predicted theoretically[48] and observed in other lipid systems too.[47] From the parallel behavior of both phosphate bands we conclude that underlying bands (e.g., $\gamma_w(CH_2)$ and CH bending) exert only a minor influence on the course of COG($\nu_{as}(PO_2^-)$).

Choline Group. The COGs of the absorption bands centered around 969 and 925 cm^{-1} differ considerably in the gel and SG_I states of DODPC, and in addition they undergo distinct changes at the SG_I/SG_{II} and SG_{II}/L_α transitions (see parts a and b of Figure 8). These bands are assigned to the antisymmetric and the symmetric $CN^+-(CH_3)_3$ stretching vibrations, ν_{as}(N−CH_3) and ν_s(N−CH_3), respectively. In the SG_I state a well-separated shoulder appears at 906 cm^{-1} assigned to the ν_s(N−CH_3) vibration of the O−C−C−N gauche conformer whereas the more intense band around 927 cm^{-1} pertains to the trans conformation[27,48] (cf. Figure 2 and Table 1). At transformation to the SG_{II} state the low-frequency shoulder weakens and the main band shifts down to 923 cm^{-1}. These spectral changes indicate probably the conformational rearrangement of the choline group of DODPC in the SG_I and SG_{II} states. A similar behavior, namely, the increase of the population of the gauche conformer, was also found in DPPC at low hydration degrees ($R_{WL} < 5$).[48]

Carbonyl Group. Upon dehydration the ν(C═O) band of DODPC shifts continuously toward higher wavenumbers accompanied by a distinct narrowing of the band shape (cf. Figures 9a and 3) . This behavior was typically found in diacyl glycerolipids investigated in the dry, partially, and fully hydrated states[32,44,48,50−52] and attributed to a multicomponent nature of the C═O absorption band. It was suggested that high- and low-frequency subbands originate from populations of free and hydrogen-bound ester carbonyl groups, respectively, explaining the band shift and narrowing, measured upon gradual drying of the lipids by the relative increase of the fraction of free C═O groups. Despite the different positions of the ν(C═O) absorption band in the IR spectra of lipids with saturated acyl chains and of DODPC, we attribute the similar spectral changes to the same molecular origin, i.e., to the desorption of H-bonded water from the vicinity of the ester carbonyl groups in both acyl chains.

The conversion into the SG_I state is accompanied by a drastic downward shift of the C═O band maximum by more than 10 cm^{-1} (cf. Table 1 and Figure 3). Subtle changes of the COG(ν(C═O)) at the SG_I/SG_{II} and SG_{II}/L_α transitions indicate that the carbonyl groups are involved in these transformations.

The ν(C═O) band of DODPC in the SG_I state shown in Figure 3 reveals a shoulder at about 1716 cm^{-1} appearing at the left-hand side of the band maximum located at 1700 cm^{-1}. Band shape analysis indicates a subband with an intensity fraction of about 35% that remains present also in the SG_{II} phase. Grdadolnik et al.[48] reported an analogous splitting of the ν(C═O) band of dry crystalline DPPC and of DPPC dissolved in dry benzene into two well-resolved peaks separated from each other by 11−12 cm^{-1}. It was suggested that the origin of these bands has to be sought in conformational differences between the *sn*-1 and *sn*-2 acyl and not in the H bonding of water to the *sn*-2 ester group. Also, fully hydrated saturated diacyl phosphatidylcholines upon transformation into the crystalline L_c phase typically show an enhancement of right-hand subbands of the ν(C═O) mode.[7,41] Close-contact interactions[41] as well as at least two different intramolecular conformations in the vicinity of the ester groups[48] are proposed as the origin of the splitting of the ν(C═O) band in dry or less hydrated PCs, and they possibly apply to DODPC too. These differences can be allocated on the *sn*-1- and the *sn*-2- octadecadienoyl chain and

also on two nonequivalent species in the crystal unit cell revealed, for example, in the crystal structure of DMPC dihydrate.[53]

Dluhy et al.[7] suggested that the dehydration of the hydrophilic region leads to a situation in which the carbonyl groups are deshielded from the negatively charged phosphate group. As a consequence, the local dielectric constant in the vicinity of the carbonyl groups increases, resulting in a downward shift of the band position. Within the frame of this hypothesis the splitting of the C═O band could be attributed to two populations of carbonyl groups exposed to a more and a less polar environment, for example, due to different distances to the phosphate group. Also, the formation of well-oriented H bonds between some trapped water molecules ($R_{WL} \approx 1-2$) and the carbonyl groups can induce the band shift observed.

Regardless of these hypotheses, the molecular origin of the C═O band splitting still remains open and requires further work. However, it remains almost unaffected during the SG_I/SG_{II} conversion where the hydration of the phosphate group increases distinctly. We conclude that the molecular packing, conformation, and/or hydration of the DODPC bilayers in the region of the carbonyl moiety differs considerably between the subgel phases on one hand and the gel and L_α states on the other and thus correlates with changes of the packing properties of the polymethylene chains.

Diene Group. Factors affecting the ν(C═O) stretching vibration are expected to influence also the ν_s(C═C) stretching vibration due to the coupling between both electronic π systems. Hence, the very similar behavior of the COG of both bands and in particular their marked shifts at the gel/SG_I and SG_{II}/L_α transitions have probably the same origin (cf. parts a and b of Figure 9).

First of all, torsional motions about the C−C bond in the ═CH−C═O fragment are expected to modify the ν(C═O) as well as the ν_s(C═C) band. In unsaturated ketones the overlap of the electronic π systems of the C═O double bond with an adjacent C═C bond results in the moderate (<10%) coupling between stretching vibrations, leading to a characteristic dependence on the torsional angle of the positions of the respective vibrational bands.[55] The cis conformer shows a larger separation between the ν(C═O) and ν_s(C═C) bands (>60 cm^{-1}) than the trans configuration.[54,55] In DODPC the separation between the band maxima ν(C═O) − ν_s(C═C) decreases during the gel/SG_I transformation from 71 to 63 cm^{-1} (see Table 1). This effect can be attributed to an increased torsional angle on the average, corresponding to a slight rotation about the CH−CO bond toward the trans conformer. At the same time the weaker ν(C═O) subband appearing as the left-hand shoulder is separated more distantly from the C═C band by 79 cm^{-1}, a tendency corresponding to rotations about the CH−CO bond in the opposite direction, i.e., toward the cis conformer. Thus, the two populations of carbonyl groups suggested above possibly represent two conformers differing in slight variations of the C═C−C═O dihedral angle. Alternatively, the coupling between the C═O and C═C vibrations may induce an in-phase and an out-of-phase mode, resulting in the splitting of the ν(C═O) band by analogy with the symmetric and antisymmetrig C═C vibration of the diene group. However, no splitting of the ν_s(C═C) and ν_{as}(C═C) bands can be detected in the subgel states (see Figure 3).

Model calculations have demonstrated that also the relative positions of the symmetric and antisymmetric C═C vibrations of the diene group exhibit a characteristic dependence on the dihedral angle in the C═C−C═C fragment.[56] At the gel/SG_I phase transformation a downward shift of both bands is observed, leaving the mean separation between them almost unchanged, i.e., 27 cm^{-1} in the gel and 28 cm^{-1} in the SG_I states (cf. Table 1). Obviously, the rigid diene moiety is not, or only scarcely, distorted by the phase transformations, as expected.

Different locations, orientations, and environments of the two diene and carbonyl groups in the *sn*-1- and *sn*-2- octadecadienoyl chains of DODPC are considered in ref 15 to appear by analogy with the nonequivalent packing of the fatty acid residues in the *sn*-1 and *sn*-2 positions typically observed in bilayers of 1,2-diacetylene phosphocholine lipids[64] where the axis of the ester group in the *sn*-2 position inclines from the membrane normal and that in the *sn*-1 position is directed along the bilayer normal. As a consequence, the long axis of the glycerol residue orients nearly parallel with respect to the bilayer normal and the *sn*-2 acyl chain bends at the C2 position in order to realize an almost parallel arrangement of the axes of both acyl chains in the hydrophobic core of the bilayer. The flexibility of the octadecadienoyl chains of DODPC is expected to be more restricted than that of the polymethylene chains of disaturated PCs because of hindered rotations about the C═C double bonds. Consequently, the spectral features of the ν(C═O) and ν_s(C═C) bands discussed above in terms of slight variations of the ═C−C═O dihedral angle reflect possibly conformational changes at the linkage between the acyl chains and the glycerol group induced by modifications of the packing of the octadecadienoyl chains on one hand and of the hydration of the polar/apolar interface on the other. However, the results presented do not allow us to differentiate between the *sn*-1 and the *sn*-2 chains, and consequently, it is not possible to assign the observed effects to the actual molecular arrangement in the various phases.

Two additional features are revealed differently in the RH dependences of the COGs of the ν(C═O) and ν_s(C═C) bands. On one hand, the L_α/gel transition affects the COG(ν_s(C═C)) more distinctly than it affects the COG(ν(C═O)). The melting of the methylene residues is expected to influence the adjacent diene group more strongly than it influences the more distant carbonyl group. The relatively high sensitivity of the diene group vibrations against the methylene chain melting transition is confirmed by sigmoidal changes of the COG of the ν(C−C) and γ_w(CH) bands (cf. parts c and d of Figure 9). On the other hand, the COG(ν(C═O)) increases stepwise at the SG_I/SG_{II} transformation, whereas the COG(ν_s(C═C)) and also the COG(ν(C−C)) do not change. This different behavior can be attributed to the fact that the diene groups are buried deeper in the hydrophobic core of the bilayer, and thus, they are probably less influenced by changes of lipid hydration than the ν(C═O) vibration.

The ═C−H out-of-plane wagging vibration and the CH_2 stretching modes are both sensitive to polymethylene chain melting, whereas lipid crystallization mostly affects the COG(γ_w(CH)). In the γ_w(CH) mode the hydrogen atoms vibrate perpendicularly to the plane formed by the carbons of the diene group. In the all-trans methylene chains an analogous motion of the hydrogen atoms with respect to the plane formed by the carbons is realized by the CH_2 rocking and bending modes, which are much more affected by modifications of the acyl chain packing than the CH_2 stretching vibrations. In view of these facts, it seems plausible that the large shift of the COG(γ_w(CH)) at the gel/SG_I transition indicates the more dense and rigid packing of the diene groups. Thus, the interchain packing of the whole octadecadienoyl chains including the methylene segments as well as the diene groups seems to be modified in the subgel phases.

4. Discussion

With increasing temperature, fully hydrated, disaturated phosphatidylcholines exhibit typically a sequence of sub-, pre-, and chain-melting transitions as the lipid converts to crystalline (L_c), gel ($L_{\beta'}$), rippled gel ($P_{\beta'}$), and liquid-crystalline (L_α) bilayer structures. The transformation of the gel into the crystalline L_c phase, often referred to as the subgel phase, is induced by prolonged annealing of hydrated samples at temperatures just above zero.[29,41,57−61] It is characterized by the rearrangement of the acyl chain packing, leading to a much more rigid packing pattern within different crystalline subcells and to a gradual dehydration of the lipid headgroup.[29,57]

An equally manifold phase behavior can be induced by application of an external hydrostatic pressure. Crystalline dipalmitoyl- and dimyristoylphosphatidylcholine (DPPC and DMPC, respectively) undergo a series of five pressure-induced structural phase transitions up to 3.0 GPa, designated as G_I−G_V.[24]

Transitions between different phases can be induced also by gradual hydration/dehydration of the lipid.[46,62] Usually, the sample is equilibrated with a reservoir of vapor assumed to obey the ideal gas law. In a crude approximation the work to desorb water into the gas phase is $w = -RT \ln a_w$,[63] where R denotes the gas constant and a_w = RH/100, the water activity. The desorption of 1 mol water is accompanied by the reduction of the condensed phase volume (lipid + water) by the molar volume of water, $v_w = 1.8 \times 10^{-5}$ m^3 mol^{-1}, assuming incompressibilty. The volume change is realized by the osmotic pressure $\Pi = w/v_w$,[25] which refers to the water vapor of a given relative humidity, i.e.,

$$\Pi = -\frac{RT}{v_w} \ln\left(\frac{\mathrm{RH}}{100}\right)$$

Within the frame of this rough approximation the RH range 92−10% corresponds to osmotic pressures of $\Pi = 0.011-0.343$ GPa at room temperature (T = 25 °C). Upon a decrease of RH, the lipid membranes are exposed to an increasing osmotic pressure, leading to an upward shift of the L_α/gel transition temperature, T_m, in accordance with the Clausius−Clapeyron relation. Under isothermal conditions at a certain osmotic pressure, Π_m, the temperature equals T_m and the liquid-crystalline lipid undergoes the phase transition to the gel upon further reduction of RH. For example, isothermal (30 °C) dehydration of fully hydrated DMPC to intermediate water content induces a lyotropic phase transition L_α/gel($L_{\beta'}$) at about RH = 90%−85%,[46] i.e., $\Pi_m \approx 0.015-0.023$ GPa. This value agrees well with the external hydrostatic pressure of $p_m = 0.015$ GPa, which should be applied to DMPC in excess water in order to induce the L_α/gel transition at 28 °C.[39]

The dehydration-induced transition from the liquid-crystalline phase to the gel of DODPC is not surprising in view of the well-known phase properties of saturated diacyl phosphatidylcholines. The osmotic pressure, $\Pi_m = 0.055-0.082$ and 0.03−0.022 GPa, corresponding to the humidity range of the freezing and melting transitions of DODPC of RH = 67−55% and 80−85%, respectively, is slightly higher than that of DMPC. The chain-melting conditions are determined mainly by the number of methylene segments per acyl chain.[64] The transition temperature of fully hydrated DODPC vesicles was found to be T_m = 21−22 °C.[13,18,65] It is very close to that of DMPC containing two saturated myristoyl chains (T_m = 24−25 °C [64]). The corresponding transition enthalpies of 24.6 kJ/mol (DODPC[65]) and 26.3 kJ/mol (DMPC[64]) differ less than the average incremental transition enthalpy per CH_2 segment of disaturated phosphatidylcholines (2.3 kJ/mol). Hence, the incorporation of the diene groups between the methylene residues and the carbonyl ester groups has only minor influence on the chain-freezing and chain-melting properties of the lipid.

Also, the IR spectroscopic features of DODPC in the L_α and gel states resemble closely those of DMPC and DPPC.[29−32,37,42,48,50] Analogous structural and dynamic properties of disaturated and dienic phosphatidylcholines can be deduced from these spectral similarities. The diene groups obviously modify the molecular arrangement in the bilayers only to a minor degree because it is determined predominantly by the conformation of the hydrophobic methylene chains as well as by the hydration degree of the phosphatidylcholine head and the carbonyl groups, i.e., by moieties common in both types of lipids.

The dehydration of DODPC is manifested spectroscopically in typical shifts of the IR absorption bands of the ν(C═O) and $\nu_{as}(PO_2^-)$ stretching vibrations, indicating the gradual desorption of water from the carbonyl and phosphate groups. On a molecular level the desorption of water from the hydrophilic moiety of the lipid decreases the spacings between the lipid molecules. This tightening of the molecular packing increases the conformational order along the CH_2 chains and finally induces the transformation of DODPC to the gel. Obviously, the freezing of the methylene chains is not accompanied by drastic changes of lipid/water interactions as indicated by only little modifications of the $\nu_{1,3}$(OH), ν(C═O), and $\nu_{as}(PO_2^-)$ bands.

After equilibration at low humidities DODPC spontaneously converts to a phase spectroscopically characterized by a sharpening of most of the absorption bands. This effect is characteristic for crystalline lipid systems where the molecules arrange into densely packed lipid lamellae. The almost complete damping of reorientations among neighboring octadecadienoyl chains becomes evident from the correlation field splitting of the CH_2 rocking and bending modes. Drastic spectral changes of the absorption bands originating from the carbonyl, phosphate, and choline groups give evidence of simultaneous changes of headgroup conformation, packing, and/or hydration. The modification in hydration is reflected in a distinct sharpening of the $\nu_{1,3}$(OH) band originating from a uniform population of 1−1.6 water molecules per lipid, which possibly form bridges between the phosphate groups or well-oriented H bonds to the C═O group. Note that the $\nu_{1,3}$(OH) band of DODPC differs essentially from that of the crystalline phase of DMPC formed at RH < 50% (T = 30 °C).[46]

DODPC undergoes a transformation to a second subgel phase, SG_{II}, at RH = 58−60% upon adsorption of one water per lipid, which occupies a broader distribution of interaction strengths with the lipid headgroup as indicated by the drastic broadening of the $\nu_{1,3}$(OH) band. Apparently, the SG_{II} state takes a position between the gel and SG_I phase; the adsorbed water resembles the properties of the water in gel state membranes, whereas the octadecadienoyl chains and the carbonyl groups are still packed like those in the SG_I phase.

Note that dry, crystalline DMPC, upon the increase of relative humidity, transforms to the liquid-crystalline phase via the gel state (phase sequence: $L_c \rightarrow L_{\beta'} \rightarrow L_\alpha$)[46] whereas DODPC undergoes a transition between two different crystalline subgel phases followed directly by the chain-melting transition ($SG_I \rightarrow SG_{II} \rightarrow L_\alpha$). The RH of the $L_c/L_{\beta'}$ transition of DMPC (∼50%) corresponds to an osmotic pressure of $\Pi_{LC/L\beta'} = 0.097$ GPa. This value agrees well with the hydrostatic pressure of 0.1 GPa of the G_I/G_{II} phase transition of DMPC measured in excess water at 28 °C.[24] The SG_I/SG_{II} transformation at RH = 60% is equivalent to $\Pi_{SGI/SGII} = 0.07$ GPa, differing from the

$\Pi_{LC/L\beta}$ of DMPC. Thus, a quite different phase behavior of both lipids was found in the low and intermediate RH range.

Besides a series of relatively similar spectral features of crystalline DODPC and disaturated phosphatidylcholines,[7,24,39,41,61] distinct qualitative as well as subtle quantitative differences give rise to the hypothesis that the diene groups cause the modification of the phase behavior due to steric and/or conformational factors acting in the vicinity of the linkage between the acyl chains and the glycerol moiety. This position is assumed to be exceptionally sensitive because of its pivotal location near the polar/apolar interface.[4] Subtle chemical modifications in this region have the potential to affect the structure and phase behavior of lipid aggregates to an extraordinary extent, for example, a relative minor change as the ester/ether substitution triggers the bilayer conversion to an interdigitated gel phase.[2]

It can be thought that in liquid-crystalline DODPC the diene groups slightly increase the degree of disordering in the hydrophobic core of the bilayer in comparison with DMPC where the polymethylene chains are attached directly to the glycerol moiety, and thus, they are sterically more restricted. Consequently, the transition to the gel state takes place at slightly lower temperatures and RHs if it is induced under isobaric or isothermal conditions. However, these deviations are small because of the equal number of methylene segments in each acyl chain. In the gel state the polymethylene chains adopt the extended all-trans configuration and thus match the 1,4 trans butadiene moieties well. Once in the frozen state, this steric matching and the smaller volume of the methyne groups in the diene moieties in comparison with the volume of the methylene groups enable a possible rearrangement into a state with more effectively packed acyl chains. The diene groups seem to promote the formation of crystalline structures that are, however, modified by the degree of lipid hydration. But with respect to the rigid diene group in the *sn*-2 position, it remains an open question how the octadecadienoyl chains align parallel within the bilayer.

When the RH is increased, the strong dispersion forces acting between the densely packed acyl chains in the subgel phases inhibit the chain melting to some degree, leading to the freezing/melting hysteresis observed. Apparently, the structural effects caused by the presence of the diene groups are intensified in the solid state as also observed in ester/ether substituted lipid systems.

Lewis and McElhany[41] gave a generalized interpretation of the crystalline subgel phases formed by *n*-saturated diacyl phosphatidylcholines upon dispersion in excess water. A particular molecular arrangement represents an optimization of the partially incompatible requirements for maximal van der Waals contacts between the hydrocarbon chains and for maximal polar interactions at the headgroups and polar/apolar interfacial regions of the lipid bilayer. Longer chain PCs exhibit less extensive hydrogen-bonding interactions at the polar/apolar interface but a tighter packing of the hydrocarbon chains than the corresponding short chain homologues. The latter are characterized by the more extensive hydration but relatively loose packing of the acyl chains. In the SG_I phase of DODPC the van der Waals forces acting in the hydrophobic core of the bilayer apparently overcome the water/lipid interactions and thus determine the molecular arrangement. In the SG_{II} phase the relation changes; lipid hydration becomes more important, but it is not sufficient to break up the dense chain packing. In view of the classification of the crystalline structures of hydrated phosphatidylcholines into three typical subgel phases formed by PCs of short ($n < 12$, L_c3), medium ($n = 13-15$, L_c2), and long ($n > 15$, L_c1) polymethylene chains,[61] we notice that the subgel phases of DODPC seem to combine some features of the polar/apolar interface of the L_c1 with the chain-packing properties of the L_c2 phases.

5. Conclusions

IR spectroscopy is well suited for detecting phase transformations as well as for characterizing the polar and apolar regions of the bilayer by means of semiempirical parameters. The present investigation gives a view of the phase properties of the dienic lipid DODPC upon graded hydration.

The most striking result is that the diene groups promote the formation of crystalline subgel phases of the lipid by structural rearrangement of the metastable gel state. Obviously, the diene moieties increase the packing density within the hydrophobic core of the bilayer, thus enabling the effective packing of the octadecadienoyl chains including the methylene units.

The balance between polar and apolar interactions in lipid aggregates stabilizes the supermolecular structure formed. Subtle changes of lipid hydration cause the transformation to a second crystalline subgel phase of DODPC.

The number of methylene segments per acyl chain determines the conditions of chain melting. Hence, the chain-melting properties of DODPC resemble those of the corresponding disaturated PC (DMPC).

The lyotropic phase behavior can be understood in terms of the osmotic pressure acting on the lipid aggregates, and thus, it shows qualitatively similar features as barotropically induced phase sequences.

The gel and subgel phases of DODPC can be used as various precursors of the polymerization reaction. Polymers obtained from 1,4-trans disubstituted polybutadienes upon solid-state reaction are of interest because of its high thermal and chemical stability.[34]

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