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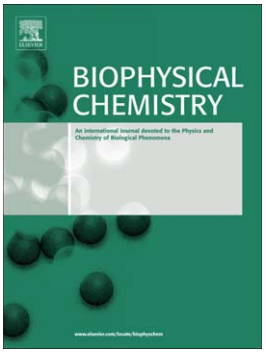

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The effect of 3-pentadecylphenol on DPPC bilayers

ATR-IR and ^{31}P NMR studies

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Abstract

The influence of 3-pentadecylphenol (PDP) on the structure and physicochemical properties of the lipid bilayers of DPPC liposomes was studied using ATR-IR and ^{31}P NMR methods. On the basis of analysis of the bands assigned to the CH_2 stretching, CH_2 scissoring, C=O stretching, and PO_2^- stretching vibrations it was revealed that PDP influences both the hydrophobic and hydrophilic parts of the DPPC liposome bilayer. Analysis of the ^{31}P NMR line-shape indicated a lamellar to non-lamellar phase transition in PDP-doped DPPC dispersions. It was shown that PDP/DPPC isotropic aggregates have similar νC=O and $\nu_{as}PO_2^-$ band positions and lower *gauche* populations in the hydrophobic chain region compared with the DPPC bilayer in the liquid-crystal phase.

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Introduction

3-pentadecylphenol (PDP) belongs to the phenolic lipids group. It is a large group of compounds of natural origin [1-5] which can interact with proteins, DNA, and biomembranes and they are antibacterial, fungicidic, and cytotoxic agents [2,6,7]. Phenolic lipids compose of an aromatic headgroup related to phenol or dihydroxybenzene connected to a hydrophobic hydrocarbon chain. The PDP compound has a phenol ring with one pentadecyl (C15) chain, see Fig. 1 A. PDP compounds are derived after the hydrogenation of unsaturated fractions of alkylphenolic oil internationally called ″cashew nut shell liquid″ (CNSL) [8]. CNSL is derived from the shell of the cashew nut (*Anacardium occidentale L.*). The CNSL oil is composed of some amount of cardanol which mainly consists of PDP with the unsaturated C15 hydrocarbon chain and small amount of saturated one [8]. The low cost of obtaining PDP from CNSL (world-wide cashew nut production is nearly 500 000 tons per year) increases the interest in this compound by industry and science. The synthesis of hydrophobic $Au_{core}Ag_{shell}$ nanoparticles in toluene proceeds by way of the interfacial reduction of silver ions by 3-pentadecylphenol followed by their deposition on hydrophobized Au nanoparticles. This leads to the formation of phase of stable and pure $Au_{core}Ag_{shell}$ nanoparticles in toluene [9]. PDP molecules are used in the formation of oriented block copolymers with anisotropic proton conduction [10]. The lamellar nanostructure present in this system is formed by PDP compounds and is responsible for characteristic organization of copolymer structures in which proton conductivity is different in three macroscopic directions. 3-Pentadecylphenol itself has various biological properties. PDP can effectively function as an anti-obesity agent by inhibiting the activity of α-glucosidase enzyme and thereby regulate animal body weight [11]. It also has quite promising antioxidant activity resulting from the presence of phenol OH group [12].

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Figure 1.

Most of the biological functions of phenolic lipids are related to modification of the physical properties of biomembranes [2]. As an amphiphilic compound, PDP can easily incorporate into the lipid bilayer, change the physicochemical properties of biomembranes, and alter the activities of different membrane proteins. The character of interactions between lipids and PDP compounds on molecular level is necessary to understand the membrane-connected biological activities of PDP molecules.

Our previous ATR-IR studies showed the influence of PDP compounds on dipalmitoylphosphatidylcholine (DPPC) multilayers in dry or partially hydrated lipid films [13]. However, the level of hydration has a crucial influence on the type of organization of the lipid bilayers and on the interactions between the lipid and doped compound [14]. The studies of liposome suspensions presented in that paper allowed us to mimic the hydration condition of natural membranes.

In this paper we focus on characterizing the effect of PDP on dipalmitoylphosphatidylcholine (DPPC) bilayers using ATR-IR (Attenuated Total Reflection Infrared Spectroscopy) and ^{31}P NMR methods. Phosphatidylcholines (PC) are the most prevalent phospholipids among those constituting the basic structure of eukaryotic cell membranes. DPPC lipid is one of the most numerous members of PC lipid group and is very often used as a model for mimic lipid-biomembrane properties. The structure of DPPC molecule is present in Fig. 1B. The ATR-IR method supplies information about the PDP-lipid interaction and characterize the influence of the new compound on the structure of the lipid bilayer under fully hydrated conditions. ^{31}P NMR provides information regarding the macroscopic architecture of the membrane phospholipids. The ^{31}P NMR spectral line-shape is diagnostic of bilayer, hexagonal H_{II}, or isotropic (micellar, cubic or rhombic) lipid

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arrangements in the fully hydrated state [15,16]. The isotropic phase in the PDP/DPPC mixtures was observed. The presence of non-lamellar structures in the PDP-doped lipid bilayer can extend the biological functions of biomembrane; for example the fusion phenomena and many other related processes are facilitated by the formation of non-bilayer lipid aggregates.

Materials and Methods

Materials

All compounds were used of the highest available purity, without further purification. The lipid dipalmitoylophosphatidylcholine (DPPC, of purity >99.8%) and 3-pentadecylphenol (PDP, of purity >95%) were obtained from Sigma-Aldrich, Germany.

Preparation of liposomes

The chloroform solution of DPPC and PDP was dried under a stream of nitrogen. Dry lipid films were hydrated by addition of 1 ml of 10 mM phosphate buffer, pH 7.2. The final concentration of DPPC was 10 mg/ml (for ATR-IR measurement) and 40 mg/ml (for ^{31}P NMR measurement). The mixtures were kept at a temperature of 10°C above the respective gel-liquid crystalline phase-transition temperature and gently shaken until optical homogeneity was obtained. The liposomal suspensions were treated 10 times in the cooling/heating process (liposomes were incubated for 10 min. at 4°C, then heated to a temperature 10 C° higher than the Tm of the doped liposomes, and incubated at this temperature, also for 10 min.). Finally, the solution was extruded through a filter of 100nm pore size (LiposoFast with polycarbonat filter, Avestin, Canada).

ATR-IR (Attenuated Total Reflection Infrared) measurements

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The ATR-IR infrared spectra were recorded on a Nicolet Avatar 360 FT-IR spectrometer. The 256 scans were collected at a resolution of 2 cm^{-1}. The liposome suspensions were prepared according to procedure described above and spread on one surface of ZnSe-ATR crystal (face angle: 45°, 6 reflections, Specac). The dry DPPC/PDP films were prepared by spreading 200 µl of chloroform solution of DPPC/PDP mixture on one surface of ZnSe-ATR crystal and evaporating the solvent under a stream of nitrogen. The concentration of DPPC in chloroform solution was 10 mg/ml. The spectra of dry film and liposome suspensions were recorded in a heating cycle from 15ºC to 80ºC. The sample temperature was equilibrated for 5 min. before acquisition of each spectrum. The data were analyzed by Grams software.

^{31}P NMR measurements

The ^{31}P NMR spectra were recorded on a Brucker 300 NMR Fourier Transform spectrometer operating at 121.49 MHz. The PDP/DPPC suspensions were prepared according to the procedure described above in a D_2O solution and were put into 5mm thin-walled NMR tubes. The relaxation delay time was 3 s. The spectra were recorded over a large spectral width (36 kHz) using broad-band proton decoupling. The data were analyzed by Grams software.

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Results and discussions

ATR-IR measurements

The alkyl chain vibration region in dry DPPC/PDP film

The ATR-IR studies of the PDP/DPPC dry films showed the interaction of the PDP compounds with the DPPC multilayer structure [13]. PDP influences both the hydrophobic and hydrophilic regions of DPPC layers. In dry DPPC film there is no phase transition in the investigated temperature range from 25 to 80ºC [17]. However, the incorporation of PDP molecules into the DPPC layers caused the appearance of a phase transition under dry conditions [13]. Analysis of the positions of the stretching and scissoring CH_2 bands, commonly used vibration indicators of conformational and alkyl chain packing changes, clearly indicates the nature of the phase transition in the PDP/DPPC mixtures as a chain-melting phase transition. For an equimolar PDP/DPPC mixture at a temperature of around 48ºC there is a characteristic shift of the stretching CH_2 bands to the higher frequency region associated with a sharp increase the amount of *gauche* conformers of the hydrocarbon chains in mixed DPPC layers (Fig. 2A). During this phase transition, conformational disordering is introduced and causes changes in lateral alkyl chain packing. The intensity, bandwidth, and position of the methylene deformation mode (δCH_2) are sensitive to the type of lateral alkyl chain packing [18-21]. In a gel phase this band is relatively sharp and intensive, with the maximum at around 1468 cm^{-1}, indicating hexagonal packing. During the melting-chain phase transition the increase in conformational disordering disturbs the hexagonal-like lipid chain packing, which is shown by an increase in bandwidth and decrease in intensity and position of the δCH_2 band. Figure 2B shows the shift of the maximum position of the δCH_2 band as a function of temperature in dry PDP/DPPC (50/50mol%) multilayer film as an example. The sharp shift of this band to the lower frequency region indicates the phase transition

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accompanied by a marked increase in *gauche* conformers and a loss of hexagonal chain packing. The temperature of the phase transition in dry PDP-doped DPPC films, derived from analysis of the $\nu_s CH_2$ and δCH_2 bands, commonly used indicators of phase transition in lipid layers, was the same.

According to numerous previous studies on the influence of hydration on phase-transition temperature in DPPC bilayers, we did not observe any phase transition in the investigated temperature range in the dry DPPC multilayers (Fig. 2).

Figure 2

The alkyl chain region in DPPC/PDP liposomes

The ATR-IR method allowed us to study the liposomal suspensions. After subtraction of the phosphate buffer's spectrum from that of the liposomal suspension, we were able to analyze some of the most important vibrational bands. The temperature dependencies of the CH_2 symmetric stretching frequency in the DPPC and PDP/DPPC (50/50mol%) liposomes are shown in Figure 3. The shift of the $\nu_s CH_2$ bands to higher frequencies arises from the increased number of hydrocarbon *gauche* conformers in the investigated liposomes induced by the increase in temperature. The line of the $\nu_s CH_2$ band positions as a function of temperature adopts for DPPC and PDP/DPPC liposomes a characteristic sigmoidal shape. The temperature of the main phase transition (Tm) of DPPC liposomes determined by this method corresponds very well with the Tm derived by *many* other techniques and was 41.9ºC. The presence of PDP molecules in the DPPC liposome wall caused an increase in Tm proportional to the increase in PDP concentration. For PDP/DPPC (50/50mol%) liposomes, the temperature of the observed phase transition rose to 50.9ºC (Fig. 3). The width of the transition region in PDP-doped DPPC liposomes is sharp and comparable to that in DPPC

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liposomes. The gel phase in both systems has a similar *trans*/*gauche* population, the positions of the CH_2 stretching bands for both the doped and undoped liposomes were approximately equal. Small differences occured only at higher temperatures, where the PDP/DPPC mixtures seem to be more rigid (Fig. 3).

Figure 3

At the phase transition temperature of the DPPC and PDP/DPPC liposomes, a sharp decrease in intensity of the methylene CH_2 deformation band (δCH_2) was observed, indicating drastic changes in lateral chain packing. In the PDP-doped liposomes, the loss of hexagonal chain packing, shown by the shift to a lower frequency region and decrease in intensity of the δCH_2 band, takes place in the observed phase transition.

The interfacial region in dry DPPC/PDP film

The interfacial region of DPPC assemblies is represented by ester group vibrations. The C=O stretching band is in the region between 1750 cm^{-1} and 1700 cm^{-1}. After spectral deconvolution or second-derivative calculations, two or more components of the νC=O mode appear. The splitting of the νC=O band is associated with the conformational nonequivalence of the C_1-C_2 bonds of the *sn*-1 (*trans*) and *sn*-2 (*gauche*) chains in DPPC molecules [22-24]. The band at 1742 cm^{-1} originates from the *sn-1* C=O group, while the corresponding band for the *sn-2* C=O group is found at 1725 cm^{-1} in the spectrum of dry DPPC layers. These two conformations of ester carbonyl groups cause differences in the extent of hydration of the ester part. In general, in hydrated samples a broad band contour is found in a lower frequency region.

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In the dry DPPC system the maximum of νC=O is centered around 1736.7 cm^{-1} and is almost stable in the 10 to 80°C temperature range (Fig. 4A). The presence of PDP molecules shifts the maximum of the νC=O band to a higher frequency. In the PDP/DPPC (50/50mol%) dry mixture this band is around 1739 cm^{-1} and shifts slightly to a higher frequency region with increasing temperature, as shown in Fig. 4A. The phase transition observed in the PDP-doped DPPC dry film (Fig. 2) is not connected with any distinct changes in the interaction and conformation state of C=O groups (Fig. 4A).

Figure 4

The interfacial region in DPPC/PDP liposomes

In buffer, the DPPC bialyer of liposomes has a fully hydrated state. Under this condition the increase in hydration of the DPPC bilayer takes place during the main phase transition and causes the characteristic shift of the νC=O band position to lower frequencies (Fig. 4B). The fluidization of the bilayer leads to more water molecules reaching the deeper part of the lipid membrane and hydration of the ester groups increases. Therefore, by monitoring the positions of the νC=O band as a function of temperature, it is possible to observe the main phase transition in DPPC liposome dispersion. The presence of PDP molecules in DPPC liposomes influences the ester group's vibrations. At a temperature lower than the phase transition the midpoint of the broad C=O stretching band is around 1738.5 cm^{-1} and is higher than in the gel state of DPPC liposomes, where the νC=O band lies near 1736 cm^{-1} (Fig. 4B). The phase transition observed in PDP/DPPC liposomes is connected with a drastic increase in the hydration of the ester groups of DPPC molecules. However, the jump in νC=O vibrations at around the temperature of the phase transition is much deeper in doped than in undoped DPPC liposomes, which suggests greater changes in the hydration of C=O

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moieties in the PDP/DPPC system (see Fig. 4B). In the higher temperature region, after the temperature of the phase transition, both the DPPC and PDP/DPPC systems have more similar νC=O band positions.

The phosphate headgroup absorption region in dry DPPC/PDP film

In the spectrum of DPPC, the phosphate moiety of the headgroup part gives rise to several characteristic vibrations in the infrared region. Asymmetric and symmetric stretching modes for the PO_2^- groups are found near 1250 cm^{-1} and 1085 cm^{-1}, respectively [25,26]. Weaker single-bond P-O stretching bands are in the 900-800 cm^{-1} region [25]. The R-O-P-O-R$^{/}$ stretching vibration is a shoulder on the $\nu_{as}PO_2^-$ band centered near 1060 cm^{-1} [26]. The band most sensitive to the state of hydration of the phospholipid bilayer is the $\nu_{as}PO_2^-$ band. In dry DPPC film it is centered at 1260 cm^{-1}, and the formation of a hydrogen bond during hydration results in a band-shift towards lower frequencies. In dry PDP/DPPC film a strong interaction by the hydrogen bond between the phenolic OH group and the PO_2^- moiety was observed. In *the* dry, equimolar PDP/DPPC mixture the $\nu_{as}PO_2^-$ band position was centered in a lower frequency region, at 1244 cm^{-1} [13]. The scheme of interaction between PDP and DPPC molecules can be proposed on the basis of theoretical calculations [27]. The structure of the PDP-DPPC complex is presented in Figure 5. The formation of PDP-DPPC complex gave 14.5 kcal/mol (PM3 method) gain of interaction energy resulting from both van der Waals forces and H-bond interactions [27]. Thus, in DPPC bilayer PDP molecules are located parallel between DPPC molecules. The phenol groups of PDP molecules locate between hydrophilic head group of DPPC molecules. The hydrocarbon chains of both molecules lays parallel to each other and form the hydrophobic region of lipid bilayer.

Figure 5

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The phosphate headgroup region in DPPC/PDP liposomes

In fully hydrated DPPC liposomes, the $\nu_{as}PO_2^-$ band reaches 1220 cm^{-1}. The $\nu_{as}PO_2^-$ frequencies are insensitive to the main phase transition in DPPC liposomes. The phase transition observed in PDP-doped DPPC liposomes is also insensitive to this band position and is centered around 1220 cm^{-1}. In the presence of water molecules, PDP is still most probably able to form a hydrogen bond with the phosphate group, but this interaction has no influence on $\nu_{as}PO_2^-$ band position. The DFT and semi-empirical calculations showed that in the presence of water molecule the H-bond between phenol and phosphate group can still exist [27].

^{31}P NMR measurements

^{31}P NMR is a useful analytical technique for the study of the polymorphic phase behavior of hydrated phospholipids in excess water. Lipid phosphorus exhibits large chemical shift anisotropy. ^{31}P NMR line-shape is sensitive to different types of motion of lipid molecules. Dynamics of phospholipid bilayer systems consist primarily of the rapid rotation of the lipid molecules about their long axes and they have a characteristic broad spectrum with a high-field peak and low-field shoulder [15,16] (Fig. 6A). In non-bilayer configurations, lateral diffusion takes place and in the micellar, cubic, or rhombic phase isotropic motion leads to a narrow, symmetric ^{31}P NMR spectrum [15,16] (Fig. 6B).

Figure 6

^{31}P NMR may be usefully applied to both model and biological membrane systems. In PDP-doped DPPC dispersions, a lamellar-nonlamellar phase transition appears. The ^{31}P NMR

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spectra obtained from aqueous (D_2O) dispersions of the equimolar PDP/DPPC mixture at different temperatures are illustrated in Fig. 7A,B. At a temperature below the temperature of the phase transition, the PDP/DPPC (50/50mol%) dispersion adopts a lamellar structure. At 25ºC the ^{31}P NMR spectrum is very broad, with a low-field shoulder and high-field peak (Fig. 7A). This line-shape is characteristic of the lamellar (anisotropic) phase of phospholipids. At a higher temperature (60ºC), above the temperature of the observed phase transition, this system adopts an isotropic phase. The ^{31}P NMR spectrum exhibits a symmetric and narrow peak centered at 0 ppm (Fig. 7B). This isotropic signal indicates the presence of regions of high curvature in which lipid diffusion can result in the complete motional averaging of the chemical shift anisotropy. Thus the isotropic phase can be represented by micellar, cubic, or rhombic structures, but the ^{31}P NMR technique does not allow us to determine which one the PDP/DPPC mixture adopts. The lamellar phase is present for DPPC liposomes both at 25 ºC and 60ºC, as was shown in Fig.7 C, D, respectively. The narrowing of the lamellar ^{31}P NMR line-shape of DPPC liposomes present at higher temperature (60ºC) derives from the increase of anisotropic motion accompanied by the rise of lipid bilayer fluidity after the main phase transition.

Figure 7

Summary

As an amphiphilic compound, 3-pentadecylphenol (PDP) easily incorporates into the DPPC bilayer. The interactions of PDP with the DPPC bilayer occur through a combination of hydrogen-bonding and hydrophobic and conformational interactions. Characteristic of PDP is that it causes the appearance of a phase transition in dry DPPC film [13]. It was a chain-

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melting phase transition accompanied by an increase in *gauche* conformers in the hydrocarbon tails and by the disorder of hexagonal chain packing. It is commonly known that the level of hydration determines the structure and physicochemical prosperities of a lipid bilayer. In order to mimic the physiological conditions of a biomembrane it is necessary to study liposomal suspensions. In PDP/DPPC liposome dispersions the phase transition was connected with changes in both the hydrophobic and hydrophilic regions of lipid aggregates. This process was accompanied by an increase in *gauche* conformers, a disturbance of hexagonal chain packing, and a rise in the hydration of the ester groups of the DPPC molecules. The sharp increase of $\nu_{as}CH_2$ and decrease of $\nu C{=}O$ and δCH_2 frequency positions were observed around the phase transition temperature.

It is quite interesting that the PDP compounds did not form hydrogen bonds with the ester part of the dry DPPC bilayer. However, in dry PDP/DPPC (50/50mol%) film the $\nu C{=}O$ band was shifted to a higher frequency region compared with that in pure DPPC layers, which suggests that changes occurred in the conformational state of these groups. The same higher-frequency shift of the $\nu C{=}O$ band was observed in the fully hydrated state of the PDP/DPPC (50/50mol%) bilayer wall of liposomes at lower temperatures, before the phase transition temperature. The state of hydration of the lipid bilayer does not influence the type of interaction between the phenolic OH group and the ester part of DPPC.

Under dry lipid film conditions, PDP compounds form hydrogen bonds with the phosphate groups of DPPC lipids, which was shown by the lower-frequency shift of the $\nu_{as}PO_2^-$ band [13] and by DFT and semi-empirical calculations [27]. In PDP/DPPC (50/50mol%) liposomes, where the PDP-concentration is very high, the $\nu_{as}PO_2^-$ vibrations are centered around 1220 cm^{-1}, which is characteristic of the fully hydrated state of phosphate groups. Most probably, the PO_2^- groups of DPPC molecules in the liposome suspension

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interact by a hydrogen bond with both water and PDP molecules. Nevertheless, the presence of doped compounds does not influence $\nu_{as}PO_2^-$ band position in a hydrated DPPC bilayer.

In DPPC liposomes at 41.9ºC, the lamellar structures go from a gel phase to a liquid-crystal phase. In PDP-doped DPPC liposomes, a lamellar-nonlamellar phase transition takes place. The ^{31}P NMR experiment showed the presence of an isotropic phase at a temperature above that of the phase transition of PDP/DPPC (50/50mol%) liposomes. The data at 60ºC for PDP/DPPC (50/50 mol%) liposomes showed a sharp and pure isotropic peak centered at 0 ppm. Several lipid-assembly architectures will produce such a spectrum including micellar, cubic, and orthorhombic phases. Many long-hydrocarbon-chain phenolic lipids can induce in higher concentrations non-layered structures of the micellar or H_{II} type in lipid membranes [28]. This is responsible for the increase in permeability of a biomembrane in the presence of phenolic lipids and it changes the activities of membrane proteins [2]. PDP molecules create an isotropic phase represented by regions of high curvature. Such structures can be involved in membrane fusion phenomena (including related processes such as exo- and endocytosis) [2,15] and transbilayer transport processes (including lipid flip-flop and facilitation of transport). The advantage of PDP over other membrane fusion compounds is the low cost of obtaining it from the commonly accessible shell of the cashew nut. Incorporation of PDP into the lipid membrane of liposomes can facilitate the fusion of liposomes with cell membrane and enable the release of drug molecules from the interior of liposomes to target cells.

The phase transition of lamellar structures to isotropic ones in PDP/DPPC (50/50mol%) dispersion is accompanied by a larger shift of the νC=O band position than in DPPC liposomes. The isotropic phase is characterized by a lower *gauche* conformer population in the hydrocarbon chains, which makes the isotropic structure more rigid than the DPPC bilayer. Isotropic PDP/DPPC aggregates reveal a character of vibrations of the phosphate and carbonyl groups closely related to that of a DPPC bilayer, which suggests a

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similar conformational and hydration state of the hydrophilic region in the pure and doped DPPC structures.

At lower temperatures, before the phase transition, a bilayer structure of PDP/DPPC mixtures was observed. The PDP/DPPC bilayer had a conformational state of the hydrophobic part similar to that of DPPC. The position of the CH_2 stretching vibrations was the same for both undoped DPPC and doped DPPC liposomes. More significant changes were observed in the hydrophilic part and they were connected with different conformational states of the carbonyl groups in the DPPC molecules.

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Figure legends:

Figure 1. Schematic representation of the structure of 3-pentadecylphenol (PDP) (left side) and dipalmitoylphosphatidylcholine (DPPC) molecule (right side). The PDP and DPPC are amphiphilic compounds with hydrophilic head-group and hydrophobic hydrocarbon-chain region.

Figure 2. (A) The maxima of the $\nu_s CH_2$ band at different temperatures for the PDP/DPPC (50/50mol%) (■) and DPPC (○) dry multilayer film. (B) Frequency of methylene deformation modes (δCH_2) of the aliphatic tails of PDP/DPPC (50/50mol%) (■) and DPPC (○) dry multilayer film as a function of temperature.

Figure 3. Wavenumber displacements of the bands due to the symmetric CH_2 stretching vibration compared for DPPC liposomes (○) and PDP/DPPC (50/50mol%) (■) depending on temperature.

Figure 4. Temperature dependence of the position of the peak maximum of the C=O stretching band contour in the infrared spectra of dry DPPC (○) and PDP/DPPC (50/50 mol%) (■) film (A) and of buffer dispersion of DPPC (○) and PDP/DPPC (50/50mol%) (■) liposomes (B).

Figure 5. The model structure of dipalmitoylphosphatidylcholine (DPPC) and 3-pentadecylphenol (PDP) complex derived from PM3 calculation. There is H-bond between P=O and phenolic OH group with 2,734 Å of O(H)···O distance. C-blue, H-grey, O-red, P-yellow, N-dark blue [27].

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Figure 6. Schematic representation of ^{31}P NMR line-shape for different lipid aggregates.

Figure 7. Proton-decoupled ^{31}P NMR spectra obtained from aqueous (D_2O) dispersions of PDP/DPPC (50/50mol%) at a temperature before (25ºC) (A) and after (60ºC) (B) the phase transition and for DPPC liposomes at 25ºC (C) and at 60ºC (D).

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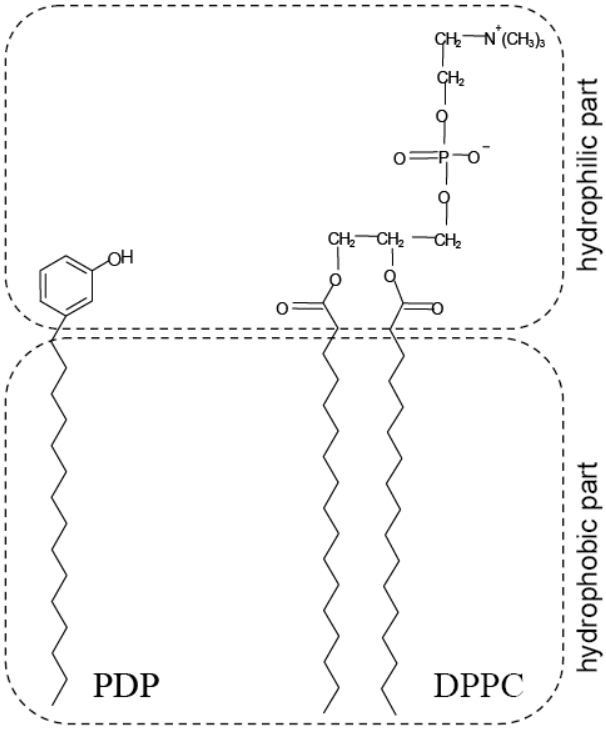

Fig. 1

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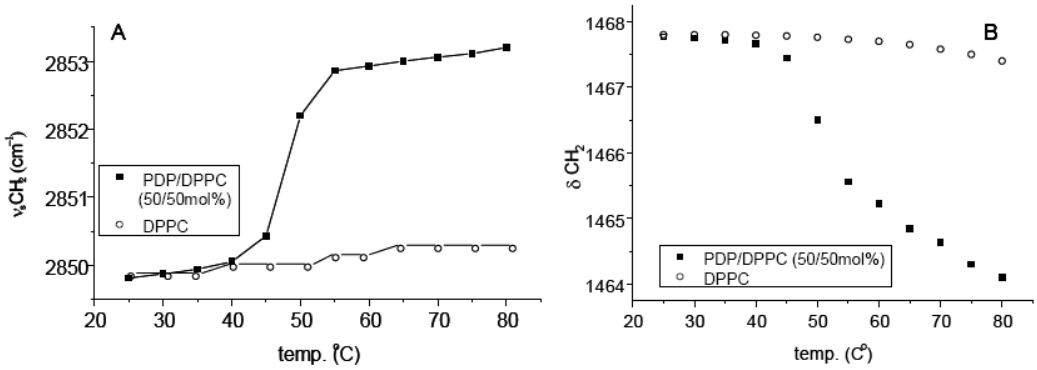

Fig. 2

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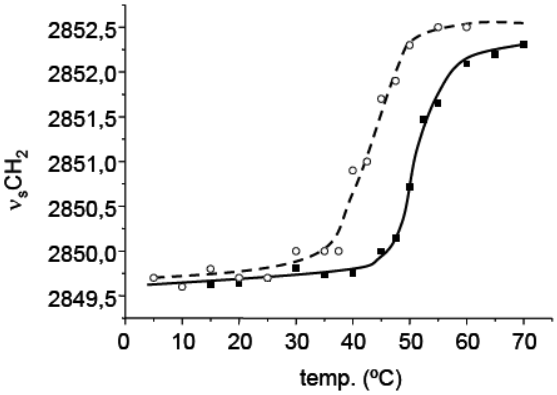

Fig. 3

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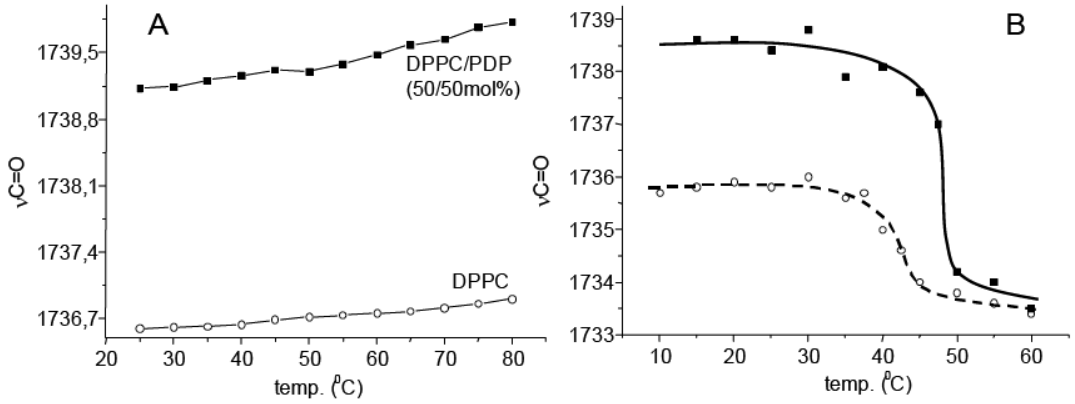

Fig. 4

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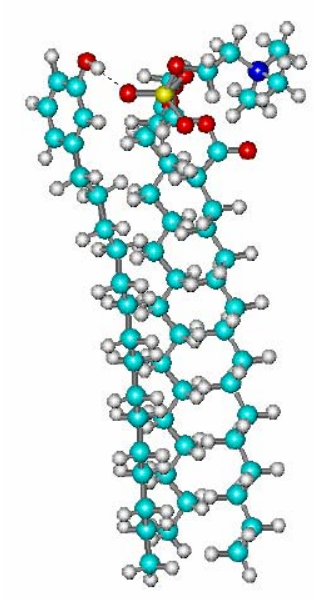

Fig. 5

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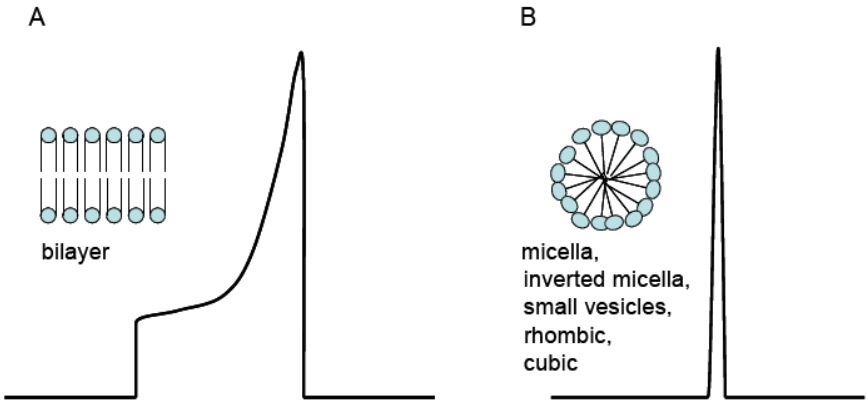

Fig. 6

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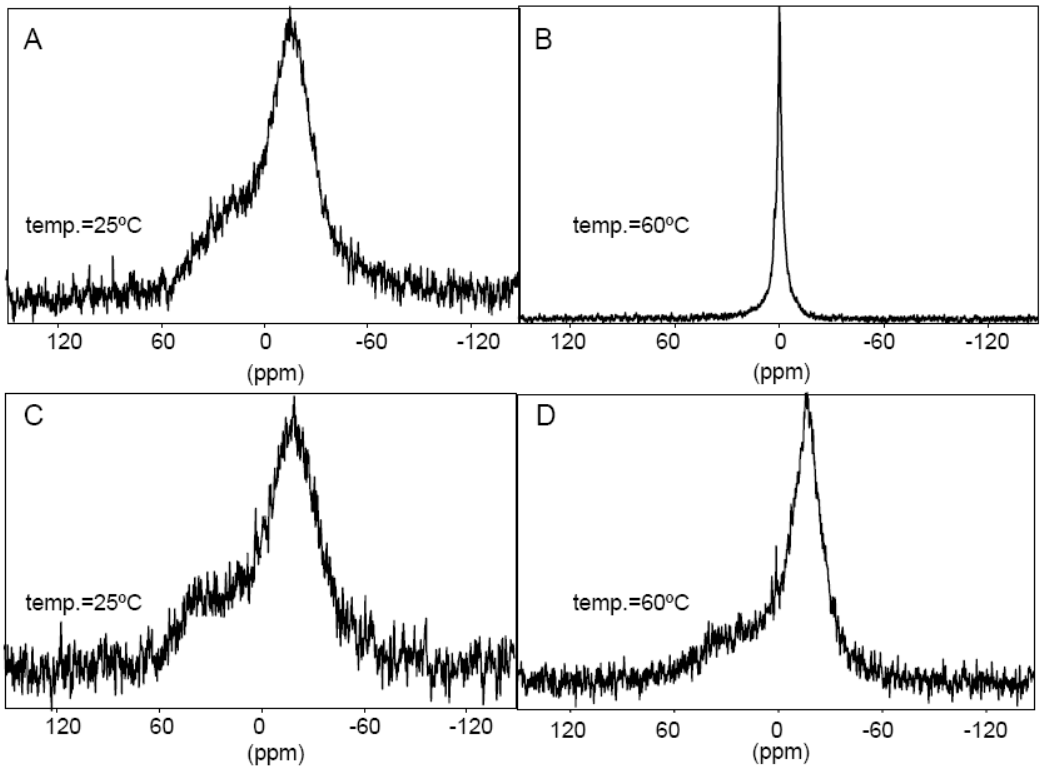

Fig. 7