Orientation Measurements on Ordered Multibilayers of Phospholipids and Sphingolipids from Synthetic and Natural Origin by ATR Fourier Transform Infrared Spectroscopy

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Infrared Spectroscopy, Attenuated Total Reflectance, Planar Membrane Multibilayer, Orientation Measurements via Dichroic Ratio, Phase Transitions

Fourier-transform infrared spectroscopy was applied to natural and synthetic planar membrane multibilayer systems made from phospholipids and sphingolipids to perform orientational measurements utilizing the attenuated total reflectance technique. Applying polarized infrared light, the dichroic ratios and the positions of the peak maxima of various infrared-active bands in dependence on temperature and degree of hydration as well as the phase behaviour were studied. Under certain assumptions – which are discussed – made for the order parameter S, the orientation of various functional groups with respect to the molecular axis or to the membrane normal can be determined. For the gel phase values for S between 0.80 ± 0.05 for the complete molecule and 0.40 ± 0.05 for the end methyl group and for the liquid crystalline phase a value of 0.50 ± 0.05 were approximated. Applying these data, a relatively precise determination of the angles of the oscillating dipole moments of various functional groups could be performed, which – for some vibrations – deviate considerably from those angles assumed earlier. Regarding the phase behaviour, it was found that not only the degree of hydration but the amount of bulk water of the sample has a strong influence on the phase transition temperature T_c (lyotrophism), but only a weak influence on the orientational data.

Introduction

Phospholipids (PL's) are the major lipid components of eukaryotic and also of most prokaryotic cell membranes. They are present in membranes in a large variety of different conformations, composition of fatty acid residues, and polarities and charges of the head-group. It is commonly assumed that membrane PL's serve as permeability barriers and provide a liquid-crystalline matrix for functional proteins. However, for this purpose only a very limited number of different PL's would be sufficient, for instance, the phosphatidylcholines (PC's or lecithins). Therefore, the other major PL's such as phosphatidylethanolamines, phosphatidylglycerols, or phosphatidylserines as well as the sphingolipids might be responsible for other functional roles as for example in membrane fusion or transbilayer movement by adopting – under certain conditions – nonbilayer structures [1].

Abbreviations: PL, phospholipid; PA, phosphatidic acid; PC, phosphatidylcholine; PE, phosphatidylethanolamine; PG, phosphatidylglycerol; CB, cerebrosid; SPM, sphingomyelin; FT-IR, Fourier transform infrared spectroscopy; ATR, attenuated total reflectance.

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The situation becomes even more complicated with the outer membrane of Gram-negative bacteria [2], which is – in respect to its lipid moiety – composed of PL's on its inner side and of lipopolysaccharide on its outer side. These latter molecules consist of a polysaccharide side chain and a covalently bound lipid component, the lipid A, anchoring the lipopolysaccharide in the membrane. The outer membrane acts as an additional permeability barrier especially against hydrophobic drugs.

As part of a comprehensive study of the polymorphism of the outer membrane lipids this paper deals with various PL's applying Fourier transform infrared (FT-IR) spectroscopy utilizing attenuated total reflectance (ATR). From the application of this technique, informations on the state of order and on the participation of the different functional groups of the – usually hydrated – lipid molecule in the different thermodynamic states are obtained. Apparently due to restrictions inherent in older IR instrumentation data on the orientational behaviour of these substances are hardly available, particularly those data concerning the behaviour of phase-specific vibrations as for instance the wagging progressions of the polymethylen chains.

We recorded FT-IR spectra from various natural and synthetic phospho- and sphingolipids at different

temperatures and degrees of hydration. From these spectra, the order parameter of the corresponding functional groups (their orientation with respect to the molecular axis and to the membrane normal) and the phase behaviour were determined via the dichroic ratio and the position of particular absorption bands (center of gravity), respectively.

The results clearly show, that (i) the water content of the lipid samples also when exceeding the fully-hydrated state, is of utmost importance, (ii) in addition to a number of hydrocarbon vibrations, molecular groups of the head-group region are strongly involved in the gel-liquid crystalline phase transition of the hydrocarbon chains and (iii) from the obtained data on the orientation of the participating dipole moments, which are partially in contrast to those presented in literature [3], a calculation of the state of order (the order parameter) can be performed in both thermodynamic states for the lipid multibilayer system. The contradictions arising from the application of the literature data for the calculation of the directions of the dipole moments will be discussed. Finally, the applicability of model functions for the distribution of the lipid chains around the bilayer normal is tested in regard to models known from literature.

Materials and Methods

Lipids

The synthetic and natural phospholipids dilauroyl-L-α-phosphatidylcholine (DLPC), dimyristoyl-L-α-PC (DMPC), dipalmitoyl-L-α-PC (DPPC), distearoyl-L-α-PC (DSPC), L-α-phosphatidylcholine from egg yolk (PC), dilauroyl-L-α-phosphatidylethanolamine (DLPE), dimyristoyl-L-α-PE (DMPE), dipalmitoyl-L-α-PE (DPPE), L-α-phosphatidylethanolamine from egg yolk (PE egg), L-α-phosphatidylethanolamine from *E. coli* (PE *E. coli*), dimyristoyl-L-α-phosphatidic acid (DMPA), dipalmitoyl-L-α-PA (DPPA), L-α-phosphatidic acid from egg yolk PC (PA), and dipalmitoyl-L-α-phosphatidylglycerol (DPPG) were purchased from Sigma (München, F.R.G.) and from Serva (Heidelberg, F.R.G.). Additionally, the sphingolipids cerebrosid from bovine brain (CB) and sphingomyelin from bovine brain (SPM) obtained from Sigma were investigated. All lipids were used without further purification. The phospholipid extract (PL extract) from *E. coli mutaflor* was obtained by the methanol/chloroform procedure [4] from bacteria grown to stationary phase in standard nutrient (Merck, Darmstadt, F.R.G.) at 37 °C.

Composition of natural lipids

The fatty acid composition of the natural lipids was investigated utilizing GC/MS. The hydrocarbon chains were cleaved by acidic alkaline hydrolysis and converted to fatty acid methyl ester by addition of diazomethane. The lipid compositions of the investigated natural lipids were as follows:

PA, PC, and PG C16:0 (relative intensity 1.0), C18:0 (0.25), C18:1 and C18:2 (0.83).

PE *E. coli* C16:0 (1.0), *cis*-9,10-methylen-C16 (0.61), *cis*-10,11-methylen-C 18 (0.16).

PE egg yolk C16:0 (0.53), C18:0 (0.78), C18:1 and C18:2 (1.0), C20:4 (0.38).

SPM C16:0 (0.52), C18:0 (0.35), C18:1 and C18:2 (0.80), C22:0 (0.20), C24:0 (0.77), C24:1 (1.0), C25:0 and C25:1 (0.51, probably branched).

CB C18:0 (0.10), 2-OH C18:0 (0.20), 2-OH C22:0 (0.18), 2-OH C24:0 (1.0), 2-OH C26:0 and C26:1 (0.28).

The PL extract consisted of 69% PE, 19% PG, 6.5% cardiolipin, while two further weak compounds could not be identified by thin-layer chromatography. The fatty acid composition of the main phospholipid component PE was as follows: C16:0 (1.0), C16:1 (0.42), *cis*-9,10-methylen-C16 (0.68), and C18:1 (0.62).

Preparation of oriented multibilayers

The preparation of oriented multilayers of non-hydrated lipids was performed according to the procedure of Fringeli [5] by spreading a 10^{-3} M solution in $CHCl_3$ with a teflon bar on the ATR plate. Hydrated multilamellar layers were prepared by spreading a 10^{-3} M lipid suspension in double destilled water on the ATR plate at a temperature above the gel-liquid crystalline phase transition T_c. The evaporation of the excess water was achieved by slow periodic movement of either a nitrogen stream or a small teflon bar along the ATR plate. Both techniques gave essentially the same results. The degree of hydration was estimated from the ratio of the peak intensities of the OH-stretch at app. 3400 cm^{-1} to the antisymmetric stretch of the methylen groups at app. 2920 cm^{-1} and was highest for the PC's (5 to 13% by weight) and sphingomyelin (app. 9%) and lowest for

the PE's (0.1 to 1%). Interestingly, the degree of hydration of PL extract was considerably higher (3%) than for pure PE's. Higher degrees of hydration (app. 90%) were achieved by repeated heating to temperatures above T_c and subsequent cooling [6]. Therefore, a lyotrophic phase behaviour should be expected especially for the lecithins, because the phase transition of these compounds has been observed to be strongly dependent on the water content [7].

Infrared spectra

The infrared measurements were performed on a Fourier transform infrared spectrometer "5DX" (Nicolet Instruments, Madison, Wisconsin, USA) applying the ATR technique [5, 8, 9]. The internal reflection plates (K. Korth Monokristalle, Kiel, F. R. G.) were in most cases from ZnSe, and in some cases from KRS-5 (a thallium bromide/iodide compound). The infrared light was polarized by a grid polarizer (Barnes Stamford, CT, USA). The reflection plates were embedded in a brass cell sealed by a 100 μm thick mylar foil and were thermostated by water with a precision of 0.5 °C measured by a thermocouple. The infrared measurements were performed by accumulation of 200 scans and box-car apodization. After Fourier transformation the absorbance spectra were smoothed according to a least square procedure as described in [10]. For each lipid sample, polarized IR spectra for parallel and vertical light were recorded at more than 3 different temperatures within the gel and the liquid crystalline phase, respectively.

Evaluation of the IR spectra

Absorbance spectra were evaluated by determining the maximum position of a band (peak position x) and by measuring the area A of a band. The peak positions of weaker absorption bands appearing as a shoulder within stronger bands (*e.g.* wagging progression bands) were determined at the points of intersection of the tangent – obtained by parallel shift of the base line – with the respective band. The precision in determining the peak position was as high as 0.1 cm^{-1}, whereas the error in calculating the band intensity (area) could be estimated to be 2% resulting essentially from the uncertainty of choosing the proper band limits.

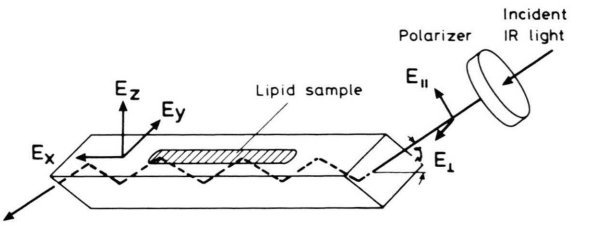


Fig. 1. Experimental design for ATR measurements; ϑ, angle of incidence; $E_{\parallel}$, $E_{\perp}$, parallel and vertical polarized components of the incident electric field; E_x, E_y, E_z, electric field components with respect to the coordinate system of the internal reflection plate.

Evaluation of the orientation measurements

The experimental design of the ATR measurements with polarized IR light is depicted in Fig. 1. In the case of weak absorption for thin layers the electric field amplitudes in the rarer medium (lipid) can be approximated [5, 9] to be

$$E_x = \frac{2 \cdot \cos\vartheta \, (\sin^2\vartheta - n_{31}^2)^{1/2}}{(1 - n_{31}^2)^{1/2} \cdot [(1 + n_{31}^2) \cdot \sin^2\vartheta - n_{31}^2]^{1/2}}$$

$$E_y = \frac{2 \cdot \cos\vartheta}{(1 - n_{31}^2)^{1/2}}$$

$$E_z = \frac{2 \cdot \cos\vartheta \cdot n_{32}^2 \cdot \sin\vartheta}{(1 - n_{31}^2)^{1/2} \, [(1 + n_{31}^2) \cdot \sin^2\vartheta - n_{31}^2]^{1/2}}$$

with $n_{ik} = n_i/n_k$ representing the refractive indices of medium i and k, n_i refractive index of the crystal, n_2 of the lipid, and n_3 of the surrounding medium. With $n_i = 2.40$ (ZnSe or KRS-5), $n_3 = 1.00$ (air), and n_2 varying between 1.55 (pure lipid) and 1.35 (lipids with 90% water content), the numeric values for the field amplitudes at $\vartheta = 45°$ are $E_x = 1.383$, $E_y = 1.556$, and $E_z = 0.712$ (pure lipid), $E_z = 0.731$ (10% water-containing lipid), and $E_z = 0.939$ (90% water-containing lipid). From the measurement of the dichroic ratio, which is defined as the ratio of the absorption coefficients for parallel and vertical polarized light $R^{ATR} = A_{\parallel}/A_{\perp}$, the molecular orientation can be obtained. In the case of lipid multibilayers the molecular axis can be assumed to be symmetrically distributed around the z-axis (normal to the membrane plane), and each hydrocarbon chain to behave independently resulting in rotational symmetry of the oscillating dipole moments around its long axis (partial axial orientation). Regarding the fact that the electric field amplitudes

$$E_{\parallel} = (E_x^2 + E_z^2)^{1/2}, \; E_{\perp} = E_y$$

are different from those defined in transmission spec-

troscopy, the following expression for the dichroic ratios can be derived [5]

$$R^{ATR} = \frac{E_x^2}{E_y^2} + \frac{E_z^2}{E_y^2 \cdot R^T}$$

$$\text{with } R^T = \frac{\sin^2\theta + S'}{2 \cdot \cos^2\theta + S'} \quad (1)$$

the dichroic ratio in transmission measurements [8] and θ the angle between the direction of the oscillating dipole moments and the molecular axis. When converting S' – the order parameter used in IR spectroscopy ($S' = 0$ for perfect ordering and $S' \to \infty$ for isotropic systems) – to the more usual order parameter S employed in ESR or fluorescence spectroscopy ($S = 0$ for isotropic and $S = 1$ for perfectly aligned systems)

$$S = 1 - 1.5 \cdot S'/(1 + 1.5 \cdot S')$$

one obtains

$$S = \frac{K - 1}{3 \cdot \cos^2\theta - 1 + K\left(1 - \frac{3}{2} \cdot \sin^2\theta\right)} \quad (2)$$

$$\theta = \arcsin \sqrt{\frac{\left(\frac{2}{3} \cdot \frac{1 - S}{S}(1 - K) + 2\right)}{K + 2}} \quad (3)$$

$$\text{with the term } k = \frac{R - \frac{E_x^2}{E_y^2}}{E_z^2/E_y^2}.$$

In Fig. 2, for pure lipid ($n_2 = 1.55$) the dichroic ratio R^{ATR} is plotted versus the order parameter S with the angle of the oscillating dipole moment θ as parameter. From equation (3), θ was calculated for measured dichroic ratios and for S-values extrapolated from literature data. The error $\Delta\theta$ was calculated from measured $R \pm \Delta R$ and $S \pm \Delta S$ (see also Fig. 2) according to Gauss' law of error propagation.

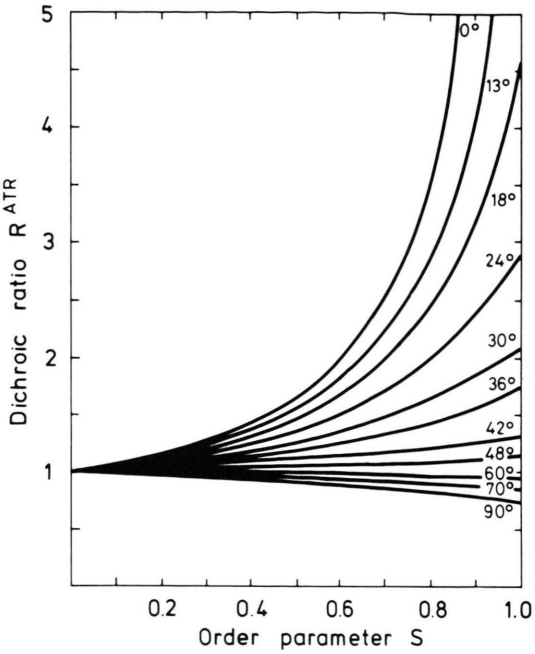


Fig. 2. Dichroic ratio R^{ATR} in dependence on the order parameter S with the angle between oscillating dipole moment and molecular axis, θ, as parameter. The calculation is based on equation (1) with $\vartheta = 45°$, $n_1 = 2.40$ (ZnSe or KRS-5), $n_2 = 1.55$ (pure lipid), and $n_3 = 1.00$ (air).

Approximation for the order parameter

A more detailed analysis of the partial axial orientation [8] yields that the order parameter S can be written

$$S = 1 - 3/2\ F, \text{ where } F = \int_0^{\pi/2} \sin^2\gamma \cdot f(\gamma) d\gamma. \quad (4)$$

The distribution function $f(\gamma)$ is normalized, *i.e.*

$$\int_0^{\pi/2} f(\gamma) d\gamma = 1.$$

While γ denotes the angle between the z-axis and the direction of the hydrocarbon chains, $f(\gamma)$ is proportional to the number of lipids pointing into the conical element around the z-axis with inner diameter γ and outer diameter $\gamma + d\gamma$. Zbinden [8] has proposed several distributions for polymers, and from these the following seem to be the most important:

1) Perfect axial distribution, *i.e.* $f(\gamma) = \delta(\gamma - \gamma_0)$, the delta function. The calculation yields: $F = \sin^2\gamma_0$. This function represents an idealized distribution with all lipid chains at constant angle γ_0 with respect to the z-axis and is applied to the gel state of the lipids ("microcrystalline ultrastructure" MCU as denoted by Fringeli [5]).

2) Kratky's model [11], describing the most realistic distribution of lipid chains obtained in a drawing process which was applied to the gel as well as to the liquid crystalline phase ("liquid crystalline ultrastructure" LCU)

$$f(\gamma) = \frac{\sin\gamma \cdot \nu^{3/4}}{(\nu^{-3/2} \cdot \cos^2\gamma + \nu^{3/2} \cdot \sin^2\gamma)^{3/2}}. \quad (5)$$

The calculation yields

$$F = 1 - \frac{v^3}{v^3 - 1} + \frac{v^3}{(v^3 - 1)^{3/2}} \arccos(v^{-3/2}),$$

where v denotes the "draw" or "extension" ratio, $v \to 1$ increasingly isotropic and $v \to \infty$ distribution around a more and more decreasing angle.

Furthermore, for different S-values the angle was calculated for which

$$I_{\%} = \int_0^{\gamma} f(\gamma')\, d\gamma', \qquad (6)$$

i.e. the angular range containing $I_{\%}$ of the lipid molecules.

Results

Orientation measurements

To check the preparation technique (planar multibilayers) and the experimental setup, polarization studies were performed with small unilamellar DPPC vesicles in excess water, a preparation, which is ordered on a submicroscopic, but isotropic on a macroscopic scale. For various functional groups in spectral ranges not showing interference with strong water vibrations dichroic ratios of 1.00 ± 0.02 were measured, *i.e.* no planar alignment could be observed.

Regarding the high number of different lipids under investigation each exhibiting a considerable number of IR active vibration bands, in the beginning the data will be interpreted exemplarily on the basis of the "standard" phospholipid DPPC.

In Fig. 3 FT-IR spectra of a water-free, *i.e.* water content (< 1% w/w), a weakly (app. 10%), and a strongly (app. 90%) hydrated DPPC sample on a ZnSe ATR plate are depicted. The assignment of the different absorption bands was performed on the basis of data taken from literature [3, 5, 12, 13]. In Fig. 4 the spectra of DPPC in the wavenumber range 1500 to 1350 cm^{-1} for parallel and vertical polarized IR light, respectively, are given showing mainly the bending vibration ("szissoring") of the methylen groups $\delta(CH_2)$ at app. 1467 cm^{-1}, the bending vibration of the α-methylen group at app. 1417 cm^{-1}, and the bending vibration ("umbrella") of the end methyl group $\delta_s(CH_3)$ at app. 1377 cm^{-1}. It can clearly be seen from Fig. 4 that the dipole moment of $\delta_s(CH_3)$ oscillates more parallel (high R-value) and that of $\delta(\alpha\text{-}CH_2)$ more perpendicular (low R-value) to the membrane normal as compared to the dipole moment of $\delta(CH_2)$ (R around 1). The bending vibration of the α-methylen group usually shows also for other lipids an abnormally low R-value being in most cases even below the minimal value of 0.79 as calculated according to equation (1). This might be due to Fermi resonance of coupled vibrations (*e.g.* with the rocking progression band at 722 cm^{-1}) but will not be considered further.

In equation (1) (see also Fig. 2) two parameters, the angle θ between the direction of the oscillating

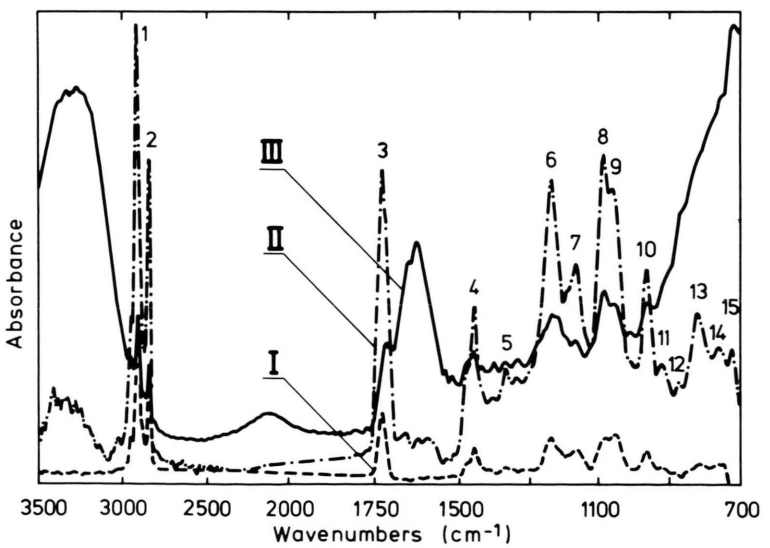


Fig. 3. IR spectra of different DPPC preparations on a ZnSe ATR plate. I: anhydrous sample; II: water content app. 10% by weight; III: water content app. 90% by weight. Assignment of main vibrations (explanation see text): (1) $\nu_{as}(CH_2)$; (2) $\nu_s(CH_2)$; (3) $\nu(C{=}O)_E$; (4) $\delta(CH_2)$; (5) $\delta_s(CH_3)$; (6) $\nu_{as}(PO_2)$; (7) $\nu(C{-}O)_E$; (8) $\nu(C{-}O)_I$; (9) $\nu(C{-}O)_{II}$; (10) $\nu_{as}(C{-}N)$ in $N{-}CH_3$; (11) $\nu_s(C{-}N)_I$ in $N{-}CH_3$; (12) $\nu_s(C{-}N)_{II}$ in $N{-}CH_3$; (13) $\nu_{as}(P{-}O)$ in $-C{-}O{-}P{-}O{-}C-$; (14) $\nu_s(P{-}O)$ in $-C{-}O{-}P{-}O{-}C-$; (15) $\gamma_r(CH_2)$.

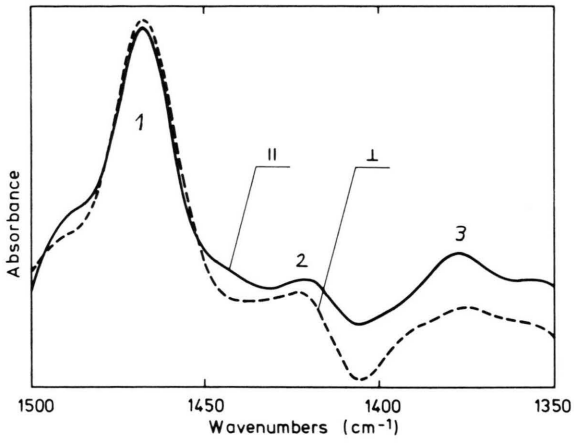


Fig. 4. IR-ATR spectra of a DPPC preparation (water content app. 10% by weight) in the spectral range 1500 to 1350 cm^{-1} for parallel (∥) and vertical (⊥) polarized light for (1) $\delta(CH_2)$, (2) $\delta(\alpha\text{-}CH_2)$, and (3) $\delta_s(CH_3)$.

dipole moment and the molecular axis and the order parameter are – a priori – freely eligible. However, they underly certain criteria of plausibility. Thus, some vibrations of the hydrocarbon moiety, as for instance the symmetric and antisymmetric stretching vibrations of the CH_2-group ν_s and $\nu_{as}(CH_2)$ would be expected to be directed more perpendicular, whereas $\delta_s(CH_3)$ should be more parallel to the molecular axis. On the other side, the order parameter S in a first approximation can be restricted to values in the range 0.4 to 0.6 for the liquid crystalline and 0.7 to 0.9 in the gel phase considering literature data from spin resonance and fluorescence polarization measurements (see Discussion).

In Table I for various important vibrations (with peak positions x) from the measured dichroic ratios R the values for θ calculated from three different values of the order parameter S (variation range $\Delta S = 0.05$) are summarized for hydrated and non-hydrated DPPC preparations. Additionally, the peak positions of several – evaluable – vibrations of DPPC vesicles (water content 99%) are listed for comparison. For water-free, 10% water-containing DPPC and for DPPC vesicles, the listed figures in Table I are mean values from several measurements at different temperatures within the respective phases, while the values of the 90% water-containing DPPC sample resulted from repeated measurements at $T = 15$ °C. The latter procedure turned out to be necessary because heating of DPPC preparations with high water content was accompanied by considerable evaporation of free and/or hydrated water even within the closed brass cell ceasing at approximately 10% water content. This behaviour could be reproducibly confirmed in several independent experiments, in which "hydration" – or better – addition of water was performed as described in [6] by repeated heating above T_c and subsequent cooling.

As can be seen from Table I, the R-values show a similar tendency for all preparations, the absolute values being very similar for anhydrous and 10% water-containing DPPC. The 90% H_2O-containing sample systematically shows significantly higher values for most vibrations, which is not surprising, because for the same values of θ and S in equation (1) R increases when lowering n_2 (higher value for E_z).

For all DPPC preparations as well as for all other investigated lipids (see Table II) the dichroic ratio of $\nu_{as}(CH_2)$ turned out to be slightly higher than that of $\nu_s(CH_2)$ (data not shown), and for both vibrations the R-values are in most cases higher in the fluid than in the gel phase. Regarding the orientational behaviour, except for the DPPC sample with the high water content these vibrations as well as $\delta(CH_2)$ and the most intense rocking progression band $\gamma_r(CH_2)$ at 722 cm^{-1} (not shown) show slight vertical polarization especially for the lecithins in the gel phase. In contrast, $\delta_s(CH_3)$ and the wagging progression bands $\gamma_w(CH_2)$ between 1350 and 1180 cm^{-1} yield values distinctly greater than unity pointing to an orientation of the respective dipole moments perpendicular to the membrane plane. Moreover, for $\gamma_w(CH_2)$ the values for R lying above 2 in the gel phase show that the lower limit for S is approximately 0.62.

Concerning the various vibrations originating from different parts of the polar head-groups, especially the stretching vibration of the ester linkage $\nu(C-O)_E$, the antisymmetric stretch $\nu_{as}(P-O)$ of the phosphate group within R′–O–P–O–R, and the 2 symmetric stretching vibrations $\nu_s(C-N)_{I,II}$ within the choline group at 876 and 926 cm^{-1} (data not shown) show R-values well above 1 expressing parallel polarization. The other investigated vibrations – stretching of the ester double bound $\nu(C=O)_E$, antisymmetric stretching of the negatively charged phosphate group $\nu_{as}(PO_2)$ in the range 1220 to 1250 cm^{-1} (data not shown), the symmetric and antisymmetric stretching vibrations of the two C–O linkages of the phosphate group $\nu(C-O)_{I,II}$ around 1080 and 1050 cm^{-1}, respectively (data not shown), and the

Table I. Peak maxima $x(\pm \triangle x)$, dichroic ratios $R(\pm \triangle R)$, and resulting directions of the oscillating dipole moments $\theta(\pm \triangle\theta)$ for 3 different values of the order parameter S for anhydrous and hydrated DPPC preparations. x and R represent the means each over 4 values obtained at different temperatures within each phase.

Vibration	S ($\pm$ 0.5)	DPPC < 1%		DPPC 10% water		DPPC 90% water w/w		DPPC vesicles
		R	x	R	x	R	x	x
		$\triangle R$ θ	$\triangle\theta$ $\triangle x$	$\triangle R$ θ	$\triangle\theta$ $\triangle x$	$\triangle R$ θ	$\triangle\theta$ $\triangle x$	$\triangle x$
		0.91	2918.3	0.93	2917.7	1.30	2917.1	2919.0
$\nu_{as}(CH_2)$		0.02	0.2	0.02	0.2	0.08	0.2	0.4
	0.7	66.9	6.8	63.3	5.4	36.8	6.7	
$T < T_c$	0.8	65.1	5.7	62.2	4.6	39.1	5.8	
	0.9	63.9	4.9	61.3	4.0	40.8	5.1	
				0.94	2918.9			2921.8
$\nu_{as}(CH_2)$				0.02	0.3			0.4
	0.4			68.5	10.6			
$T > T_c$	0.5			65.4	7.6			
	0.6			63.4	6.0			
		0.95	1738.8	1.06	1736.9			1736.5
$\nu(C{=}O)_E$		0.03	0.3	0.03	0.6			1.7
	0.7	61.1	5.0	50.8	4.4			
						n.e.		
$T < T_c$	0.8	60.3	4.3	51.3	3.9			
	0.9	59.6	3.8	51.7	3.5			
				1.15	1736.8			1738.8
$\nu(C{=}O)_E$				0.03	0.2			0.5
	0.4			37.9	6.1			
$T > T_c$	0.5			41.3	4.8			
	0.6			43.4	4.0			
		0.96	1467.4	0.92	1467.7	1.05	1467.8	1467.8
$\delta(CH_2)$		0.10	0.3	0.02	0.4	0.05	0.2	0.4
	0.7	52.1	6.5	64.7	5.8	50.5	7.6	
$T < T_c$	0.8	52.4	5.7	63.4	4.9	51.0	6.6	
	0.9	52.7	5.1	62.3	4.3	51.5	5.9	
				0.93	1467.3			1465.9
$\delta(CH_2)$				0.03	0.3			0.3
	0.4			71.4	18.5			
$T > T_c$	0.5			67.4	12.6			
	0.6			64.9	9.8			
		1.31	1377.2	1.27	1376.7	2.35	1376.1	1377.8
$\delta_s(CH_3)$		0.20	0.2	0.06	0.2	0.20	0.3	1.6
			+27.5					
	0.4	1.0		26.4	11.3	[a]		
			+25.8					
$T < T_c$	0.5	21.3	−21.3	32.7	8.1	[a]		
	0.6			36.5	6.3	16.1	7.2	
						(S_{min} = 0.52)		
				1.22	1376.0			1378.9
$\delta_s(CH_3)$				0.06	0.2			1.5
	0.3			21.4	19.6			
$T > T_c$	0.4			31.1	11.3			

Table I. (continued)

Vibration	S (± 0.5)	DPPC < 1% R $\triangle R$ θ	x $\triangle\theta$ $\triangle x$	DPPC 10% water R $\triangle R$ θ	x $\triangle\theta$ $\triangle x$	DPPC 90% water w/w R $\triangle R$ θ	x $\triangle\theta$ $\triangle x$	DPPC vesicles x $\triangle x$
		2.36	[b]	2.10	[c]	2.97	[d]	
$\gamma_w(CH_2)$		0.71		0.70		1.71		
			+25.2		+31.6		+46.7	
	0.7	9.9		15.8		15.6		
			−9.9		−15.8		−15.6	
			+21.6				+29.9	
$T < T_c$	0.8	19.3		22.6	20.4	22.5		
			−19.3				−22.5	
	0.9	24.4	15.9	27.0	16.0	26.9	23.4	
		2.27	1180.6	2.20	1180.1	2.10	1173.4	1177.5
$\nu(C-O)_E$		0.10	0.2	0.40	0.1	0.12	1.0	0.9
					+18.9			
	0.7	8.4	7.1	13.6		28.3	4.4	
					−13.6			
$T < T_c$	0.8	18.6	3.4	21.2	11.3	32.0	3.7	
	0.9	24.0	2.7	25.9	8.7	34.6	3.2	
				1.52	1176.5			1176.4
$\nu(C-O)_E$				0.17	0.2			1.0
	0.4			[a]				
$T > T_c$	0.5			33.5	8.6			
	0.6			36.0	7.4			
		1.44	822.2	1.27	819.1	1.08	823.7	
$\nu_{as}(P-O)$		0.05	2.6	0.03	2.2	0.20	1.2	
in								
R−O−P−O−R	0.7	31.6	3.6	38.2	2.8	59.1	26.1	
	0.8	34.7	3.1	41.1	2.4	58.5	22.6	
$T < T_c$	0.9	37.0	2.7	42.6	2.2	58.1	19.9	
				1.45	811.2			
				0.05	1.0			
$\nu_{as}(P-O)$					+18.0			
	0.4			2.7				
					−2.7			
	0.5			21.6	6.3			
$T > T_c$	0.6			28.2	4.4			

n.e. not evaluable.
[a] No mathematical solution possible.
[b] From the bands at 1199, 1221, 1283, 1310, and 1343 cm^{-1}.
[c] From the bands at 1222, 1264, 1285, and 1308 cm^{-1}[1].
[d] From the bands at 1198, 1221, 1265, 1285 cm^{-1}.

antisymmetric stretching vibration of the choline group $\nu_{as}(C-N)$ at 969 cm^{-1} (not shown) – have no pronounced or only a slight parallel (DPPC with 90% H_2O) orientation. It should be mentioned that in two recent publications [12, 14] the band at app. 1095 cm^{-1} was assigned to the symmetric stretch of the charged phosphate group $\nu_s(PO_2)$ and not to $\nu(C-O)_I$. A more detailed investigation of this region, however, showed that the slight shoulder at 1100 to 1110 cm^{-1} is present in all phospholipids, but

Table II. Peak maxima $x(\pm \triangle x)$ and dichroic $R(\pm \triangle R)$ for a number of vibrations in the wavenumber region 3000 to 700 cm^{-1} for different lipid preparations.

Vibration		DMPC	PC egg	DMPE	PE *coli*	PA egg	CB	SPM	PL extract
	x	2918.1	2921.1	2919.1	2922.1	2923.1	2919.0	2918.2	2922.4
$\nu_{as}(CH_2)$	$\triangle x$	0.3	0.5	0.5	0.5	0.2	0.2	0.3	0.6
	R	0.89	0.96	1.10	1.05	1.43	0.93	0.92	1.05
$T < T_c$	$\triangle R$	0.03	0.02	0.03	0.03	0.07	0.03	0.03	0.04
	x	2920.0	2923.6	2920.3	2923.1	2923.2		2919.8	2923.6
$\nu_{as}(CH_2)$	$\triangle x$	0.2	0.3	0.2	0.2	0.1	[c]	0.1	0.3
	R	0.94	1.15	1.12	1.12	1.40		0.91	1.15
$T > T_c$	$\triangle R$	0.03	0.04	0.03	0.04	0.04		0.03	0.04
	x	1738.0	1736.3	1736.4	1739.4	1739.4		app.	1737.0
$\nu(C=O)$	$\triangle x$	0.3	0.2	0.3	0.4	0.2		1736 [b]	0.1
	R	1.08	1.04	1.24	1.12	1.25	–	app.	1.15
$T < T_c$	$\triangle R$	0.03	0.02	0.03	0.03	0.03		1.8	0.03
	x	1738.0	1736.8	1736.6	1740.0	1740.0		app.	1738.0
$\nu(C=O)$	$\triangle x$	0.1	0.3	0.5	0.3	0.1		1735 [b]	0.1
	R	1.02	1.15	1.31	1.17	1.24	–	app.	1.22
$T > T_c$	$\triangle R$	0.09	0.05	0.03	0.05	0.04		0.9	0.03
	x	1467.6	1466.8	1466.4	1463.7	1463.6	1466.8	1467.5	1462.2
$\delta(CH_2)$	$\triangle x$	0.2	0.2	0.2	0.4	0.2	0.3	0.1	0.3
	R	0.96	0.91	1.03	0.94	1.13	0.95	1.01	1.09
$T < T_c$	$\triangle R$	0.05	0.03	0.05	0.05	0.12	0.03	0.03	0.03
	x	1467.3	1464.5	1466.3	1462.3	1463.1		1467.1	1460.9
$\delta(CH_2)$	$\triangle x$	0.2	0.2	0.2	0.5	0.3	[c]	0.2	0.1
	R	0.86	1.16	1.00	1.01	1.13		0.95	1.20
$T > T_c$	$\triangle R$	0.03	0.04	0.03	0.04	0.05		0.05	0.03
	x	1377.0	1376.7	1375.8	1376.0	1376.6	1375.5	1376.7	1375.9
$\delta_s(CH_3)$	$\triangle x$	0.1	0.3	0.2	0.4	0.2	0.2	0.3	0.2
	R	1.49	1.12	1.27	1.35	1.27	1.38	1.37	1.38
$T < T_c$	$\triangle R$	0.05	0.05	0.06	0.10	0.04	0.12	0.04	0.03
	x	1376.5	1376.2	1375.7	1374.8	1376.4		1376.5	1375.4
$\delta_s(CH_3)$	$\triangle x$	0.1	0.2	0.1	0.3	0.2	[c]	0.3	0.1
	R	1.63	0.97	1.25	1.30	1.28		1.25	1.27
$T > T_c$	$\triangle R$	0.10	0.03	0.03	0.05	0.03		0.03	0.05
$\gamma_w(CH_2)$	$\triangle x$	[a]	[a]	[a]	[a]	[a]	[a]	[a]	[a]
	R	1.62	1.67	2.03	1.40	2.21	1.89	1.58	2.20
$T < T_c$	$\triangle R$	0.08	0.35	0.60	0.32	0.80	0.37	0.05	0.75
	x	1177.9	1175.0	1177.3	1177.2	1176.5		1176.5	1173.2
$\nu(C-O)$	$\triangle x$	0.07	0.3	0.2	0.1	0.1		0.4	0.2
	R	1.47	1.34	1.83	1.71	1.56	–	1.50	1.42
$T < T_c$	$\triangle R$	0.04	0.05	0.32	0.18	0.04		0.10	0.03
	x	1177.6	1172.8	1175.5	1169.9	1176.7		1175.0	1172.9
$\nu(C-O)$	$\triangle x$	0.1	0.2	0.2	0.3	0.1		0.3	0.2
	R	1.40	1.34	2.05	2.21	1.40	–	0.76	1.25
$T > T_c$	$\triangle R$	0.04	0.04	0.20	0.08	0.15		0.08	0.03
	x	825.6	820.6	826.3	822.0	818.9		830.3	824.6
$\nu_{as}(P-O)$	$\triangle x$	0.9	1.2	0.5	2.3	0.2		2.0	0.3
	R	1.74	1.93	1.23	1.13	1.35	–	1.24	1.50
$T < T_c$	$\triangle R$	0.06	0.21	0.05	0.20	0.13		0.03	0.10
	x	823.2	812.5	822.8	819.1	817.9		823.0	822.7
$\nu_{as}(P-O)$	$\triangle x$	0.6	1.2	1.7	0.2	1.5		1.5	0.2
	R	1.55	1.63	1.42	0.61	1.35	–	1.25	1.75
$T > T_c$	$\triangle R$	0.03	0.08	0.13	0.02	0.03		0.04	0.09

Table II. (continued)

Vibration		DMPC	PC egg	DMPE	PE *coli*	PA egg	CB	SPM	PL extract
	+	758.7	762.2	757.7	761.5			762.1	763.1
$\nu_s(P-O)$	$\triangle x$	1.6	0.5	1.2	0.5			1.4	0.1
	R	0.90	1.23	1.44	1.15	–	–	1.30	0.95
$T < T_c$	$\triangle R$	0.03	0.10	0.05	0.10			0.08	0.20
	x	753.5	760.0	756.8	762.5			756.0	761.2
$\nu_s(P-O)$	$\triangle x$	0.9	0.8	1.8	0.2			1.2	0.5
	R	0.93	n.e.	2.06	0.84	–	–	1.50	1.25
$T > T_c$	$\triangle R$	0.03		0.20	0.06			0.20	0.10

[a] Mean values from at least two progression bands.
[b] No precise evaluation possible.
[c] No transition in the temperature range 7 to 60 °C.

absent in cerebrosid, and should therefore rather represent $\nu_s(PO_2)$ according to the former assignment [3]. The latter vibration is not considered here, because due to the weakness of the band, except for water-free DPPC (x = 1112.7 ± 0.2 cm^{-1} and R = 1.36 ± 0.11), no precise evaluation could be performed.

In Table II data for the dichroic ratio R and the position of the peak maximum x (in cm^{-1}) are summarized for different lipids measured on a ZnSe plate. Several experiments with a KRS-5 ATR plate gave essentially the same results, but led to R-values lying systematically above (max. 5%) those measured with ZnSe. From Table II and equation (3) the angle θ can be obtained for different S-values. The results in Table II point out that common to all investigated lipids and for both phases – gel and liquid-crystalline state – the vibrations $\delta_s(CH_3)$, $\gamma_w(CH_2)$, $\nu(C-O)_E$, and to a lower extent also $\nu(C=O)_E$ are preferentially excited parallel to the membrane normal. Interesting results were obtained from a more detailed evaluation of the $\nu(C-O)_E$-vibration. The dichroic ratio was found to depend strongly on the water content especially for the PE's, *i.e.* R was significantly higher at lower water content apparently because of different hydrogen bonding. Furthermore, for most lipids a weak splitting of this vibration into 2 bands was observed.

The antisymmetric stretch of the charged part of the phosphate group $\nu_{as}(PO_2)$ shows – except for PA – a vertical polarisation in the fluid state (data not shown), where no disturbances by wagging progressions should occur, thus showing a dipole moment oscillating perpendicular to the molecular axis. This is in accordance to the assumption of Akutsu *et al.* [15] who investigated built-up films from PE's.

In addition to the vibrations listed in Table II which – as far as possible – were evaluated for all lipids under investigation, further absorption bands can be observed which are specific for the individual lipids due to their particular chemical structure. The evaluation of $\nu_s(C-N)$ at app. 876 cm^{-1} shows in contrast to SPM a significant parallel polarisation for the lecithins (DMPC, DPPC, PC egg yolk) being even higher in the L_α phase. For the PE's the bending vibration of the NH_3 group at app. 1550 cm^{-1} gives evidence for nearly parallel orientation of the respective dipole moments to the chain axis. This can be deduced from the high R-values lying typically above 3. From this, a high degree of parallel alignment may be concluded for the polar parts of these compounds.

It should be noted also that for cerebrosid and sphingomyelin the amid II band at 1543 cm^{-1} – which represents coupled N–H deformation and C–N stretching modes [16] – exhibits in the gel phase a considerably higher R-value than the amid I vibration at 1644 cm^{-1} which is predominantly resulting from the C = O stretch.

Phase transitions

Beside the determination of orientational data for various functional groups the phase behaviour of the respective vibrations were examined. In general, no uniform dependence for the different vibrations and lipids on the degree of hydration can be stated. Thus, for example, for lecithins $\nu_s(CH_2)$ and $\nu_{as}(CH_2)$ show a strong dependence on the water content as well concerning the peak position as the observation of the gel-liquid crystalline transition and the value of T_c. In Fig. 5 the peak position of $\nu_{as}(CH_2)$ is plot-

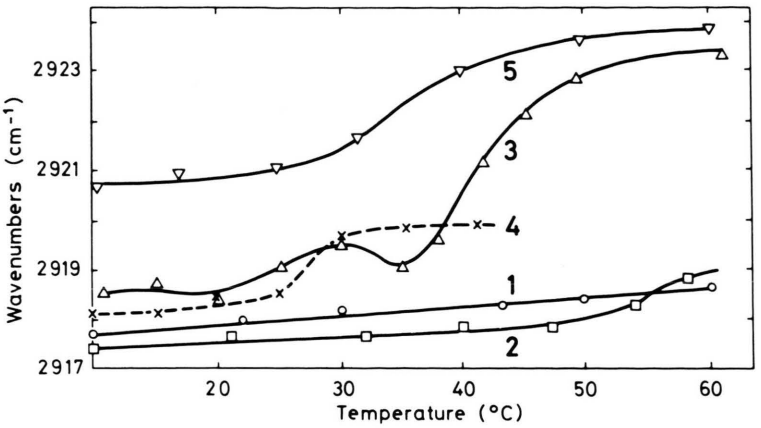


Fig. 5. Peak position of the antisymmetric stretching vibration $\nu_{as}(CH_2)$ in dependence on temperature for different lecithin preparations: (1) DPPC, anhydrous; (2) DPPC, water content app. 10% by weight; (3) DPPC, water content app. 90% by weight; (4) DMPC, water content app. 10% by weight; (5) PC egg yolk, water content app. 10% by weight.

ted versus temperature for PC preparations showing an increase in T_c with decreasing water content and no transition at all for the anhydrous sample within the investigated temperature range. Furthermore, the shift in peak position between the two phases is higher at increasing water content. These observations point out that when comparing different membrane systems – natural or artificial – it is of utmost importance to assure that they are in a comparable physical state, *e.g.* both below or above T_c.

From Table II it can be taken whether the main phase transition can be detected at all from changes in x and R for the various functional groups. However, obviously no clear-cut distinction between head-group and acyl chain behaviour can be made. As transition temperatures T_c for the lipids listed in Table I and II and for some other investigated compounds following values were determined with a precision of 1 centigrade: PA 25, DMPA 55, PC 34, DMPC 27, DPPC with 10% water content 50, DPPC vesicles 41, PE coli 25, PE egg 34, DLPE 32, DMPE 55, DPPG 46, SPM 35, and PL extract 30 °C.

As important examples for vibrations of the hydrocarbon chains the wagging progression bands $\gamma_w(CH_2)$ should be pointed out. These vibrations are assumed to be completely observable in the gel phase, because here the polymethylene chains should be mainly in the *all-trans* configuration [17]. They should decrease with increasing temperature dependent on the number of melted CH_2 groups – and finally vanish in the fluid state. The number of progression bands should be half the number of methylen groups in the respective acyl chains [18]. At first glance, this proved to be true for example for the lecithins, because 6, 7, 8, and 9 absorption bands in the wavenumber region 1350 to 1180 cm^{-1} could be observed for DLPC, DMPC, DPPC, and DSPC, respectively.

A detailed analysis of the peak positions for PL's at fixed hydrocarbon chain lengths, but with different polar parts, *i.e.* PA, PC, PE, and PG gave the following results: The peak positions are nearly independent from the kind of polar head-group which is true for the 14:0 and 16:0 phospholipids. The values for DMPA, DMPE, DMPC lie approximately at 1202, 1228, 1255, 1280, 1300, and 1326 cm^{-1} with a maximum deviation for the single species of 2 cm^{-1}. An additional band can be observed between 1355 and 1340 cm^{-1}, exhibiting no uniform behaviour for the different lipids. The values for DPPA, DPPC, DPPE, and DPPG are app. 1202, 1222, 1244, 1264, 1284, and 1306 cm^{-1}, whereas two further bands lie between 1326 and 1355 cm^{-1} at different positions for each lipid. Further evaluation of these bands did not show a uniform phase behaviour. Some bands (for DPPC the bands at 1201 and 1221 cm^{-1}) exhibit a pronounced decrease in intensity with temperature, which is, however, uniform over both phases, whereas only few bands (for DPPC the bands at 1284 and 1308 cm^{-1}) show the expected phase behaviour in that they vanish at $T > T_c$ (Fig. 6). The other absorption bands, for DPPC for instance the band at 1343 cm^{-1}, shows only a small (Fig. 6) or even no decrease in intensity as well in the gel as in the fluid phase. From Fig. 6 it becomes obvious that different values for T_c can be derived for different bands which is also typical for other phospholipids. This indicates that the various wagging progression bands originate

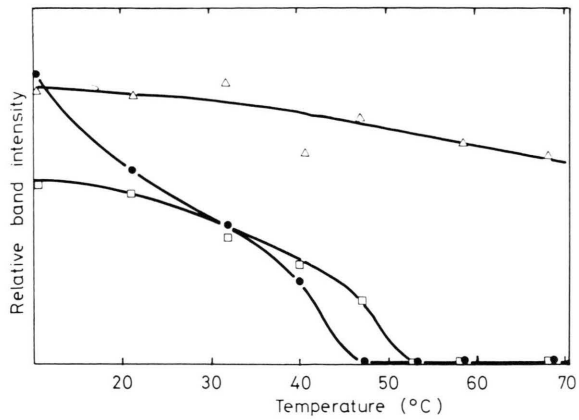


Fig. 6. Temperature dependence of the intensity of different wagging progression bands at 1284 cm^{-1} (●), 1308 cm^{-1} (□), and 1343 cm^{-1} (△) for 10% water-containing DPPC.

from different parts of the hydrocarbon moiety thus expressing the fluidity gradient along the acyl chain within the transition range.

The vanishing of the absorption band around 1285 cm^{-1} (± 6 cm^{-1}) at $T > T_c$ turned out to be common to all synthetic and natural lipids under investigation. This band can therefore be taken as phase-specific probe. It should be noted that the evaluation of the orientational behaviour showed that some of the vibrations assigned above as wagging progression bands have oscillating dipole moments deviating considerably from a parallel orientation to the hydrocarbon chains. This observation and the behaviour described above point to a possible misassignment of the respective bands to wagging vibrations. This could be true especially for the band at the highest frequency (for DPPC at 1343 cm^{-1}).

Discussion

Generally it can be stated that the application of the ATR technique in IR spectroscopy allows the determination of orientational data of comprehensive validity. As was to be expected [7], particularly for the lecithins a shift in the peak position in dependence on water content was observed and the phase transition – if it was detectable at all – was shifted to higher temperatures with decreasing water content. However, it could be shown for a larger number of vibrations that these observations obviously had little influence on the calculated orientational data. This can be taken from Table I, where the results for DPPC at three different water concentrations are compared. It must be mentioned that all lipid form planar multibilayer structures even at a water content of 90%.

State of order

The ATR technique allows the comparison of the state of order of different lipids in a fairly simple way if this is performed under identical preparative conditions. Experimental proof for a closer valuation of S than the approximation made in Table I can be taken from the dichroic ratio of $\gamma_w(CH_2)$ which has values around 2 for most investigated lipids. Because the wagging vibrations are coupled with the twisting and/or twisting/rocking progressions, the latter having been observed as weak absorption bands by Cameron *et al.* [19], it was to be expected that in the average θ should have values above zero (at least 15 to 20°). With Fig. 2 this allows an estimate of S to be around 0.75 in the gel phase. This value should represent a lower limit, because for the acyl chains it is known that even in the gel phase the terminal CH-groups exhibit relatively high fluidity resulting *e.g.* from *gauche*-conformers. The stretching vibration of the ester bound $\nu(C-O)_E$ typically has R-values around 1.5. The peak maximum is shifted to lower frequencies with respect to the value of 1180 cm^{-1}, which corresponds to a planar configuration of the −C−C−O−C− group [5]. This implies that the direction of the resulting dipole moment must deviate from the molecular axis. The angle θ can be estimated to be 30° using $S = 0.75$ and $R = 1.5$. As an example for the fact that $S = 0.75$ is rather a low estimate DPPC may be taken (Table I), for which R-values above 2.1 are observed. Application of Fig. 2 – modified for the different water content – only leads to a fit for $S > 0.75$. This estimate is in good agreement with values of $S = 0.7$ to 0.9 obtained with fluorescence polarization measurements applying diphenylhexatriene (DPH) as a probe [20–21]. McDonald *et al.* [22] found – except for the methyl end group – a value of $S = 0.83$ from ^{19}F-NMR measurements of polar lipids of *Acholeplasma laidlawii*, while the ESR measurements by Hartmann *et al.* [23] with DPPA and DPPC yielded $S = 0.90$ and $S = 0.73$, respectively. It must be noticed, however, that indirect techniques represent the state of order of the applied marker molecule (averaged over its

length) which can be quite different from that of the undisturbed bilayer.

The evaluation of our measured data for the "umbrella" vibration $\delta_s(CH_3)$ shows that for the typical values of R (app. 1.40) an order parameter $S \approx 0.40$ would follow representing the state of order of the bilayer center in the gel phase. This value seems realistic since for this vibration only minor disturbances by other vibrations are to be expected. A direct comparison with literature data could not be performed because of the apparent lack on measured values for the order parameter of the end methyl group. An indirect comparison using measurements of the gradient of S along the hydrocarbon chain yields that deuterium NMR [24] as well as ^{19}F-NMR [22] measurements on DPPC and on *A. laidlawii* membranes, respectively, and ESR measurements with 5-, 12-, and 16-oxyl stearic acids on outer and cytoplasmic membrane preparations of *Pseudomonas halosaccharolytica* [25] show a decrease in order parameter of app. 50% towards the end of the hydrocarbon chains. A similar result can be obtained from the investigation of Thulborn *et al.* [26] applying fluorescence polarization measurements on DPPC preparations, if an extrapolation of their results for 2-, 6-, 9-, and 12-(9-anthroyloxy) fatty acids to the position of the 16th carbon atom is performed. Therefore, the above derived values of $S = 0.80 \pm 0.05$ for the molecule in the average and a value of $S = 0.40 \pm 0.05$ for the bilayer center seem to reflect a very suitable approximation for the state of order of the measured lipids in the gel state.

Analysing the situation in the liquid crystalline state analogeously, for $\delta_s(CH_3)$ a S-value of 0.35 can be calculated from $R \approx 1.30$ for most of the lipids. As the terminal methyl group has a relatively high mobility in the fluid state, it is assumed that $S = 0.35$ represents a lower limit. For the stretching vibration of the ester bond $\nu(C-O)_E$ S-values in the range from 0.40 to 0.55 are obtained, for PE's even values around 0.60. The latter can be explained by the comparably low degree of hydration and with that, a weaker hydrogen bonding to the ester groups. However, from the observation of a splitting of this absorption band it can be concluded that the 2 ester bonds in β- and γ-position are in different configurations. In the fluid state from the dichroic ratio of $\nu_{as}(PO_2)$ lying app. at 0.90 and from the limiting value $\theta = 90°$ a minimum value for S of 0.47 can be calculated.

From the above discussion it seems reasonable for most of the investigated lipids to assume $S = 0.50 \pm 0.05$ for the liquid crystalline state.

The approximation of S in both − the gel and the liquid − phases now allows the calculation of the direction of the oscillating dipole moments for all vibrations listed in Tables I and II according to equation (3). The respective calculation for the 4 vibrations of the hydrocarbon moiety $\nu_s(CH_2)$, $\nu_{as}(CH_2)$, $\delta(CH_2)$, and $\gamma_r(CH_2)$ yields values of 50 to 70° for PC's, for the other lipids even distinctly lower. Fringeli [5], Fringeli and Günthard [3], and Akutsu and co-workers [15] assumed a value of $\theta = 90°$ for phospholipids. However, with this θ-value, Fringeli found R-values especially for DPPE not being consistent with each other and followed that the model should be inadequate.

Assuming $\theta = 90°$, Table II clearly shows an inconsistency of the R-values for the listed hydrocarbon vibrations $\nu_{as}(CH_2)$ and $\delta(CH_2)$, but also for other vibrations. This observation is not surprising if one considers that this θ-value was obtained from theoretical calculations or from experimental data for paraffins, fatty acids, and fatty acid derivatives [17, 18, 27], the phase behaviour of which cannot a priori be expected to be identical with the polymorphism of phospho- and sphingolipids. It must furthermore be noticed that for simultaneously occurring vibrations the resulting θ-values of each single vibration might deviate from the value of the undisturbed vibration. Thus, for example, the value of θ for the $\nu_{as}(CH_2)$ vibration is reduced by the interference of the twisting (or twisting/rocking) vibrations which are about twice as slow as the former leading to a reduction of θ by 20% as calculated by Zbinden for polymethylene [8]. On the basis of a similar argumentation, also for $\nu_s(CH_2)$ the deviation of θ from 90° can be explained by the existence of the wagging progressions. At this point it should be emphasized that to our knowledge measured values for θ have not been published up to now, although they are of fundamental importance when interpreting orientational data *e.g.* of even more complex systems like lipopolysaccharides or whole membranes.

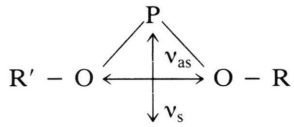
Single vibrations

The small shift in peak position between the gel and fluid phase especially for $\nu_s(CH_2)$ and $\nu_{as}(CH_2)$ at low water content (see Fig. 5) is apparently con-

nected with the "hydrophobic hydration" of the methylen chains observed by Blume [28], *i.e.* water molecules are not excluded from moving within the hydrophobic moiety. At higher water content a stronger coupling of the methylen groups with water molecules takes place – being different in both phases – which causes a larger shift in the peak position than at low water content.

The peak position of $\nu_{as}(PO_2)$ appearing at significantly lower values for the PE's and the PL extract indicates a much stronger hydrogen bonding of these compounds. On the other hand, for most of the other parameters, the PL extract despite its high PE content (app. 70%) does not behave like PE, but – as indicated also by the higher degree of hydration – the character of pure PE in biological membranes is altered considerably by the addition of other PL's.

The results described for the lecithins regarding the behaviour of the symmetric and antisymmetric C–N-stretches of the choline part indicate that in the fluid state to a greater extent than in the gel state the choline group is directed towards the molecular axis, *i.e.* the molecule extends when entering the liquid crystalline state. In a similar way, the analysis of the symmetric and antisymmetric P–O-stretch within R′–O–P–O–R shows a non-linear 3-dimensional arrangement such as



whereas the other phospholipids have a more linear conformation.

The evaluation of the wagging progression bands clearly shows their diagnostic value in regard to the phase specifity. Moreover, from the fluctuations in regard to the appearance and position of T_c and to the band intensities in dependence on temperature the phase behaviour of the wagging progression bands can be taken as a direct measure for the fluidity gradient along the hydrocarbon chain within and outside the transition range. Thus, a more detailed investigation of this spectral region should be worth while.

Model distributions

From the determination of the order parameter the distribution of the directions of the lipid chains can now be calculated applying the model distributions presented in Methods, namely the MCU (microcrystalline ultrastructure, a distribution according to a delta function) and the LCU (liquid crystalline ultrastructure, distribution obtained in a drawing process). In Fig. 7 Kratky's distribution $f(\gamma)$ – showing the probability of individual chains lying at an angle γ_o in respect to the z-axis – is plotted for the S values determined for the gel and liquid crystalline state, and also the distribution of the lipid chains according to the MCU for $S = 0.8$, *i.e.* the gel state.

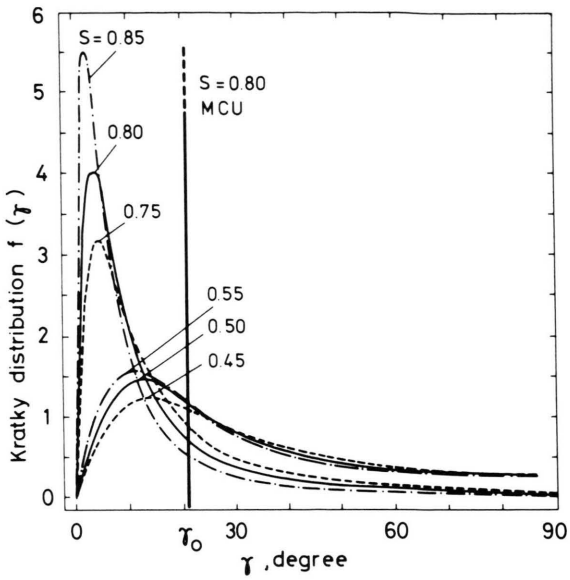


Fig. 7. Kratky distribution $f(\gamma)$ according to equation (5) for the state of order calculated for the gel ($S = 0.80 \pm 0.05$) and the liquid crystalline ($S = 0.50 \pm 0.05$) phases. Also, the distribution according to a delta function (MCU) for $S = 0.80$ is included.

The comparison of the calculated $\gamma_o = 21.4°$ with those values given by Fringeli [5] and Akutsu *et al.* [15] show – as far as comparable – similar, but slightly lower values. However, it can be stated that because of the existence of dynamic molecular interactions the idealized model distribution according to the MCU is only a rough approximation. In the case of the Kratky distributions of the lipid chains according to Fig. 7 no data seem to be available. If it is assumed, however, that the chain order parameter S_α – derived from NMR experiments and specified by Petersen and Chan [29] – can be correlated with the order parameter S of the present paper, which seems reasonable from their data, the relation

$$S_\alpha = \tfrac{1}{2}(\cos^2\Delta\alpha + \cos\Delta\alpha)$$

can be used, where $\Delta\alpha$ denotes the angular range within which an individual chain can fluctuate in respect to the bilayer normal. In this model the lipid chains are assumed to be equally distributed within $\Delta\alpha$ and outside with probability 0. Using $S = 0.80 \pm 0.05$ and $S = 0.50 \pm 0.05$, the calculation yields angular ranges of $\Delta\alpha = (30.7 \pm 4.1)°$ and $(51.8 \pm 3.3)°$, respectively. The comparison with the plots of Fig. 7 applying equation (6) shows that these angular ranges would contain 84 and 78% of the lipid chains, respectively, when distributed according to a Kratky function. It may be stated, therefore, that the two approaches discussed here – the model derived from NMR measurements and the Kratky model established for IR investigations – show a remarkable agreement. Assuming a distribution of lipids as it is obtained in a drawing process, however, seems to be more realistic than by assuming the lipid chains to be equally distributed within a certain angular range.

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