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ScienceDirect
Chemistry and Physics of Lipids 144 (2006) 108–116
CPL
CHEMISTRY AND PHYSICS OF LIPIDS
www.elsevier.com/locate/chemphyslip

Effect of the antibiotic azithromycin on thermotropic behavior of DOPC or DPPC bilayers

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Received 6 April 2006; accepted 8 August 2006
Available online 15 August 2006

Abstract

Azithromycin is a macrolide antibiotic known to bind to lipids and to affect endocytosis probably by interacting with lipid membranes [Tyteca, D., Schanck, A., Dufrene, Y.F., Deleu, M., Courtoy, P.J., Tulkens, P.M., Mingeot-Leclercq, M.P., 2003. The macrolide antibiotic azithromycin interacts with lipids and affects membrane organization and fluidity: studies on Langmuir–Blodgett monolayers, liposomes and J774 macrophages. J. Membr. Biol. 192, 203–215]. In this work, we investigate the effect of azithromycin on lipid model membranes made of 1,2-dioleoyl-*sn*-glycero-3-phosphocholine (DOPC) or 1,2-dipalmitoyl-*sn*-glycero-3-phosphocholine (DPPC). Thermal transitions of both lipids in contact with azithromycin are studied by ^{31}P NMR and DSC on multilamellar vesicles. Concerning the DPPC, azithromycin induces a suppression of the pretransition whereas a phase separation between the DOPC and the antibiotic is observed. For both lipids, the enthalpy associated with the phase transition is strongly decreased with azithromycin. Such effects may be due to an increase of the available space between hydrophobic chains after insertion of azithromycin in lipids. The findings provide a molecular insight of the phase merging of DPPC gel in DOPC fluid matrix induced by azithromycin [Berquand, A., Mingeot-Leclercq, M.P., Dufrene, Y.F., 2004. Real-time imaging of drug–membrane interactions by atomic force microscopy. Biochim. Biophys. Acta 1664, 198–205] and could help to a better understanding of azithromycin–cell interaction.

Keywords: DSC; NMR; FT-IR; Azithromycin: DOPC; DPPC

1. Introduction

With the growing interest in lipidic cell membranes, there is an increase in the number of investigations on interactions between drugs and lipids, e.g. in relation to cellular drug uptake and membrane traffic. In this

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doi:10.1016/j.chemphyslip.2006.08.002

context, incorporation of an amphiphilic drug in membrane may be viewed as the "bilayer couple concept" initially developed by Sheetz and Singer (1974). The uptake of drugs into biological or model membranes increases the required area of the outer monolayer, whereas the inner monolayer tends to maintain its required area if the solute does not translocate into it. Such an area imbalance between the two monolayers is one of the key properties of biological membranes, actively regulated by transmembrane pumping (e.g. efflux pumps, flippase) or directed synthesis of, e.g. lipids. It governs deformations initiating endocytosis, exocytosis or other physiologically required shape changes of cells (Rauch and Farge, 2000) as well as changes in biophysical membrane properties induced by external stress or drugs.

Three years ago, we demonstrated that azithromycin, a macrolide dicationic antibiotic (Djokic et al., 1987; Bright et al., 1988), which is a semi-synthetic acid-stable erythromycin with an expanded spectrum of activity and improved pharmacokinetic characteristics (Dunn and Barradell, 1996), markedly inhibits fluid-phase endocytosis (Tyteca et al., 2001, 2002), probably by interacting with lipid membranes (Tyteca et al., 2003). However, the precise mechanism underlying its effect is not well established and studies for this purpose are in progress.

Using supported lipid bilayers of DOPC:DPPC that mimic biological membranes, we reported by atomic force microscopy, that azithromycin leads to the erosion and the disappearance of DPPC gel phase domains surrounded by a fluid matrix of DOPC (Berquand et al., 2004, 2005). One potential explanation might be the increase of fluidity induced by azithromycin at the hydrophilic/hydrophobic interface (Berquand et al., 2005).

From these observations and because changes in thermotropic properties of phase transition like lipid temperature transition and changes in enthalpy values are critical in several cellular and physiological processes including endocytosis (Heerklotz, 2001), we explored the thermodynamic parameters that govern molecular associations between azithromycin and lipids.

The aim of the present work is therefore to further study the effect of azithromycin on the thermotropic behavior of DOPC and DPPC using Differential Scanning Calorimetry (DSC) on multilamellar vesicles. We also used ^{31}P Nuclear Magnetic Resonance (^{31}P NMR) for probing the thermally dependent dynamics of the headgroup of these two phospholipids. The phosphorous atom with its isolated $\frac{1}{2}$ spin is subject to chemical shift anisotropy ($\Delta\sigma$) in lamellar phases and, therefore, represents a well-defined intrinsic probe of molecular motion. In parallel, the thermotropic phase behavior of DPPC in presence of azithromycin was examined by monitoring the temperature dependent changes in the symmetric CD_2 stretching band ν(C–D) of the phospholipids hydrocarbon chains by Attenuated Total Reflection Fourier Transform Infra-Red (ATR-FT-IR).

2. Experimental procedure

1,2-Dioleoyl-*sn*-glycero-3-phosphocholine (DOPC) and 1,2-dipalmitoyl-*sn*-glycero-3-phosphocholine (DPPC) were purchased from Sigma–Aldrich (Saint-Louis, MO) and used without further purification. Azithromycin (potency = 94.4%, MW = 785 g mol^{-1}) was supplied as dihydrate free base by Pfizer (Groton, CT). Azithromycin is sparingly soluble in water at pH 7.4. A stock solution was prepared by dissolving 22.5 mg of the free base in 1 ml 0.1 M HCl (28.6 mM) and further diluted in Tris buffer (10 mM, pH 7.4) for experiments. Tris (Tris-hydroxymethyl-aminomethan) was purchased from Merck (Darmstadt, Germany).

2.1. MLVs preparation

Multilamellar vesicles (MLVs) of DOPC or DPPC were prepared by dissolving the lipids in chloroform:methanol 2:1 at a concentration of 20 mg ml^{-1} for DSC experiments, and at a concentration of 30 mg ml^{-1} for ^{31}P NMR experiments. Azithromycin dissolved in acetone (1.5 mg ml^{-1}) was mixed with lipid solutions to obtain different ratios of azithromycin:lipid. The organic solvents were evaporated with rotative dessicator, the lipid–azithromycin film was dried overnight in a dessicator and then hydrated with Tris buffer (10 mM, pH 7.4). The MLVs obtained were then submitted to five cycles of freeze/thawing to increase the homogeneity of the vesicles size.

2.2. Enthalpy change of thermal transitions induced by azithromycin determined by DSC on MLVs

DSC experiments were performed on a DSC 2920 TA (TA Instrument, New Castle, DE) using sealed hermetic aluminium pans of matched mass (i.e. the empty sample and reference pans were of equal mass to within ±0.10 mg). The analyzed sample mass was generally about 10 mg. Temperature and enthalpy reading were calibrated with indium (H: 28.7 J g^{-1}, T_{onset}: 156.6 °C) and eicosane (H: 247.4 J g^{-1}, T_{onset}: 36.8 °C). The DSC cell was purged with 100 ml min^{-1} dried nitrogen. The sample was heated from 20 °C up to 70 °C and from −50 °C up to 20 °C for DPPC and DOPC, respectively

with a heating rate of 2.5 °C min^{-1} and after equilibration of the system at the minimal analysis temperature for 2 min. Initially, two heating rate scans were tested: 1.0 °C min^{-1} and 2.5 °C min^{-1}. The heating rate of 2.5 °C min^{-1} was slow enough to give a good resolution of the thermogram, and fast enough compared to the rate of 1 °C min^{-1} to increase the signal to noise ratio. After a 2 min isotherm, the sample was then cooled at the same rate. Thermal cycles were repeated three times to ensure constancy and good repeatability of the data and to erase the thermal history of the sample. Due to the supercooling phenomenon and in accordance with Videira et al. (1999), accurate thermotropic transitions were evaluated from heating curves. Consequently, only heating scans have been used throughout this work. The temperature at the peak maximum was defined as the transition temperature. Calorimetric enthalpies were calculated by integrating the peak area after baseline adjustment and normalization to the amount of sample analyzed. DSC curves were obtained by Thermal Advantage software and analyzed by using Universal Analysis 2000 software, both provided by TA Instrument. To obtain the value of the enthalpy change of transitions (ΔH) per mole of lipid, the lipid concentration was determined by the phosphorus assay (Bartlett, 1959). The azithromycin:lipid ratio varied from 0.016 to 0.71.

2.3. Interaction of azithromycin with phospholipid polar heads determined by ^{31}P NMR on MLVs

^{31}P NMR of phospholipid dispersion gives both information on the local order and the mobility of the phosphate part of the lipid headgroup and the overall organization of the aggregate structure (Smith and Ekiel, 1984). MLVs ^{31}P NMR spectra show the characteristic lamellar lineshape with a high field maximum ($\sigma_\perp$) and a low field shoulder ($\sigma_\parallel$). The effective chemical shift anisotropy ($\Delta\sigma$) can be directly measured on the spectra by taking the difference between $\sigma_\parallel$ and $\sigma_\perp$.

The $\Delta\sigma$ value depends on the phosphate group motion and, because most motional processes are thermally activated, the $\Delta\sigma$ decreases when the sample temperature increases (Montenez et al., 1996; Tyteca et al., 2003). Moreover, the gel to liquid crystal transition temperature of pure phospholipids can be detected by a significant drop of $\Delta\sigma$ (Dufourc et al., 1992). Because the low field shoulder on our spectra was not well defined, we deduced $\Delta\sigma$ according to the expression:

$$\Delta\sigma = 3(\sigma_i - \sigma_\perp)$$

where σ_i is the isotropic chemical shift of DPPC in MLVs (Seelig, 1978).

The lineshape of ^{31}P NMR spectra also reflects the mobility of the phosphate heads and the spectral second moment is a quantitative measurement of the shape of the signal related to the various motions of the headgroup.

The spectral second moments were calculated according to:

$$S_2 = \frac{\int_{-\infty}^{+\infty} \nu^2 f(\nu)\,d\nu}{\int_{-\infty}^{+\infty} f(\nu)\,d\nu}$$

where ν is the frequency with respect to ν_i, the isotropic frequency corresponding to σ_i and $f(\nu)$ is the lineshape.

DPPC and DOPC MLV suspensions were prepared in Tris buffer and in a mixture of Tris buffer and ethylene glycol 1:1 respectively. Ethylene glycol was used to obtain NMR measurements below 0 °C. Five hundred microliters of samples were heated between 35 °C and 50 °C for DPPC and from −30 °C to 0 °C for DOPC liposomes. Thermal regulation was accurate at 0.1 °C. Samples were analyzed in 5 mm NMR tubes and added to 0.1 ml D_2O for locking on the deuterium NMR signal. A molecular ratio azithromycin:lipid of 0.71 was used to study the effect of the drug.

^{31}P spectra were run on a Bruker AVANCE 500 spectrometer at 202.5 MHz with proton broadband decoupling. Typical Fourier Transform parameters were: 45° pulse (6 μs); 50 kHz spectral width; 8 K data points; 0.6 s repetition time. Five thousand scans were accumulated and a line broadening of 70 Hz was applied to the free induction decays before Fourier transformation. ^{31}P spectra were run at different temperatures with 15 min of equilibration before data acquisition at the next temperature.

2.4. Infrared spectroscopy

Attenuated Total Reflection Fourier Transform Infra-Red (ATR-FT-IR) is particularly well suited for the study of membranes (Goormaghtigh et al., 1999). Spectra were obtained on a Bruker IFS55 FT-IR spectrophotometer (Ettlingen, Germany) equipped with a MCT detector (broad band 12,000–420 cm^{-1}, liquid N_2 cooled, 24 h hold time) at a resolution of 2 cm^{-1} with an aperture of 3.5 mm and acquired in the double-sided, forward-backward mode. The spectrometer was placed on vibration-absorbing sorbothane mounts (Edmund Industrial Optics, Barrington, NJ). Two levels of zero filling of the interferogram prior to Fourier transform allowed encoding the data every 1 cm^{-1}. The spectrometer was continuously purged with dry air (Whatman 75–62, Haverhill, MA). For a better stability, the purging of the spectrometer's optic compartment (5 l min^{-1})

and of the sample compartment (10–20 l min^{-1}) were dissociated and controlled independently by flowmeters (Fisher Bioblock Scientific, Illkirch, France). The internal reflection element was a 52 mm × 20 mm × 2 mm trapezoidal germanium ATR plate (ACM, Villiers St. Frédéric, France) with an aperture angle of 45° yielding 25 internal reflections. The germanium crystals were washed in Superdecontamine (Intersciences, AS, Brussels, Belgium), a lab detergent solution at pH 13, rinsed with distilled water, washed with methanol, then with chloroform and finally placed for 2 min in a plasma cleaner PDC23G (Harrick, Ossining, NY) working under reduced air pressure. Thin films were obtained by slowly evaporating a sample containing 60 μg of DPPC on one side of the ATR plate under a stream of nitrogen. Under these conditions a well ordered multilayer stack is formed as proven by AFM (Ivanov et al., 2004) and the stack is stable under a buffer flow (Scheirlinckx et al., 2004). The germanium crystal was then placed in an ATR holder for liquid sample with an in- and out-let (Harrick, Ossining, NY). The liquid cell was placed at 45° incidence on a Specac vertical ATR setup. Temperature was controlled with temperature-regulated water flowing in a cavity of the steal cell (not in direct contact with the sample). Temperature was raised degree by degree. After 3 min for equilibration, spectra were recorded. An elevator under computer control made it possible to move the whole setup along a vertical axis (built for us by WOW Company SA, Nannine, Belgium). This allows the crystal to be separated in different lanes. Here, one such lane contained the membrane film; the other was used for the background. Peak position were determined according to a classical peak picking method as described by Isaksson et al. (2003). A curve fitting is performed with third order polynomials on 11 data points around the maximum. All the software used for data processing was written under MatLab 7.0 (Mathworks Inc., Natick, MA). A ratio between azithromycin and DPPC of 0.71 was used.

3. Results

3.1. Change in thermotropic behavior of DOPC or DPPC induced by azithromycin

Differential Scanning Calorimetry (DSC) was used to characterize the thermotropic behavior of DOPC and DPPC multilamellar dispersions at increasing azithromycin:lipid ratios (Fig. 1) and to determine the corresponding changes in enthalpy (Fig. 2). Both cooling and heating curves have been determined. The enthalpy values were within the expected experimental errors and cooling calorimetric plots corroborated heating results with cooling cycles presenting only a slight and constant decrease of the fusion temperature by about 1–2 °C due to the calorimeter finite response time. So, in the present study, we only considered the heating curves.

For DOPC MLVs, the L_c–L_α phase transition appears at -18.4 ± 0.2 °C (Fig. 1A, curve 5) with an enthalpy variation of 42 kJ mol^{-1} (Fig. 2). For an azithromycin:DOPC ratio of 0.063 (Fig. 1A, curve 3), a shoulder appears at the end of the main transition temperature that shows a weak shift towards the lower temperatures. The relative intensity of the shoulder increases as the main peak decreases for azithromycin:DOPC ratios ranging from 0.063 (Fig. 1A, curve 3) to 0.196 (Fig. 1A, curve 2). For higher azithromycin content, thermal transitions are markedly reduced (Fig. 1A, curve 1). Consequently, the enthalpy variation corresponding to the sum of the areas of the two peaks decreases from 42 kJ mol^{-1} to 25 kJ mol^{-1} with increasing quantities of azithromycin (azithromycin:DOPC from 0 to 0.455) (Fig. 2).

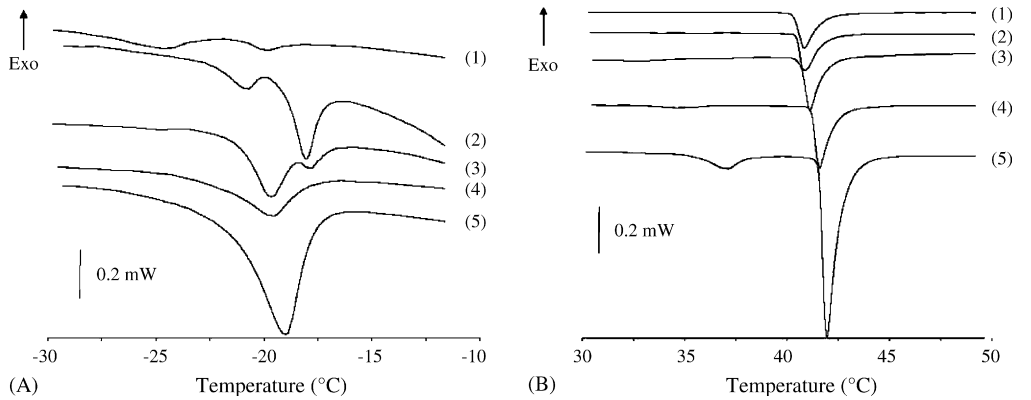

Fig. 1. Effect of azithromycin concentration on DSC thermograms of DOPC (panel A) and DPPC (panel B); A: azithromycin:DOPC ratio: 0.455 (1); 0.196 (2); 0.063 (3); 0.016 (4); pure DOPC (5); B: azithromycin:DPPC ratio: 0.435 (1); 0.263 (2); 0.034 (3); 0.024 (4); pure DPPC (5).

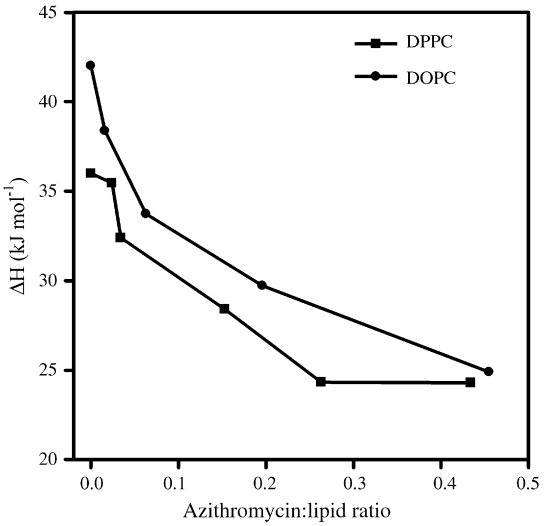

Fig. 2. Effect of azitromycin on enthalpy change ΔH (kJ mol^{-1} of lipid). Circles symbols: azithromycin + DOPC; squares symbols: azithromycin + DPPC. The mean of three independent experiments is plotted on the figure.

For DPPC MLVs, two endothermic transitions are exhibited in the 20–70 °C range (Fig. 1B, curve 5). The first peak at 37 °C (T_p) is broad and corresponds to the conversion of a lamellar gel phase to a rippled gel phase ($L_{\beta'}$–$P_{\beta'}$) pretransition. The second sharp peak, at 42 °C, reflects the conversion from a gel phase to a lamellar liquid-crystal ($P_{\beta'}$–L_α) corresponding to the main transition (T_m) (Lichtenberg et al., 1984). The associated enthalpy variations are 3.4 kJ mol^{-1} (ΔH_p) and 32.7 kJ mol^{-1} (ΔH_m) for the pretransition and the main transition respectively. For an azithromycin:lipid ratio of 0.024 (Fig. 1B, curve 4), the pretransition was still detectable, but it was not possible to determine precisely its temperature due to its large range and low intensity. By increasing the azithromycin:lipid ratio from 0.034 (Fig. 1B, curve 3) to 0.435 (Fig. 1B, curve 1) the pretransition peak vanishes and the transition temperature is slightly shifted (from 42 °C to 41 °C). The enthalpy variation corresponding to the sum of the pretransition and the main transition areas decreases from 35 kJ mol^{-1} to 25 kJ mol^{-1} when the proportion of azithromycin increases (azithromycin:lipid ratios from 0 to 0.435) and reaches a plateau around 0.25 (Fig. 2).

3.2. ^{31}P NMR analysis on DOPC and DPPC MLVs

^{31}P NMR experiments were first performed on DOPC MLVs containing or not azithromycin. Effective chemical shift anisotropy $\Delta\sigma$ were determined in presence of ethylene glycol from −30 °C to 0 °C. The standard deviation on $\Delta\sigma$ determinations is about 1 ppm indicating that the differences in $\Delta\sigma$ between control and azithromycin containing samples are not significant (Fig. 3A) as confirmed by the spectral second moments (Fig. 3B).

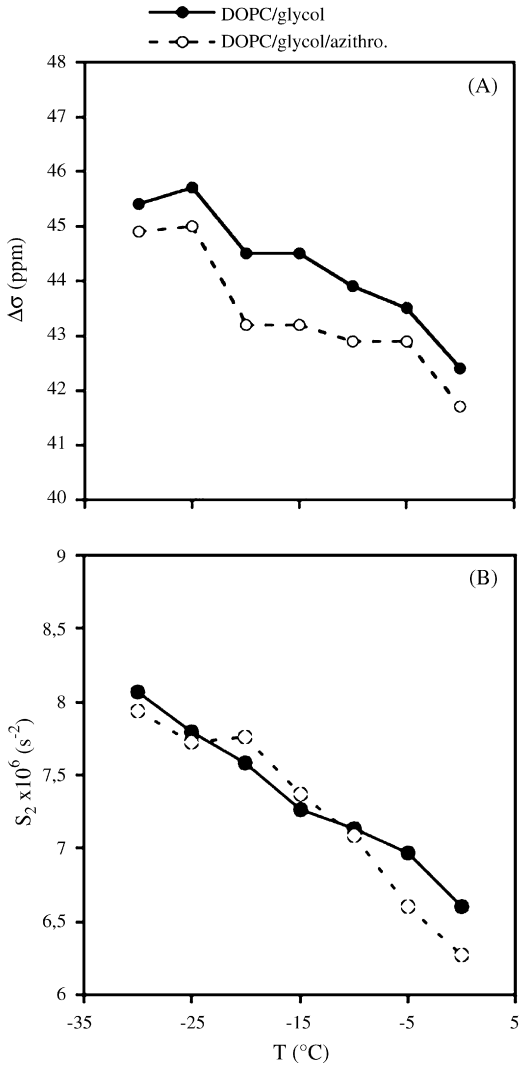

Fig. 3. Temperature-dependence of chemical shift anisotropy $\Delta\sigma$ (S.D. of 1 ppm) (panel A), and of the second moment S_2 (S.D. of 0.5×10^6 s^{-2}) (panel B) of ^{31}P NMR spectra of DOPC in presence of ethylene glycol. Closed symbols: DOPC; open symbols: azithromycin:DOPC ratio 0.71. Results are the mean of five measurements. For sake of clarity the S.D. are not shown.

Focusing on DPPC, modifications of lineshapes were observed after addition of the antibiotic, at 40 °C and higher (Fig. 4B) while no significant changes in $\sigma_\perp$ were noticed (Fig. 4A and B). The $\Delta\sigma$ variation as a function of temperature of control and azithromycin containing liposomes did not show significant differences (Fig. 5A). However, in presence of azithromycin,

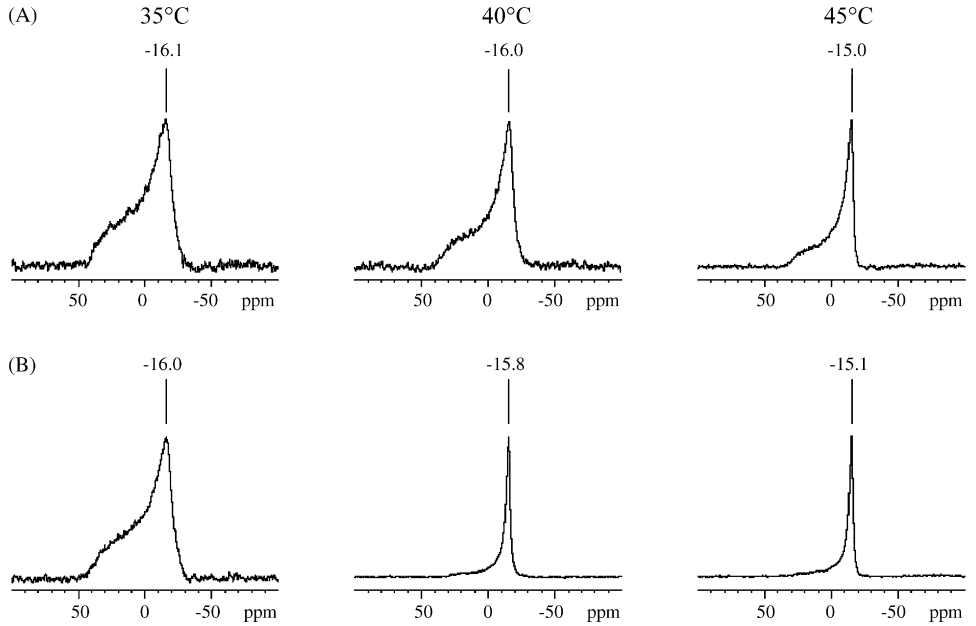

Fig. 4. ^{31}P NMR spectra of DPPC control samples (A) and of azithromycin containing samples (B) as a function of temperature (T = 35 °C, 40 °C and 45 °C). Azithromycin:DPPC ratio 0.71.

the spectral second moments are significantly decreased around transition temperature of DPPC (Fig. 5B).

3.3. Phase transition in DPPC: azithromycin monitored by infrared spectroscopy

As depicted in Fig. 6, IR spectroscopy showed that DPPC-D_{62} undergone a highly cooperative chain-melting phase transition, as indicated by an abrupt increase in the νs(C–D) frequency from values near 2089 cm^{-1} at temperature below the transition temperature to values near 2094 cm^{-1} at higher temperatures. Fig. 6 also shows that comparable temperature induced changes in νs(C–D) frequency also occurred with azithromycin. At high temperature the frequencies of the CH_2 stretching band were slightly lower than those of the pure lipid. This effect, which is probably not significant, might be due to a slight ordering of the all-*trans* hydrocarbon chains of DPPC caused by azithromycin. With DOPC, no transition could be observed in the temperature range available for these experiments.

4. Discussion

AFM experiments recently published (Berquand et al., 2004, 2005) showed a strong eroding effect of the macrolide antibiotic azithromycin on the DPPC gel phase domains in DOPC:DPPC bilayers confirming the effect of azithromycin on membrane dynamics suggested previously on cellular models (Tyteca et al., 2003). In the present study, we investigated in detail the effect of azithromycin on lipid phase transition and order of either DOPC or DPPC as a function of temperature and azithromycin concentration using different approaches including DSC, ^{31}P NMR, and FT-IR.

First, considering the azithomycin influence on DOPC vesicles, we observed in presence of an azithromycin:lipid ratio of 0.063, the unfolding of the phase transition peaks and a dose dependent decrease of enthalpy variation. This probably corresponds to a lateral phase separation into azithromycin rich domains as a result of an aggregation of the antibiotic in the membrane (peak at higher temperature), coexisting with DOPC rich domains (peak at lower temperature). Such a phenomenon has already been observed earlier (Mavromoustakos et al., 1995; Gandhi et al., 2002). These results might be explained by the insertion of the azithromycin into lipid layers, resulting in a drawing aside effect of the lipid molecules and a disorder which broadens the phase transition. This hypothesis is in good agreement with our previous results (Berquand et al., 2005) that showed a fluidity increase in regions where TMA-DPH is located suggesting that azithromycin accumulates in the upper 10 carbons of the acyl chain, i.e. in the cooperative region. The absence of significant effect of azithromycin on $\Delta\sigma$ of DOPC around the transition temperature, might be due to the presence of ethylene glycol, which has a marked effect on the $\Delta\sigma$ and

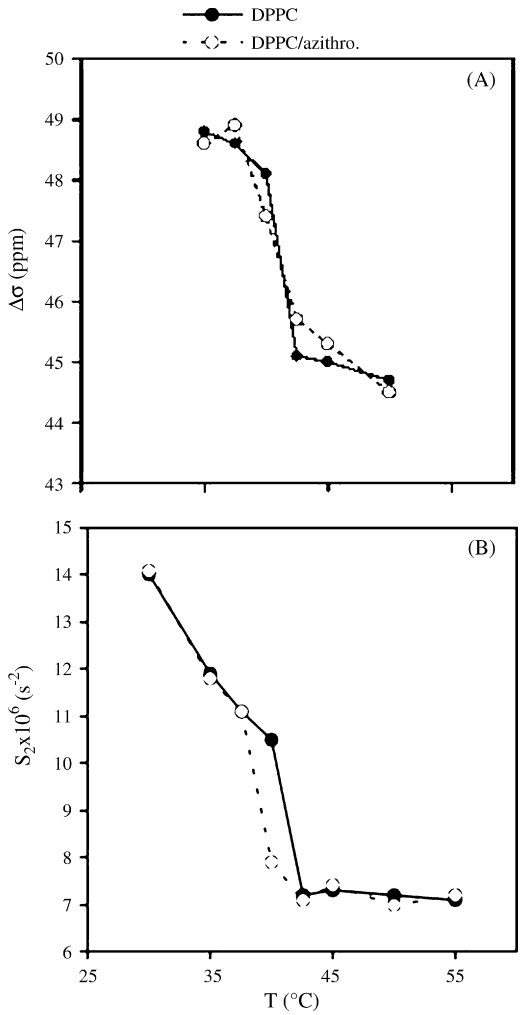

Fig. 5. Temperature-dependence of chemical shift anisotropy $\Delta\sigma$ (S.D. of 1 ppm) (panel A), and of the second moment S_2 (S.D. of 0.5×10^6 s^{-2}) (panel B) of ^{31}P NMR spectra of DPPC. Closed symbols: DPPC; open symbols: azithromycin:DPPC ratio 0.71. Results are the mean of five measurements. For sake of clarity the S.D. are not shown.

S_2 values, probably by surrounding the polar headgroups as reported in the literature (Kaneshina et al., 1998).

Second, since DOPC differs from DPPC by the length of acyl chain but also by its insaturation resulting in a rearrangment into a less compact structure, we compared the effect of azithromycin on DOPC and DPPC MLVs by DSC experiments. The enthalpy variation associated to the transition of DPPC is found in good agreement with those reported elsewhere for a pure lipid system (Janiak et al., 1976; Ruocco and Shipley, 1983; Tahir et al., 1999; Videira et al., 1999). The pretransition of DPPC, which is considered very sensitive to the incorporation of various small molecules into the bilayer (Lohner, 1991), is suppressed in presence of azithromycin. This behavior

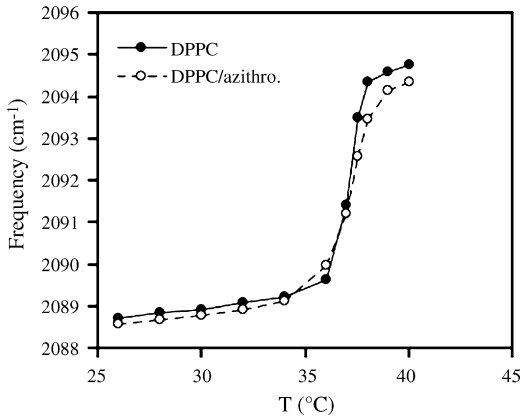

Fig. 6. Evolution of the frequency of the maximum of the $\nu_{(C-D)}$ band as a function of temperature in the presence (open symbols) and in the absence of azithromycin (closed symbols). The mean of three independent experiments is plotted on the figure. The azithromycin:DPPC ratio was 0.71.

is common with that encountered with other molecules or drugs interacting with lipids like endosulfan (Videira et al., 1999), several carotenoids (Kostecka-Gugala et al., 2003), dodecyl-2-(*N*,*N*-dimethylamino)propionate, a skin penetration enhancer (Wolka et al., 2004), fluorinated alcohols used for novel pulmonary drug delivery (Lehmler and Bummer, 2005), cannabinoid drugs (Mavromoustakos et al., 2001), melatonin (Severcan et al., 2005), progesteron (Korkmaz and Severcan, 2005) or tamoxifen (Engelke et al., 2002). Again, this fact is interpreted as a consequence of the drug insertion into the bilayer of the membrane (Mavromoustakos et al., 2001), disturbing the hydrophobic interactions between the lipid molecules. Such an effect causes simultaneous variation in the transition temperature and ΔH values. Again, our observations are in accordance with previous fluorescence polarization studies (Berquand et al., 2005). So, the insertion of the drug between the polar heads of DPPC could favor the development of a liquid crystalline phase less ordered than the gel phase and slightly decreases the gel to liquid crystal phase transition temperature as observed by DSC and by ^{31}P NMR. The absence of significant effect on the phase transition as measured by FT-IR, while a definite effect is recorded by DSC and ^{31}P NMR, can be tentatively related to the difficulty for the azithromycin to insert in planar bilayers but also to the difference in the physical properties measured by both methods, number of gauche conformers for FT-IR, cooperative transition units for DSC. In the case of a large number of small cooperative units (microdomains), DSC would measure a lower transition for interfacial molecules (at the border of the microdomains) and the usual transition for the molecules present in the bulk

of the microdomains. The DSC curve appears then as a mix between two transitions whose temperatures are relatively close. The overall effect is a decrease in the transition temperature and in the enthalpy variation. On the other hand, interfacial molecule transition might go unnoticed in FT-IR because the molecules from the interface already possess a number of gauche C–C bonds. In turn, only the bulk of the microdomain would participate to the transition curve obtained by FT-IR.

At this point, the azithromycin:lipid interactions may be viewed as: (i) a transfer from the bulk aqueous solution to the membrane surface and (ii) the incorporation of the antibiotic molecule into the hydrophobic membrane core that results in a decrease of ΔH values. The force which drives azithromycin into the lipid layer is probably an hydrophobic effect, i.e. the release of water molecules from the hydrophobic chain upon membrane incorporation, which is essentially an entropy driven process.

Moving to the significance of these results, the high amount of azithromycin used in this study and necessary to observe an effect on lipid behavior may be questioned. However, these concentrations are in the range used in other studies for the characterization of interactions between molecules like salicylic acid (Panicker and Mishra, 2006), diterpene (Matsingou et al., 2005), phenothiazine (Hendrich et al., 2002) with zwitterionic phospholipids. Moreover, presence of high amounts of drugs in specific domains is not so surprising since it is well known that partitioning drugs accumulate heterogeneously in the membrane, suggesting that high local concentrations may be achieved in specific lipid domains. In this respect, to better mimic biological membranes, it would be interesting to investigate the effect of the drug on lipids present in biological membrane domains (rafts) like sphingomyelin and cholesterol or on negatively-charged lipids like phosphatidylserine.

In conclusion, we have shown that the thermotropic behavior of lipid bilayers made of DOPC or DPPC were affected by the presence of azithromycin. Calorimetric studies showed a decrease of the ΔH associated to the transition of the DOPC and DPPC. These effects may be due to an increase of the available space between hydrophobic chains, allowing an enhancement of their mobility. Together, all the results may explain why azithromycin facilitates the phase merging of DPPC gel in DOPC fluid matrix. These findings provide a molecular insight of the interaction between a macrolide antibiotic and lipids, which could help to a better understanding of azithromycin–cell interaction. Especially, since variations in enthalpy are associated not only with membrane traffic but also with changes in membrane permeabilization (Wieprecht et al., 2000; Heerklotz, 2001) and adhesion (Simon et al., 1994; Chan and Wan, 2002), our results could be a first step to new interesting developments.

Acknowledgements

This work was supported by the Région wallonne (Grant #011/5020 to M.-P. M.-L.) and by the Belgian Fonds National de la Recherche Scientifique Médicale (grant #3.4546.02). The authors thank Dr. Blecker for the use of the calorimeter and the film balance at the Unité de Technologie des Industries Agro-alimentaires (Gembloux, Belgium). Dr. Deleu is Research Associate of the National Fund for Scientific Research (FNRS). Dr. Goormaghtigh is Research Director at the Belgian National Fund For Scientific Research.

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