

Study by infrared spectroscopy of the conformation of dipalmitoylphosphatidylglycerol monolayers at the air–water interface and transferred on solid substrates

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Abstract

A parallel investigation of the conformational changes of dipalmitoylphosphatidylglycerol (DPPG) monolayers induced by surface pressure has been studied in situ at the air water interface using polarization modulation external infrared reflection absorption spectroscopy (PM-IRRAS) and on solid substrate by attenuated total reflection (ATR) spectroscopy. For both the ATR and PM-IRRAS spectra of DPPG, the non-linear increase of the intensity of the antisymmetric methylene stretching band of DPPG with molecular surface density, indicates that the compression of the DPPG monolayer induces a change of the conformation and/or orientation of the acyl chains of the phospholipid. At surface pressures between 7 and 20 mN/m, the acyl chains of the DPPG monolayer are found to be more ordered on the germanium substrate than at the air–water interface. At higher surface pressures, the conformation of the lipid acyl chains of DPPG on both substrates are almost similar. In the liquid-condensed phase, the acyl chains are in all-*trans* conformation and their tilt angle with respect to the normal of the film is approximately 30°. Our results also suggest the presence of DPPG solid domains in the liquid-expanded phase. Investigation of polar head group region indicates that at low surface pressures, the carbonyl groups were more oriented on the water surface than the acyl chains. Finally, the present study shows that intermolecular hydrogen bonding probably occur between the glycerol hydroxyl and the phosphate or carbonyl groups of the phospholipid. © 1998 Elsevier Science Ireland Ltd. All rights reserved.

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1. Introduction

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The Langmuir–Blodgett (LB) technique has been widely used to characterize monomolecular

films of biological molecules (Möhwald, 1990; Ulman, 1991). This method is particularly attractive when it is coupled with spectroscopic techniques such as attenuated total reflectance (ATR) infrared spectroscopy since information could be obtained about the conformation and orientation of the molecules in the film (Takenaka et al., 1971; Okamura et al., 1985; Rana et al., 1994; Subirade et al., 1995). However, this technique presents some disadvantages. For example, it has been reported that the rate of deposition of monomolecular films of phospholipids on a solid substrate may influence the final conformation of the hydrocarbon chains of the lipid molecules (Rana et al. 1994). Moreover, Gregory et al. (1994) have mentioned that the transfer of a monolayer onto a solid substrate may alter its uniformity.

The study of organized monolayers of biological molecules directly on the water surface, not only eliminates problems inherent to their transfer onto solid substrates, but allows for a better simulation of their native environment. Characterization of molecules at the air–water interface has been achieved by various techniques including ellipsometry (e.g. Lukes et al., 1992) and epifluorescence microscopy (e.g. Subirade et al., 1995). Infrared spectroscopy has also been used successfully to study such monolayers. External infrared reflection absorption spectroscopy (IRRAS) is a powerful technique to obtain direct structural and conformational information about molecules at the air–water interface (Dluhy et al., 1988; Mitchell and Dluhy, 1988; Flach et al., 1993, 1996, 1997; Gericke et al., 1993, 1996; Gericke and Hühnerfuss, 1994a,b,c, 1995; Mendelsohn, et al., 1995; Pastrana-Rios et al., 1995; Riou et al., 1997). However, because of the spectral interferences due to the water vapor absorption between 1400 and 1800 cm^{-1} , IRRAS spectra in this region may be strongly affected by humidity variations (Mendelsohn et al., 1995). Polarization modulation infrared reflection absorption spectrometry (PM-IRRAS) has been shown to be an appropriate technique to overcome this drawback. Indeed, this technique, originally used to increase the sensitivity of IRRAS on solid substrates (Golden et al., 1981; Buffeteau et al., 1991), has

been applied recently to the study of organic monolayers spread on the water surface (Blaudez et al., 1993). It has been used extensively by Desbat's research group (Blaudez et al., 1992, 1993, 1996; Cornut et al., 1996) and more recently in our laboratory (Xiao et al., 1998) to characterize lipids, proteins and polymers at the air–water interface. This non-destructive differential technique is based on the rapid modulation of the polarization of the incident electromagnetic radiation on the sample. Under such conditions, strong absorption of water vapor and instrumental fluctuations are minimized (Blaudez et al., 1996).

Phosphatidylglycerols (PG) are found at high concentration in the chloroplast of plants membranes (Watts et al., 1981). They also constitute a component of bacterial membranes (Scherphof, 1993). The thermotropic behavior of negatively charged phospholipids is dependent on both the pH and the concentration of ions (Dijck et al., 1978). Hence, the state of ionization of the PG polar head group and the nature of the neutralizing ions strongly influence the molecular packing of the lipid (Tocanne et al., 1974). Sacré and Tocanne (1977) have shown that the ionic properties of the PG derivatives at the air–water interface depend on the glycerol moiety and on the molecular packing of the lipid.

In the present study, PM-IRRAS has been used to study *in situ* at the air–water interface the conformation and orientation changes of both the polar head group and the acyl chains of dipalmitoylphosphatidylglycerol (DPPG) induced by the surface pressure applied to the films. The results obtained have been compared with the ATR spectra of LB monolayers in order to examine the influence of the transfer.

2. Material and methods

2.1. Material

1,2-dipalmitoyl-*sn*-glycero-3-phosphoglycerol (sodium salt) was purchased from Avanti polar lipids (Alabaster, AL) and used without further purification. Chloroform and methanol (HPLC grade), purchased from Sigma–Aldrich, were used

as spreading solvents. Sodium chloride (analytical reagent, BDH) subphase solution was prepared with deionized water (Barnstead NanopurII, Boston, MA; resistivity 18.3 M Ω /cm).

2.2. LB films technique

All experiments were performed with a KSV-3000 film balance apparatus. The Teflon trough (8 × 58 × 1.5 cm) trough was cleaned either with a 10% sulfuric acid or a 3% Hellmanex II (Hellma GMBH, Müllheim) solution and rinsed several times with distilled water. It was then filled with a solution of 150 mM NaCl, pH 5.7. The temperature of the subphase was controlled to 17.0 ± 0.3°C. ATR germanium parallelograms (50 × 20 × 2 mm, cut at 45° angle) were used as substrates for the transfer of the films. The crystals were cleaned with a chloroform/MeOH solution in a bath sonicator for 5–10 min, rinsed with distilled water and finally cleaned with a plasma cleaner (Harrick, NY). The substrates were then lowered into the subphase at a depth of 10 mm. Monomolecular films of DPPG were formed by spreading 80 μ l of a 0.5 mg/ml DPPG stock solution in chloroform/methanol: 9/1 (v/v) onto the subphase. After an equilibration period of 15 min for solvent evaporation, the surface pressure-area isotherms were recorded with a compression speed of 2.5 \AA^2 /molecule per min. The change of the monolayer surface pressure was monitored by the Wilhelmy method using a platinum plate. In order to transfer the monolayer on the germanium substrate, the DPPG film was compressed to the desired surface pressure which was then held constant. After an period of about 10 min, the substrate was raised vertically from the subphase through the compressed monolayer film at a rate of 2 mm/min. This process resulted in the transfer of one monolayer on both faces of the ATR crystal with a transfer ratio close to unity, except at low surface pressure where the transfer ratio was less reproducible.

2.3. Multilamellar DPPG dispersion sample

DPPG was dispersed in 150 mM aqueous NaCl solution to a final concentration of 10% w/vol.

Homogenization of the sample was achieved by heating the lipid dispersion under vigorous vortexing at about 20°C above the gel-to-liquid crystalline phase transition temperature and quenched in liquid nitrogen at 77 K. This treatment was performed thrice.

2.4. Infrared spectroscopy

Infrared spectra of transferred monolayers were recorded with a Magna 550 (Nicolet Instrument, Madison, WI) Fourier transform infrared spectrometer equipped with a narrow-band liquid nitrogen cooled Mercury–Cadmium–Telluride detector. A motorized rotating polarizer (Specac, Orpington, UK) located inside the sample compartment was used to obtain polarized spectra without breaking the purge of the spectrometer. Reference spectra of the bare germanium crystal were recorded after those of the transferred monolayers. An average of 1000 scans at 4 cm^{-1} resolution using a triangular apodization were collected for each spectrum.

The order parameter $\langle P_2 \rangle$ for a given vibration was calculated from the electric field amplitudes and the experimental dichroic ratio. The amplitudes of the electric field of the infrared radiation along the x , y and z directions (E_x , E_y and E_z) were calculated using the Harrick thin film equations (Harrick, 1967; Fringeli and Günthard, 1981) with an angle of incidence of 45°. The refractive indices used for the germanium parallelogram, phospholipid film and air were 4, 1.44 and 1, respectively.

The dichroic ratio is defined as:

$$R = A_p/A_s \quad (1)$$

where A_p and A_s are the absorbances measured with the infrared radiation polarized parallel and perpendicular to the plane of incidence, respectively.

The order parameter $\langle P_2 \rangle_\gamma$ characterizing the orientation of a given transition moment uniaxially oriented at an angle γ relative to the normal to the crystal is given by the relation (Fringeli and Günthard, 1981):

$$\langle P_2 \rangle_\gamma = \frac{RE_y^2 - E_x^2 - E_z^2}{RE_y^2 - E_x^2 + 2E_z^2} \quad (2)$$

From the Legendre addition theorem, it is possible to obtain the order parameter $\langle P_2 \rangle$ related to the axis of the acyl chains and the normal to the ATR crystal (Lafrance et al., 1995):

$$\langle P_2 \rangle = \langle P_2 \rangle_\gamma / P_2(\cos \beta) \quad (3)$$

The angle β between the transition moment and the acyl chains axis was set at 90° for the CH_2 stretching modes.

For a narrow distribution of orientation, the average tilt angle of the acyl chains in all-*trans* conformation relative to the normal to the ATR plate can be calculated from the order parameter using the following equation (Lafrance et al., 1995):

$$\theta = \arccos \sqrt{\frac{2\langle P_2 \rangle_\gamma}{3P_2(\cos \beta)} + \frac{1}{3}} \quad (4)$$

Transmission spectra of multilayers dispersions of DPPG were obtained as a function of temperature as described previously (Dicko et al., 1998). Briefly, 20 μl of the DPPG dispersion was spread between two BaF_2 windows separated by a 12 μm mylar sheet. For each spectrum, 250 scans were collected at 2 cm^{-1} using Happ–Genzel apodization. The Spectra Calc software (Galactic Industries, Salem, NH) was used for all data treatment. The frequency of the different bands was determined from the center of gravity at 80% of their height (Cameron et al., 1982).

2.5. Polarization modulation infrared spectroscopy

A small homemade Teflon trough ($5 \times 36 \times 0.5 \text{ cm}$) sitting on the side of the infrared spectrometer was used for these experiments. The surface pressure was measured with a precision of $\pm 1 \text{ mN/m}$ with a Riegler and Kirstein film balance (Germany) using as Wilhelmy plate a filter paper ($3 \times 8 \text{ mm}$, Whatman paper, no. 1). The paper plate was cleaned as described by Mitchell and Dluhy (1988). The experimental conditions were similar to those reported above for the LB films technique. To obtain the same starting molecular area as for LB films, 37 μl of the DPPG solution (0.5 mg/ml) was spread on the subphase. After an equilibration period of 15

min, the film was then compressed to the desired surface pressure. The hydrophilic barriers were then stopped and the PM-IRRAS spectra were recorded using a Magna 850 (Nicolet, Madison, WI) spectrometer. The experimental setup used was similar to that described by Blaudez et al. (1996). The infrared radiation modulated in intensity by the Michelson interferometer was polarized with a ZnSe wire grid polarizer (Specac). The beam was then passed through a Hinds (type II) ZnSe photoelastic modulator which modulates the polarization of the infrared radiation between the linear parallel and perpendicular polarization states at a fixed frequency of 74 kHz. The angle of incidence of the infrared beam with respect to the normal to the water surface was 76° . The reflected infrared radiation was collected with a ZnSe lens and focused on a narrow-band liquid nitrogen cooled MCT detector (Bylov Technologies). The signal at the output of the detector was first amplified with a low-noise preamplifier (Graseby Infrared). The low-frequency (intensity) and high-frequency (polarization) modulations were separated as described previously (Blaudez et al., 1993; Bufeteau and P  zolet, 1996) using two dual channel electronic filters (Stanford Model 650) and a digital signal processing lock-in amplifier (EG&G Instruments Model 7260) with a time constant of 80 μs . The frequency of the modulator was adjusted at 1400 cm^{-1} and the velocity of the interferometer moving mirror was set at 0.5 cm/s. PM-IRRAS spectra were recorded at a spectral resolution of 4 cm^{-1} using triangular apodization. For each spectrum, 1000 scans were co-added over a total acquisition time of about 25 min. Normalized PM-IRRAS signals were obtained using the following expression:

$$\Delta S/S = S(d) - S(0)/S(0) \quad (5)$$

where $S(d)$ and $S(0)$ are the PM-IRRAS signals of the covered and uncovered subphase, respectively. The signal to noise ratio was further improved by averaging several PM-IRRAS spectra. The theoretical aspect of the technique was described elsewhere (Blaudez et al., 1993) and is not presented here.

3. Results and discussion

3.1. Surface pressure-area isotherm

The surface pressure-area isotherm of DPPG at 17°C is reproduced in Fig. 1. This isotherm is similar to those obtained by Sacré and Tocanne (1977), Sacré et al. (1979) and Subirade et al. (1995) using either phosphate buffer or salt solutions as subphase. Four distinct regions are seen in this isotherm. At zero surface pressure, the lipid film is in a gaseous state (G). On compression of the monolayer, the lipid passes progressively to the liquid-expanded state (LE) from ca. 100–75 Å²/molecule. In the region between 75 and 50 Å²/molecule, there is a plateau at 7 mN/m where the LE and liquid-condensed phases (LC) are in equilibrium. This two-phase coexistence (LE–LC) transition region was shown to be sensitive to several parameters such as temperature and ionic strength (Helm et al., 1987; Möhwald, 1990). Above 50 Å²/molecule, the system is completely in the LC phase and the surface pressure

increases rapidly with the compression of the film. The extrapolation of the liquid-condensed region of the isotherm to zero surface pressure gives an area of about 48 Å² per molecule, in full agreement with the area reported by Watts et al. (1981) for charged hydrated DPPG bilayers. This value is also close to the packing cross-section reported by Pascher et al. (1987) for crystalline DPPG (44 Å² per molecule). Infrared spectra of the DPPG monolayers at different points of the isotherm (shown by arrows) were recorded either directly at the air–water interface or after transferring the films on germanium ATR substrates.

3.2. The 2800–3000 cm⁻¹ region

Fig. 2 shows the ATR and the PM-IRRAS spectra in the C–H stretching mode region between 2800 and 3000 cm⁻¹ of monolayers of DPPG at surface pressures between 0 and 40 mN/m. Results are not presented for LB films at 0 mN/m since it was not possible to transfer DPPG monolayers from the gaseous phase. As

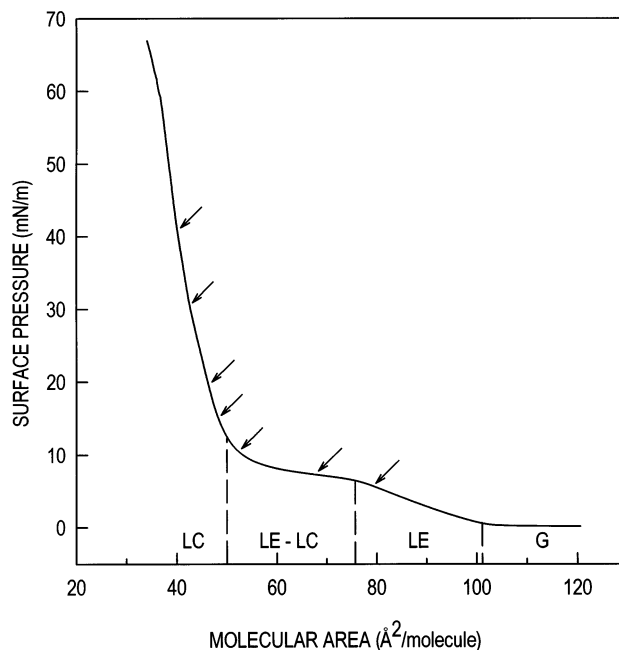


Fig. 1. Pressure–area isotherm of DPPG at 17°C on a 150 mM NaCl subphase. The arrows indicate the surface pressures at which films were transferred and PM-IRRAS spectra were recorded. Regions corresponding to different phases are separated by dashed lines.

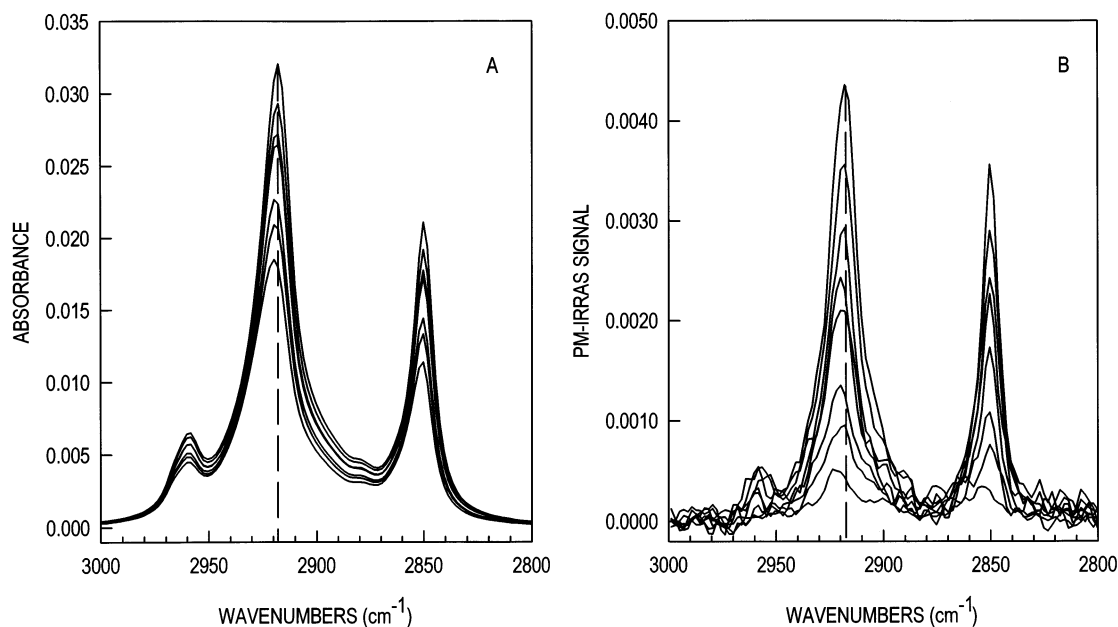


Fig. 2. (A) ATR (P polarization) and (B) PM-IRRAS spectra in the 3000–2800 cm^{-1} region of DPPG at different surface pressures.

seen on this figure, the use of the multiple internal reflection technique (24 reflections for the ATR crystals used) results in spectra of transferred single monolayers with a remarkable signal-to-noise ratio. Such a quality of spectra could not be achieved by PM-IRRAS at the air–water interface since in this case the spectra are obtained from a single reflection of the infrared beam on the covered water surface. The CH_2 stretching mode region of the infrared spectra of phospholipids is dominated by two strong bands with their maximum around 2918 and 2850 cm^{-1} , due to the antisymmetric and symmetric CH_2 stretching vibrations, respectively. The small bands at about 2959 and 2879 cm^{-1} are due to the antisymmetric and symmetric stretching modes of the terminal methyl groups, respectively. This spectral region is particularly useful since it is well known that the frequency and width of the methylene bands are sensitive to the conformation of phospholipid acyl chains (Casal and Mantsch, 1984). Fig. 2 shows that as the surface pressure increases, the methylene bands in both the ATR and PM-IRRAS spectra increase in intensity, become narrower and shift slightly to lower frequencies. All

these changes show clearly that the system becomes more ordered when the surface pressure is increased. The variation of the intensity of the ATR and PM-IRRAS methylene bands of DPPG at 2918 cm^{-1} with molecular surface density, ρ , is shown in Fig. 3. This parameter was chosen for the X -axis since the intensity should vary linearly with ρ if the intensity variation is only due to the increase of the number of molecules per unit area. As seen in Fig. 3, this is clearly not the case for DPPG and there is a break at around $2 \cdot 10^{14}$ molecules/ cm^2 that corresponds to a surface pressure of about 10–15 mN/m (end of the plateau region). This indicates that the change of the conformation and orientation of the acyl chains of DPPG with the increase of the surface density is more important above $2 \cdot 10^{14}$ molecules/ cm^2 leading to a steeper variation of the spectral intensity with the surface density. It is interesting to note here that such a break was not observed for LB monolayers of dipalmitoylphosphatidylcholine (DPPC) (Okamura et al., 1985). The latter behavior was associated to the existence of islands of DPPC with acyl chains in the all-*trans* conformation, even at low surface pressure. In the case of

DPPG, a significant change of the structure of the monolayer seems to occur even when all lipids molecules are in the liquid-condensed phase.

The variation of the frequency of the antisymmetric CH_2 stretching vibration of DPPG with the surface pressure is displayed in Fig. 4. As can be seen, the frequency of this band decreases as the surface pressure of the film increases due to the increase of the number of *trans* conformers along the lipid chains. For the film in the gaseous state at 0 mN/m, the PM-IRRAS band due to the antisymmetric CH_2 stretching vibration of DPPG appears at 2922 cm^{-1} which is slightly lower than the frequency observed for a multilamellar dispersion of DPPG in 150 mM NaCl above the gel-to-liquid crystalline phase transition temperature (Fig. 5). Therefore, even in the gaseous phase, the acyl chains of the DPPG monolayers are not as disordered as in the liquid crystalline phase of the bulk lipid dispersion. This result suggests that lipid domains of DPPG are present at low surface pressure. Epifluorescence microscopy experiments (not shown) have confirmed the presence of solids

domains in the LE phase as also observed by Subirade et al. (1995). Moreover, as shown below, the bands due to the wagging methylene progression are present even at low surface pressure, indicating the presence of acyl chains with the all-*trans* conformation in the LE phase. However, the decrease of the frequency of the 2918 cm^{-1} band with the increase of the surface pressure in the LE phase reveals that the conformation of the lipid changes with compression and that the increase of the surface pressure when the film is compressed is not only due to the fusion of ordered domains.

The results shown in Fig. 4 indicate that, at 5 mN/m, the acyl chains are less ordered on the germanium substrate than at the air–water interface. On the contrary, Rana et al. (1994) reported that at low surface pressure and slow transfer rate ($< 10\text{ mN/m}$), the acyl chains of DPPC were more ordered on the germanium crystal than at the air–water interface. Our results should, however, be interpreted with caution since at low surface pressures, the PM-IRRAS spectra are

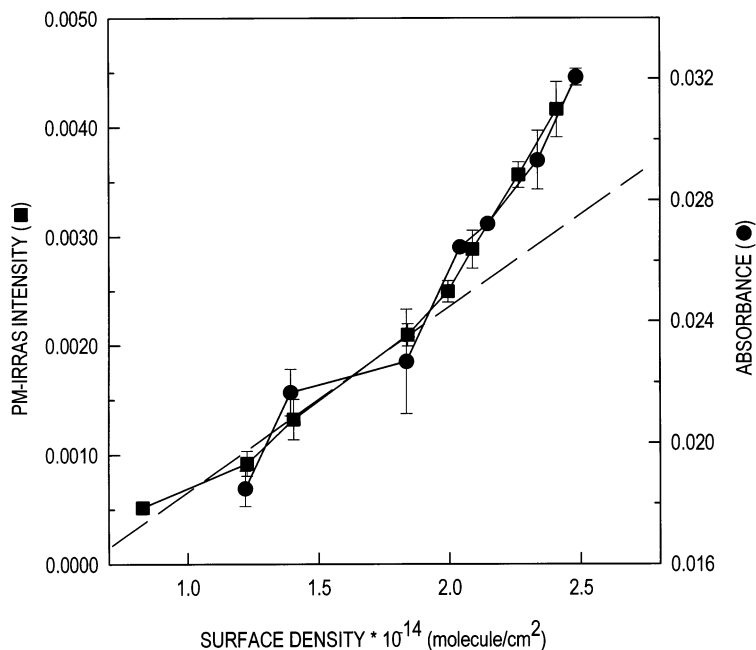


Fig. 3. Effect of the molecular surface density on the peak height intensity for the antisymmetric CH_2 stretching band in the ATR spectra of LB films (●) and in the PM-IRRAS spectra at the air–water interface (■). The data points are the mean of 3–7 values. Errors bars are the standard errors of the mean.

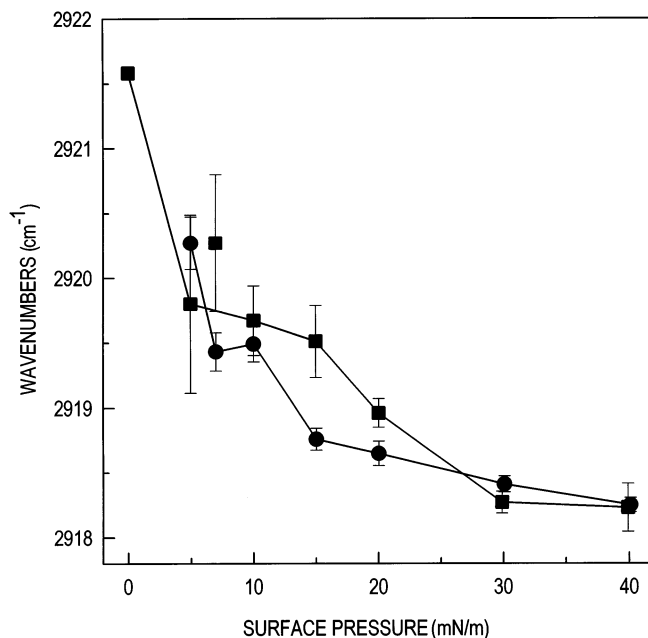


Fig. 4. Variation of the frequency of the antisymmetric CH_2 stretching band of DPPG with surface pressure for both LB films (●) and films at the air-water interface (■). The data points are the mean of 3–7 values. Errors bars are the standard errors of the mean.

weak and the error on the frequency is quite important. Okamura et al. (1985) have reported that monolayers of DPPC in the LE state are not homogeneous. The inhomogeneity and the coexistence of fluid and solid phases can also contribute to the large standard deviation of the mean frequency for the antisymmetric stretching vibration (Mitchell and Dluhy, 1988). In fact, we were not able to obtain a good transfer of DPPG films on solid substrates at low surface pressures. The transfer ratio at 5 and 7 mN/m was always around 1.5. The reason for this high transfer ratio is still unclear. However, this could be due, as seen below, to the ordering of the lipid when it is transferred on the ATR crystal since the transfer ratio decreases from 1.5 at 5–7 mN/m to 1.1 at 30–40 mN/m. These problems are most likely at the origin of the less linear behavior of the intensity of the 2918 cm^{-1} band with the lipid surface density below $2 \cdot 10^{14}$ molecules/ cm^2 (Fig. 3) compared with the PM-IRRAS results. Fig. 4 also shows that in the plateau region of the PM-IRRAS spectra, between 5 and 15 mN/m, the fre-

quency of the 2918 cm^{-1} band is, within experimental errors, fairly constant. We believe that in this surface pressure range, the size of the lipid domains increases without major changes in the conformation of the acyl chains until the liquid-condensed phase is completely formed. As the surface pressure is increased from 15 to 30 mN/m, the frequency of the 2918 cm^{-1} band still decreases indicating further conformational ordering of the acyl chains of DPPG. Above 30 mN/m, the frequency of the 2918 cm^{-1} band is constant and is very close to that of DPPG multilayers in the gel phase (Fig. 5).

As seen in Fig. 4, for surface pressures between 7 and 25 mN/m, the frequency of the antisymmetric stretching vibration of DPPG obtained by ATR technique is always lower than that measured for transferred films by the PM-IRRAS. This suggests that the process of transferring the monomolecular film of DPPG on a solid substrate induces some ordering of the acyl chains. As reported above, similar observations were made by Rana et al. (1994) for DPPC. This ordering

effect may explain the absence of a well defined plateau for LB films between 5 and 15 mN/m as observed for PM-IRRAS. At higher surface pressures, (30 and 40 mN/m) our results revealed that the conformation of the hydrocarbon part of DPPG on a solid substrate was, within experimental errors, similar to that at the air–water interface.

Table 1 gives the acyl chains order parameter $\langle P_2 \rangle$ calculated for the polarized ATR spectra of transferred films of DPPG at different surface pressures. As the surface pressure increases, the order parameter increases gradually. Below 30 mN/m, this increase could be due to both the increase of the population of *trans* conformers with compression as shown in Fig. 4 and the reorientation of the lipid acyl chains. For that reason, the acyl chain tilt angle was not calculated at surface pressure below 30 mN/m (Table 1). On the other hand, Fig. 4 shows that at 30 mN/m and above, the frequency of the 2918 cm^{-1} band remains almost constant since the acyl chains are in the all-*trans* conformation, the order parameter still increases. The tilt angle calculated from the order parameter is then 30° at 30 mN/m and 27° at 40

mN/m. Watts et al. (1981) reported that the acyl chains of DPPG in the charged state are tilted with an angle of 30° relative to the bilayer normal, in good agreement with that obtained in the present study in the liquid-condensed state. Under our experimental conditions (pH 5.7), the DPPG head group is ionized (Sacré et al., 1979), giving rise to film expansion (Sacré and Tocanne, 1977). The electrostatic repulsions between DPPG head groups is most likely at the origin of the tilt of the hydrocarbon chains. In the case of DPPC, Okamura et al. (1985) have found that independently to the surface pressure, the acyl chains are in the all-*trans* conformation and oriented perpendicular to the crystal. Similar observations were made by Rana et al. (1994) for DPPC at low and high surface pressures. However, both groups have used the two-phase approximation (Citra and Axelsen, 1996) to calculate the electric field amplitudes used in Eq. (2). A recent study in our laboratory (Picard et al., 1998) has demonstrated that the optical properties of thin films have to be taken into account for accurate orientation determination in thin films by ATR spectroscopy.

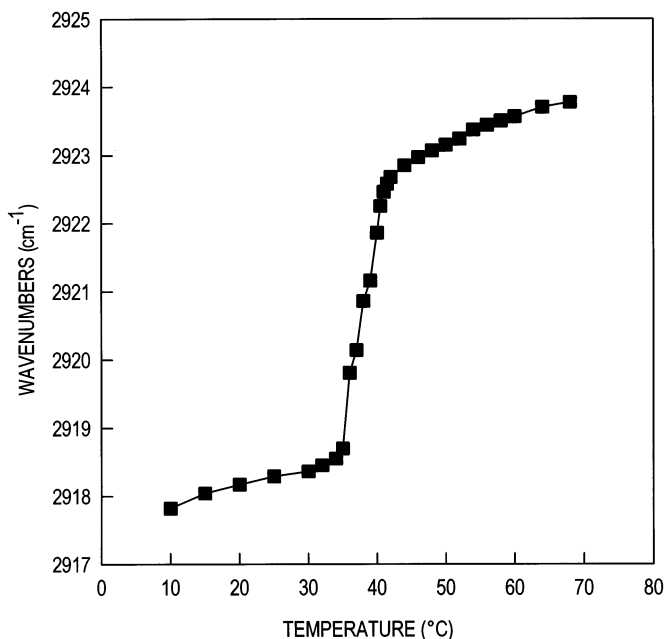


Fig. 5. Temperature dependence of the frequency of the antisymmetric CH_2 stretching band in the transmission spectra of a multilamellar dispersion of DPPG.

Table 1

Effect of surface pressure on the order parameter and average acyl chain tilt angle for DPPG calculated from the band due to the antisymmetric stretching vibration

Surface pressure (mN/m)	Frequency (cm ⁻¹)	Order parameter <P ₂ >	Average tilt (θ) ^a
5	2920.3	0.42	—
7	2919.4	0.48	—
10	2919.5	0.50	—
15	2918.8	0.53	—
20	2918.7	0.56	—
30	2918.4	0.62	30
40	2918.3	0.67	27

^a Tilt angle were not calculated below 30 mN/m because of the presence of gauche bonds along the acyl chains.

Surface selection rules for PM-IRRAS spectra are different from those of classical IRRAS spectra. Blaudez et al. (1996) have shown from spectral simulations of PM-IRRAS spectra that vibrations with transition moments oriented at an angle between 90 and 39° relative to the normal to the water surface should give positive bands, while vibrations with transition moments oriented at less than 39° relative to the normal of the interface should appear as negative bands. Consequently, the fact that the bands due to the CH₂ stretching vibrations are positive indicates that the orientation of acyl chains is closer to the normal of the water surface. Determination of the exact tilt angle of the acyl chains at the air–water interface is currently under investigation in our laboratory.

3.3. The 1000–1800 cm⁻¹ region

3.3.1. Carbonyl stretching vibration

The carbonyl stretching band around 1740 cm⁻¹ is often used to investigate the interfacial region of phospholipids. Its frequency is sensitive to hydrogen bonding and thus gives information about the state of hydration of the lipid. The spectra of transferred LB films of DPPG in this spectral region (Fig. 6A) display a single band centered around 1739 cm⁻¹, independently of the surface pressure. This band has been attributed to free carbonyl groups by Blume et al. (1988), suggesting that the lipid head group in the transferred films is essentially in a dehydrated state over the entire isotherm. Similar results were also

reported by Subirade et al. (1995) for DPPG. Moreover, no bands characteristic of the O–H stretching vibrations were observed in the spectra, confirming that the films are in a water-free state. The results of Fig. 6 show that the carbonyl band is actually asymmetric on the low frequency side. The presence of a low-frequency shoulder around 1720 cm⁻¹ could be due to the presence of hydrogen bonds between the lipid ester groups and the glycerol O–H groups of adjacent DPPG molecules (Blume et al., 1988). Such hydrogen bonding is more likely to occur at low surface pressure due to the higher disorder in the DPPG film. This is confirmed by the more pronounced asymmetry of the carbonyl band at low surface pressures.

The frequency of the carbonyl band at the air–water interface was difficult to measure with accuracy because of the low signal to noise ratio in the spectra. Nevertheless, Fig. 6B shows a gradual increase of frequency of the C=O band with the compression of the film from about 1730 cm⁻¹ at 0 mN/m to 1737 cm⁻¹ at 40 mN/m. This most likely corresponds to a progressive decrease of the degree of hydration of the glycerol moiety when the surface pressure increases. Interestingly, our results indicate that the frequency of the carbonyl band at the air–water interface is always lower than that of completely dehydrated DPPG on a solid substrate. The negative dip around 1660 cm⁻¹ observed in the PM-IRRAS spectra is due to the bending mode of water and has been associated with the difference in the optical response of the covered and uncovered water surface (Blaudez et al., 1993).

The variation of the intensity of the DPPG carbonyl bands with surface density for both PM-IRRAS and LB films is depicted in Fig. 7. As seen, the behavior of the carbonyl band is similar to that of the band due to the antisymmetric CH_2 stretching vibration. The discontinuity observed at around $2 \cdot 10^{14}$ molecules/ cm^2 (corresponding to about 10–15 mN/m) indicates a change of the conformation and/or orientation of the carbonyl group. The parallel behavior of the intensity of the carbonyl and the acyl chains bands suggests that the lipid molecules behave as a whole unit upon monolayer compression. Considering the selection rules mentioned above, the fact that the carbonyl bands in the PM-IRRAS spectra are positive suggests that the carbonyl groups are almost parallel to the water surface.

Comparison of Fig. 7 and Fig. 3 shows that, at all surface pressures, the bands due to the methylene stretching vibrations in the LB films spectra are more intense than the carbonyl bands, as generally observed in bulk lipid spectra. The relative intensity of this region in the PM-IRRAS spectra is however quite different. Below 15 mN/m ($2 \cdot 10^{14}$ molecules/ cm^2), the carbonyl band of DPPG at the air–water interface is more intense

than the CH_2 stretching bands while the opposite is observed at higher surface pressures. This indicates that at low surface pressures, the lipid ester groups are well anchored in the water subphase and better oriented than the acyl chains at the air–water interface.

3.3.2. Methylene scissoring mode

This mode provides information about acyl chains packing (Cameron et al., 1980). At low surface pressure, the PM-IRRAS spectra exhibit a broad and poorly defined CH_2 scissoring band, confirming that the acyl chains are partially disordered. As the surface pressure increases, a band at around 1468 cm^{-1} grows up, indicating that the chains are self organized into a subcell of hexagonal symmetry (Cameron et al., 1981). In addition, particularly for the spectra of LB films, this band has a shoulder around 1460 cm^{-1} . Watts et al. (1981) have reported that the lateral packing of charged DPPG gives rise to a distorted hexagonal structure in the plane perpendicular to the chain axes. Such a packing of the acyl chains could be at the origin of the 1460 cm^{-1} shoulder at high surface pressure.

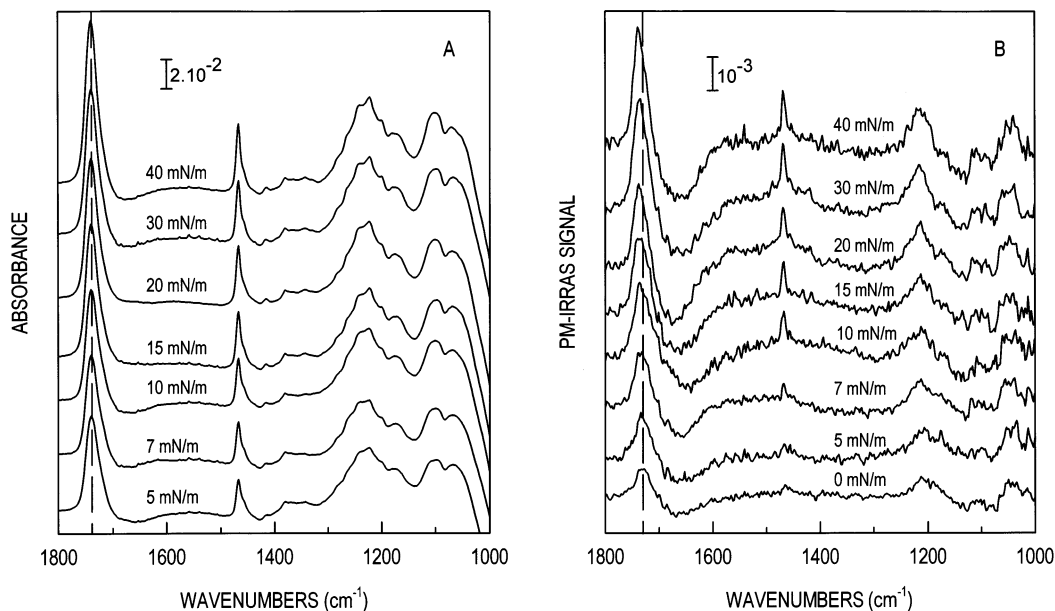


Fig. 6. (A) ATR (p polarization) and (B) PM-IRRAS spectra of DPPG in the $1800\text{--}1000 \text{ cm}^{-1}$ region at different surface pressures.

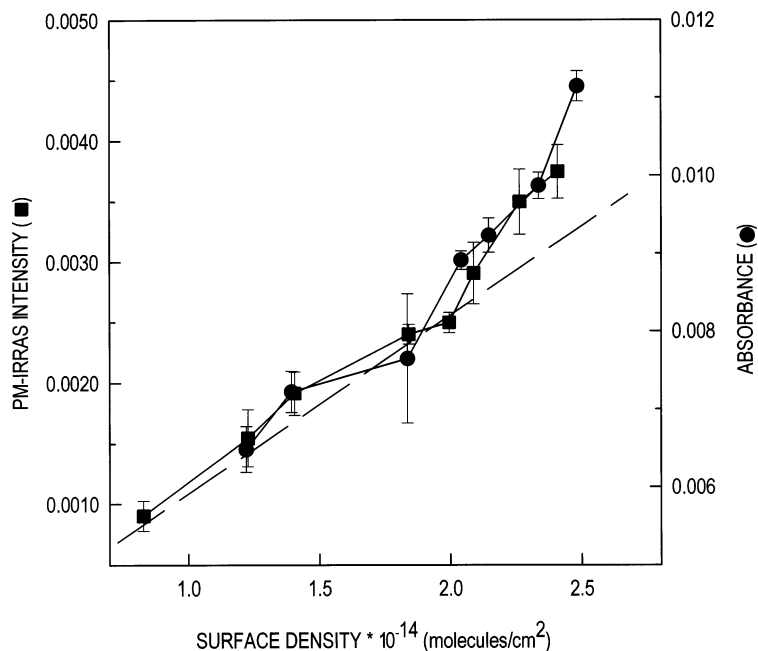


Fig. 7. Effect of the molecular surface density on the intensity of the carbonyl band in the ATR spectra of LB films (●) and in the PM-IRRAS spectra at the air–water interface (■). The data points are the mean of 3–7 values. Errors bars are the standard errors of the mean.

3.3.3. Phosphate stretching vibration

The frequency of the bands due to the phosphate group is fairly independent of the surface pressure, for both the LB films and the monolayer on the water surface (Fig. 6). The absorption maximum of the antisymmetric stretching band is around 1223 cm^{-1} for LB films. This value appears to be rather low for dry DPPG molecules. As reported above, the frequency of the carbonyl bands indicates that these groups were not hydrogen bonded to water and no band due to water was observed in the spectra. The phosphate moiety of PG molecules are thought to be involved in a strong intermolecular hydrogen bonding with the glycerol hydroxyl group of neighboring molecules ((Zhang et al., 1997) and references cited therein). These authors have also suggested that the PG hydroxyl groups may partially mimic the solvation properties of water. This behavior of PG molecules is most likely at the origin of the rather low frequency for the antisymmetric stretching vibra-

tion of the phosphate group, even for the dry lipid. In agreement with this hypothesis, the same frequency was also observed for fully hydrated phosphatidylserine (Choi et al., 1991) and dimirystoylphosphatidylcholine bilayers (Ter-Minassian-Saraga et al., 1988).

Spectra of the transferred films show CH_2 progression wagging bands even at low surface pressure, revealing that the acyl chains are not totally disordered. The wagging bands are more visible at surface pressures above 15 mN/m , in good agreement with our proposal that above this surface pressure, the acyl chains are almost in the all-*trans* conformation. Despite the fact that the bands were not well resolved at the air–water interface, the antisymmetric stretching band of the phosphate group seems to appear at lower frequency than for LB films (around 1215 cm^{-1}). This is consistent with a higher degree of hydration of this group and/or stronger hydrogen bonds with neighboring molecules on the water surface.

4. Conclusion

The present study shows that PM-IRRAS and ATR spectroscopy are powerful methods to study conformation of phospholipid molecules at the air–water interface and on solid substrates, respectively. ATR spectroscopy of a transferred single monolayers on a germanium crystal gives spectra with better signal-to noise ratio than that observed in the PM-IRRAS spectra. On the other hand, the transfer of the monolayer could lead to a reorganization of the lipid film. For DPPG, our results shows that between 7 and 25 mN/m, the acyl chains are more ordered on the germanium crystal than at the water surface. Both ATR and PM-IRRAS reveal that as DPPG is compressed, a change of the conformation and/or orientation of the acyl chains takes place at around 10–15 mN/m leading to a steeper increase of the spectral intensity with the increase of the molecular surface density. The presence of DPPG solid domains, even in the liquid-expanded phase, seems also likely. In addition, the occurrence of hydrogen bonding between the phosphate and the glycerol hydroxyl groups on both the water and germanium surfaces was inferred from the observed behavior of the DPPG polar head group.

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