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# THE SYNTHESIS AND USE OF THIONPHOSPHOLIPIDS IN $^{31}$P-NMR STUDIES OF LIPID POLYMORPHISM

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**(1) Dipalmitoyl- and dioleoylthionphosphatidylcholine, which are phosphatidylcholine analogues in which the double bonded oxygen of the phosphate group is replaced by a sulfur atom, have been synthesized in 50–60% yields by condensation of diacylglycerol with phosphorus thionchloride in the presence of choline toluene-sulfonate. Dioleoylthionphosphatidylethanolamine has been prepared by the phospholipase D-catalyzed base exchange reaction. (2) Freeze-fracturing of aqueous dispersions of the thionphospholipids reveals that the thionphosphatidylcholines are organized in extended bilayers whereas dioleoylthionphosphatidylethanolamine above 0°C forms the hexagonal $H_{II}$ phase similar to dioleoylphosphatidylethanolamine. The gel → liquid crystalline phase transition of the dipalmitoylthionphosphatidylcholine occurs at 44°C which is only slightly higher than the transition temperature of dipalmitoylphosphatidylcholine which together with other data demonstrates that the thionphospholipids closely resemble the natural phospholipids in physicochemical behaviour. (3) Proton decoupled $^{31}$P-NMR spectra of aqueous dispersions of thionphosphatidylcholines have the characteristic asymmetrical line-shape with a low-field shoulder and a high-field peak typical of phospholipids organized in extended bilayers in which the phosphate group can undergo fast axial rotation. The $^{31}$P-NMR spectrum of the thionphosphatidylethanolamine in the hexagonal $H_{II}$ phase has a line-shape with a reversed asymmetry and an effective chemical shift anisotropy half of that of thionphospholipids organized in bilayers which is caused by fast lateral diffusion of the lipids around the cylinders of the hexagonal $H_{II}$ phase as has been observed for the corresponding phosphatidylethanolamines. (4) Since the $^{31}$P-NMR resonance of the thionphospholipids is completely separated from that of natural phospholipids, these lipids can be used to study by $^{31}$P-NMR the motional and structural properties of individual lipids in mixed systems. This is demonstrated for various lipid mixtures in which non-bilayer lipid structures have been induced by variations in composition, temperature and presence of divalent cations. It is shown that bilayer → non-bilayer transitions can be modulated by gel → liquid crystalline phase transitions and that typical bilayer forming lipids can be incorporated into non-bilayer structures such as the hexagonal $H_{II}$ phase.**

## Introduction

In the last years it has become increasingly clear that the fluid mosaic model of biological membranes [1] cannot explain some basic properties of membrane lipids. In particular, this model does not account for the abundant occurrence of various non-bilayer lipids in membranes which suggests that these lipids and the structures they can form are actively involved in several membrane processes [2]. This has led to the proposal of an extension of this model of biological mem-

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branes in which these structural and functional abilities of non-bilayer lipids have been included [2,3].

In particular, it has been the use of the $^{31}$P-NMR technique in conjunction with freeze-fracture electron microscopy which has revealed many aspects of lipid polymorphism. The line-shape of the $^{31}$P-NMR spectra of lipids organized in extended bilayers, or cylindrical structures such as those found in the hexagonal $H_{II}$ phase, or in structures in which the lipids can undergo rapid isotropic motion are distinctively different allowing quantitative determinations of these structures in model and biological membranes [2].

One disadvantage of the $^{31}$P-NMR technique is that the chemical shift differences of $^{31}$P resonances of different phospholipids are small when compared to the width of the spectrum of the lipids present in large aggregates. Therefore, it is impossible to resolve the $^{31}$P-NMR spectrum and thus discriminate between the structure of individual lipids in mixed systems. One way to overcome this problem is to modify chemically the polar head group such that the $^{31}$P resonance of the modified molecule is well-separated from that of the natural phospholipids. Obviously, such a chemical modification should not lead to a gross change in the physico-chemical properties of the lipid.

In this paper we report the synthesis and characterization of various thionphospholipids which are phospholipid analogues in which the double bonded oxygen atom of the phosphate group is replaced by a sulfur atom. It will be shown that the $^{31}$P resonance of these lipids is well-separated from that of conventional phospholipids which they closely resemble in structure. The usefulness of these thionphospholipids in detecting the structural organization of individual lipids in mixed model membranes will be illustrated in various systems in which non-bilayer structures are induced by variations in composition, temperature and divalent cations.

## Materials and Methods

### *Lipids*

1,2-Dipalmitoyl-*rac*-glycero-3-thionphosphocholine (16 : 0/16 : 0-thionphosphatidylcholine) and 1,2-dioleoyl-*rac*-glycero-3-thionphosphocholine (18 : $1_c$/18 : $1_c$-thionphosphatidylcholine) were synthesized by condensation of the diacylglycerol with phosphorus thionchloride and choline toluenesulfonate (Fig. 1). This latter compound was prepared by mixing 1 mole of choline chloride (Merck, Darmstadt) with 1 mole of toluenesulfonic acid (Merck, Darmstadt). Water and hydrochloric acid were removed by repeated evaporation with toluene. The choline toluenesulfonate was crystallized from acetone and dried in vacuum over $P_2O_5$. The final yield was 95%. For an alternative method of synthesis see Ref. 4. 1,2-Dipalmitoyl-*rac*-glycerol and 1,2-dioleoyl-*rac*-glycerol were synthesized using triphenylsilyl as a protective group as described before [5]. To 1.808 ml (17.4 mmol) $PSCl_3$ (Fluka, Buchs) in a 500 ml roundbottom flask equipped wit magnetic stirrer, gas drying tube and reflux condensor we added at room temperature dropwise under $N_2$ within 5 min, 8 g (14.1 mmol) of 1,2-dipalmitoyl-*rac*-glycerol dissolved in 100 ml ethanol-free chloroform containing 2.48 ml dry triethylamine. The temperature was then raised to 45°C for 30 min. After cooling, 10 ml of dry pyridine and 6.68 g (23.7 mmol) of choline toluenesulfonate were added whereafter stirring was continued for 18 h. Due to the low reactivity of $PSCl_3$ with diacylglycerol as compared to $PSCl_3$ with choline toluenesulfonate the 30 min at 45°C incubation of the diacylglycerol and $PSCl_3$ was essential to obtain satisfactory yields.

To stop the reaction 3 ml of water was then added and the mixture was stirred for another 30 min. Lipids were extracted from the reaction mixture with 300 ml chloroform. The chloroform phase was washed subsequently with 3% (w/v) $Na_2CO_3$ in water, 5% HCl in water and water. After drying over anhydrous $Na_2SO_4$, and removal of the chloroform, 11.0 g of crude product was obtained. The 16 : 0/16 : 0-thionphosphatidylcholine was obtained in a pure form by quantitative HPLC on silica gel. Final yield of the pure compound was 5.7 g (60% of theoretical yield). The lipid ran in TLC on silica gel, using as a solvent chloroform/methanol/water/ammonia (68 : 28 : 2 : 2, v/v), as a single spot with an $R_F$ value of 0.63 as compared to 0.5 for 16:0/16:0-phosphatidylcholine. $^1$H-NMR on a $C^2HCl_3$ solution of the lipid resolved the following peaks: 0.9 ppm (tri-

plet, $-CH_3$, fatty acids), 1.3 ppm (singlet, $-CH_2$, chain protons), 2.3 ppm (triplet, $-CH_2-\overset{\overset{O}{\|}}{C}-$), 3.4 ppm (singlet, $N^+(CH_3)_3$), 4.2 ppm (multiplet, $CH_2-O$ and $CH_2-N$), and 5.2 ppm (multiplet, $CH-O$). The chemical shift of the $^{31}P$ resonance in $C^2HCl_3$ was $-56.4$ ppm from the resonance of $H_3PO_4$.

18 : $1_c$/18 : $1_c$ -Thionphosphatidylcholine was synthesized similarly in a 50% final yield. The $R_F$ of the lipid on TLC in the basic system was 0.63. The chemical shift of the $^{31}P$ resonance of the lipid dissolved in $C^2HCl_3$ was $-56.4$ ppm from $H_3PO_4$, whereas in the $^1H$-NMR spectrum of the lipid in this solvent the additional triplet at 5.8 ppm of the olefinic protons was observed.

1,2-Dioleoyl-*rac*-glycero-3-thionphosphatidylethanolamine (18 : $1_c$/18 : $1_c$ -thionphosphatidylethanolamine) was prepared in a 40% yield from the corresponding thionphosphatidylcholine by the base exchange reaction [6] catalyzed by purified [7] phospholipase D from Savoy cabbage. The $R_F$ on silica gel in the basic system was 0.78 as compared to 0.60 for 18 : $1_c$/18 : $1_c$-phosphatidylethanolamine. The chemical shift of the $^{31}P$-NMR resonance of this lipid dissolved in $C^2HCl_3$ was $-58.0$ ppm from $H_3PO_4$.

1,2-Dipalmitoyl-*sn*-glycero-3-phosphocholine (16:0/16:0-phosphatidylcholine) and 1,2-dioleoyl-*sn*-glycero-3-phosphoethanolamine (18 : $1_c$/18 : $1_c$ -phosphatidylethanolamine) were prepared as described before [6,9]. Cholesterol was obtained from Fluka (Buchs, Switzerland). The sodium salt of cardiolipin isolated from bovine heart was prepared as described before [9].

*Techniques*

Samples were prepared by dispersing above the transition temperature 50–150 μmol of lipid dried from chloroform in 1.0 ml of 10% $^2H_2O$ containing 100 mM NaCl, 10 mM Tris-HCl, pH 7.0 buffer as described before [6]. In some cases $Ca^{2+}$ was added in aliquots from a 100 mM solution. Sometimes glycerol (10%, v/v) was added to the samples to prevent freezing and to prevent freeze-damage during freeze-fracturing which was carried out as described in Ref. 10. Differential scanning calorimetry (DCS) measurements were performed on a Perkin-Elmer DSC 2 equipped with intracooler II using 17 μl sample pans and a 5°C/min scan rate [11]. High power (18 W input) proton noise-decoupled $^{31}P$-NMR spectra of aqueous lipid dispersions were obtained at 36.4 MHz as described before [6]. Proton noise-decoupled (input power 20 W) $^{31}P$-NMR spectra of dry phospholipid powders (stored overnight over $P_2O_5$ under high vacuum) were obtained at 81.0 MHz on a Bruker WP-200 using a 50 kHz sweepwidth and a 10 μs delay time. In all cases signal to noise ratios were improved by multiplying the free induction decay with an exponential function resulting in a 50 Hz line broadening. The 0 ppm position in the spectrum corresponds to the resonance position of $H_3PO_4$. $^{31}P$-Spin-lattice relaxation times of aqueous dispersions of 16 : 0/16 : 0-thionphosphatidylcholine and 16:0/16:0-phosphatidylcholine were measured at 45°C with the inversion recovery method and were found to be 0.3 and 0.2 s, respectively.

## Results and Discussion

*Preparation and characterization of thionphospholipids*

Thionphosphatidylcholines have been synthesized as described in the experimental section in yield of 50–60% by condensation of the diacylglycerol, with phosphorus thionchloride and choline toluenesulfonate according to the scheme presented in Fig. 1. Synthesis of these compounds has been reported before using a 4-step procedure involving isolation of the intermediate products [12]. The present method is appreciably faster and has a higher yield predominantly because no intermediate products need to be isolated.

Aqueous dispersions of the thionphosphatidylcholines formed extended multilayered liposomes as revealed by freeze-fracturing (data not shown). The gel → liquid crystalline phase transition of 16:0/10:0-thionphosphatidylcholine was found by DSC from heating scans to be 44°C as compared to 41°C for 16:0/16:0-phosphatidylcholine. No pretransition could be detected in the thermogram of the thionphosphatidylcholine liposomes. 18 : $1_c$/18 : $1_c$ -Thionphosphatidylethanolamine was organized in the hexagonal $H_{II}$ phase as shown by freeze-fracturing after quenching the sample from 20°C

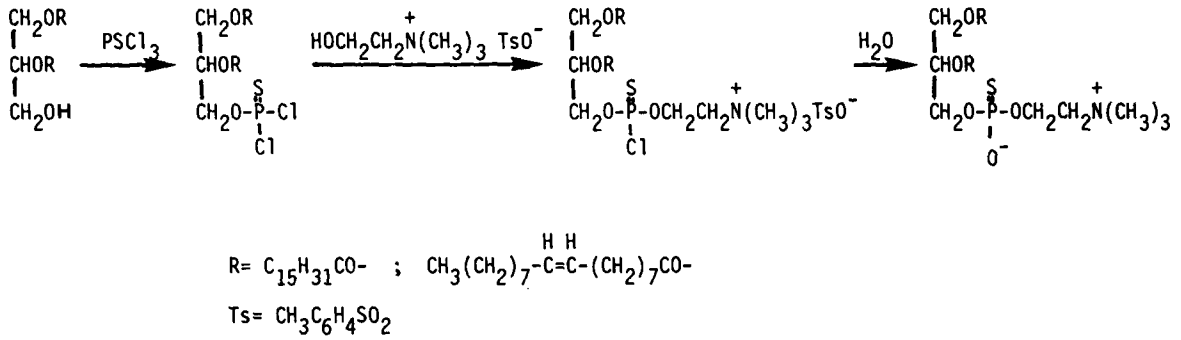


Fig. 1. Scheme of thionphosphatidylcholine synthesis.

(Fig. 2). This $H_{II}$ phase formation is typical of unsaturated phosphatidylethanolamines [2]. In Fig. 3A and B, the $^{31}$P-NMR spectra of dry powders of 16 : 0/16 : 0-thionphosphatidylcholine and 16 : 0/16 : 0-phosphatidylcholine are compared.

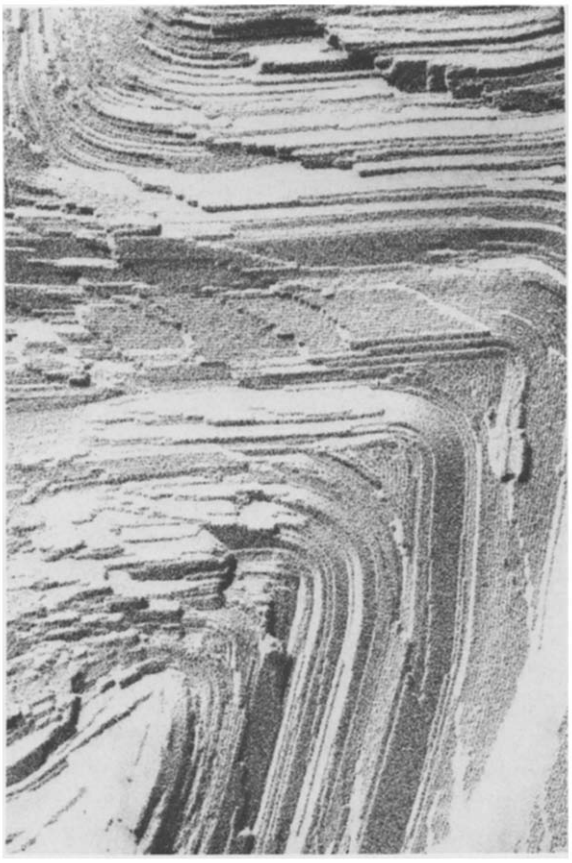

Fig. 2. Freeze-fracture electron microscopy of an aqueous 18 : $1_c$/18 : $1_c$-thionphosphatidylethanolamine dispersion. Quench temperature 20°C. Final magnification 95000×.

Two differences are immediately apparent. Firstly, the spectrum of the thionphospholipids is ±50 ppm shifted to lower field and secondly, the values of the $^{31}$P chemical shielding tensors are different for the two lipids. For the thionlipid they can be estimated to be: $\sigma_{11} = -123$ ppm, $\sigma_{22} = -96$ ppm, and $\sigma_{33} = +54$ ppm as compared to $\sigma_{11} = -81$, $\sigma_{22} = -21$ and $\sigma_{33} = +108$ for the natural phospholipid. These differences reflect the different electronic configurations of the two types of phosphate group. Hydrating these lipids at 45°C with excess water leads to the formation of bilayer structures having $^{31}$P-NMR spectra of a characteristic asymmetrical lineshape with a high-field peak

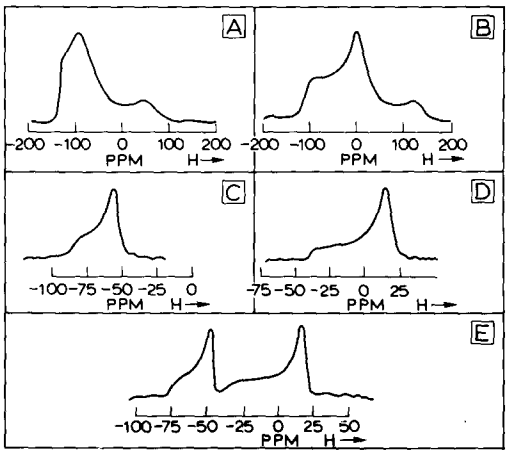


Fig. 3. Comparison of the $^{31}$P-NMR characteristics of 16 : 0/16 : 0-thionphosphatidylcholine (A, C) and 16 : 0/16 : 0-phosphatidylcholine (B, D) model systems. (A and B) 81.0 MHz $^{31}$P-NMR spectra at 25°C of the dry powders of these lipids. (C and D) 36.4 MHz $^{31}$P-NMR spectra at 45°C of aqueous lipid dispersions. (E) 36.4 MHz $^{31}$P-NMR spectrum at 45°C of an equimolar mixture of these lipids in buffer.

and a low-field shoulder (Fig. 3C, D). The effective chemical shift anisotropy $\Delta\sigma$ is 30 and 45 ppm for the two lipids, respectively. This latter value is in good agreement with previously [13] reported values of $\Delta\sigma$ of 16:0/16:0-phosphatidylcholine.

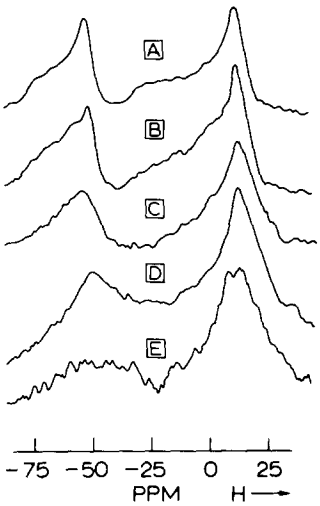


Fig. 4. Temperature dependence of the 36.4 MHz $^{31}$P-NMR spectra of an aqueous equimolar dispersion of 16:0/16:0-thionphosphatidylcholine and 16:0/16:0-phosphatidylcholine. A, 45°C, B, 40°C; C, 38°C; D, 35°C and E, 25°C.

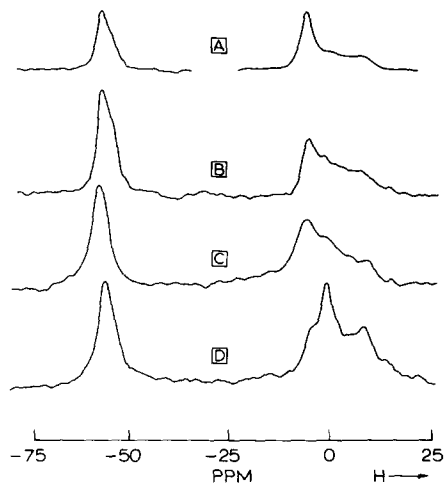


Fig. 5. 36.4 MHz $^{31}$P-NMR spectra of aqueous dispersions of 18:$1_c$/18:$1_c$-thionphosphatidylethanolamine (A, left) and 18:$1_c$/18:$1_c$-phosphatidylethanolamine (A, right) at 25°C and of an aqueous dispersion of an equimolar mixture of these lipids at 10°C (B), 0°C (C) and −10°C (D). The last spectrum was obtained from a sample which contained 30% (v/v) glycerol.

These lineshapes are typical for lipids in which the chemical shift anisotropy of the $^{31}$P is only partially averaged predominantly by rapid axial rotation of the phosphate group [13]. Using the approach of Seelig [13] to calculate $\Delta\sigma$ from the values of the chemical shift anisotropy tensors it can be estimated that the relatively small value of $\Delta\sigma$ for the thionlipids is predominantly caused by the differences in values of the tensors and not by a decreased order of the thionphosphate group.

The $^{31}$P-NMR resonance of the thion-lipid is shifted approx. 55 ppm to lower field as compared to the natural lipid. This chemical shift difference is sufficient to completely separate the signals in an equimolar mixture of the two lipids (Fig. 3E). Phospholipids in the gel state have an $^{31}$P-NMR spectrum with a greatly increased linewidth [13,14] due to an increase in dipolar coupling [13,14], which under our conditions is only partially removed by the decoupler. This is shown in Fig. 4 for an equimolar mixture of 16:0/16:0-thionphosphatidylcholine and 16:0/16:0-phosphatidylcholine. Upon cooling below 45°C a large increase in linewidth is observed. The broading of the resonance of thionphosphatidylcholine starts at a slightly higher temperature which is in agreement with the somewhat higher gel → liquid crystalline transition temperature of that lipid. Also for the 18:$1_c$/18:$1_c$-thionphosphatidylcholine typical 'bilayer' type lineshape proton decoupled $^{31}$P-NMR spectra were observed with a $\Delta\sigma$ at 25°C of 30 ppm.

Fig. 5 shows the $^{31}$P-NMR spectra of aqueous dispersions of 18:$1_c$/18:$1_c$-thionphosphatidylethanolamine and 18:$1_c$/18:$1_c$-phosphatidylethanolamine in a pure form and in an equimolar mixture. At 25°C where both lipids are in the hexagonal $H_{II}$ phase, the spectrum has a reduced width and a reversed asymmetry typical of lipids organized in cylindrical structures in which the lipids can undergo rapid diffusion around the cylinders [6,13]. The thionphosphatidylethanolamine has again a smaller $\Delta\sigma$ than the natural phosphatidylethanolamine which makes the asymmetry of the lineshape less pronounced. Upon cooling below 0°C the $^{31}$P-NMR spectrum of the 18:$1_c$/18:$1_c$-phosphatidylethanolamine changes partially to that typical of lipids organized in a bilayer which is in agreement with previous data

[2]. This effect is less pronounced in the thionlipid. The $^{31}$P-NMR spectrum at this low temperature indicates more isotropic motion of the lipid.

*Thionlipids and lipid polymorphism*

Since the lineshape of the $^{31}$P-NMR resonance of the thionphospholipids is like that of natural phospholipids, e.g., dependent on the macroscopic structure adopted by these lipids, these phospholipid analogues can be used to obtain structural information on the phase behaviour of individual lipid species in mixed systems. Therefore, we have used these lipids to answer several questions on the polymorphism of lipid mixtures. In the first place we were interested to see whether the reported bilayer stabilization of phosphatidylethanolamine by phosphatidylcholine [15,16] is dependent upon the gel → liquid crystalline transition of the phosphatidylcholine, to detect possible structural phase separations in such a mixture and to see whether the isotropic 'phase' associated with inverted micellar lipidic particles [17,18] occurring in mixed phosphatidylethanolamine/phosphatidylcholine systems contains both lipids.

Fig. 6 shows the $^{31}$P-NMR spectra of an aqueous dispersion of 16 : 0/16 : 0-thionphosphatidylcholine and 18 : $1_c$/18 : $1_c$-phosphatidylethanolamine at different temperatures. At 35°C (Fig. 6C), the spectrum of the thionphosphatidylcholine demonstrates that this molecule is in a liquid crystalline lamellar phase (compare Fig. 4) which stabilizes the bilayer phase of the phosphatidylethanolamine, which at this temperature prefers the hexagonal $H_{II}$ phase (compare Fig. 5) in agreement with previous observations [15,16]. Upon cooling, the hydrocarbon chains of the thionlipid crystallize and the thionlipid adopts the lamellar gel phase (Fig. 6D, see also Figs. 4 and 5). Clearly the bilayer stabilization is now absent as the phosphatidylethanolamine is organized in the hexagonal $H_{II}$ phase. Thus, the decrease in temperature led to a structural separation of those lipids. Heating the mixture above 35°C leads to the gradual occurrence of an isotropic signal for both lipids (Fig. 6A, B), demonstrating the formation of structures in which both lipids can undergo rapid isotropic motion. This temperature dependent behaviour was fully reversible. Although the exact nature of this isotropic 'structure' is unknown, several studies (see Ref. 19) have indicated that this structure is related to the occurrence of a new non-bilayer structure, the inverted micellar lipidic particle. These data could therefore be taken to suggest that typical bilayer forming lipids can participate in such a non-bilayer structure, in agreement with previous suggestions [15,17,20]. A

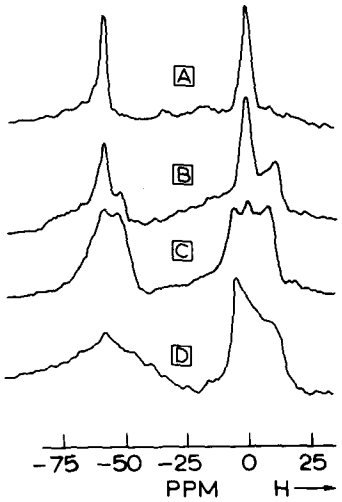


Fig. 6. 36.4 MHz $^{31}$P-NMR spectra of an aqueous dispersion of an equimolar mixture of 16 : 0/16 : 0-thionphosphatidylcholine and 18 : $1_c$/18 : $1_c$-phosphatidylethanolamine at 60°C (A), 45°C (B), 35°C (C), and 25°C (D).

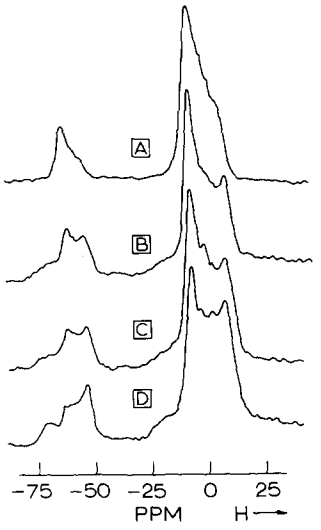


Fig. 7. 36.4 MHz $^{31}$P-NMR spectra of an aqueous dispersion of a mixture of 16 : 0/16 : 0-thionphosphatidylcholine/18 : $1_c$/18 : $1_c$-phosphatidylethylamine/cholesterol (1 : 3 : 2) at 60°C (A), 45°C (B), 35°C (C) and 25°C (D).

direct proof for the formation of non-bilayer structures by bilayer prefering lipids is given in Fig. 7, which shows the $^{31}$P-NMR spectra of an aqueous dispersion of the 16 : 0/16 : 0-thionphosphatidylcholine/ 18 : $1_c$/18 : $1_c$-phosphatidylethanolamine/cholesterol (1 : 3 : 2) mixture. The polymorphic phase behaviour of such a mixture made of natural phospholipids has been extensively studied [17,21]. The main result of this experiment is that at 25°C both phospholipids are predominantly organized in a liquid-crystalline bilayer organization and that by increasing the temperature there is a gradual increase in the amount of hexagonal $H_{II}$ phase of both lipids. Thus, at 60°C it can be clearly observed that at this temperature also the 16 : 0/16 : 0-thionphosphatidylcholine is organized in this non-bilayer phase.

In addition to variations in composition and temperature, non-bilayer structures can be induced by divalent cations in several negatively charged phospholipid containing model membranes [2]. One such lipid is bovine heart cardiolipin which in buffers aqueous containing monovalent cations adopts a bilayer configuration which changes to a hexagonal $H_{II}$ type of organization by the addition of divalent cations such as $Ca^{2+}$ [22,23]. In mixed phosphatidylcholine-cardiolipin systems, $Ca^{2+}$ addition results in the formation of an isotropic structure with associated lipidic particles [17,24]. To answer some of the above questions in a system in which the non-bilayer structure is induced by divalent cations we studied the phase behaviour of a mixed 16 : 0/16 : 0-thionphosphatidylcholine/ cardiolipin (5 : 1) dispersion in response to the addition of $Ca^{2+}$. In the absence of $Ca^{2+}$ at 25°C both phospholipids are organized in bilayers which, due to the high linoleic acid content of bovine heart cardiolipin [9], is in the liquid crystalline state for both lipids (Fig. 8A). Addition of $Ca^{2+}$ ($Ca^{2+}$/cardiolipin = 2 : 1) to these liposomes at 25°C results in pronounced phase separation as the 16 : 0/16 : 0-thionphosphatidylcholine is mainly

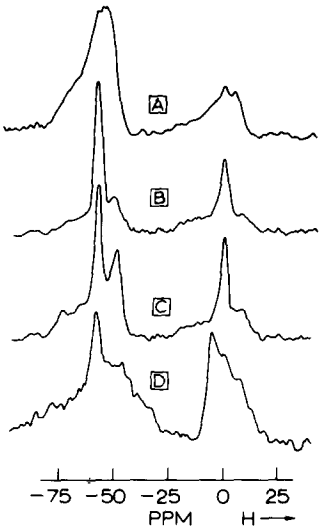


Fig. 8. 36.4 MHz $^{31}$P-NMR spectra of an aqueous dispersion of a mixture of 16 : 0/16 : 0-thionphosphatidylcholine/cardiolipin (5 : 1) in the absence of $Ca^{2+}$ at 25°C (A) or in the presence of $Ca^{2+}$ ($Ca^{2+}$/cardiolipin = 2 : 1, $|Ca^{2+}|_{total}$ = 0.04 M) at 50°C (B), 40°C (C), and 25°C (D).

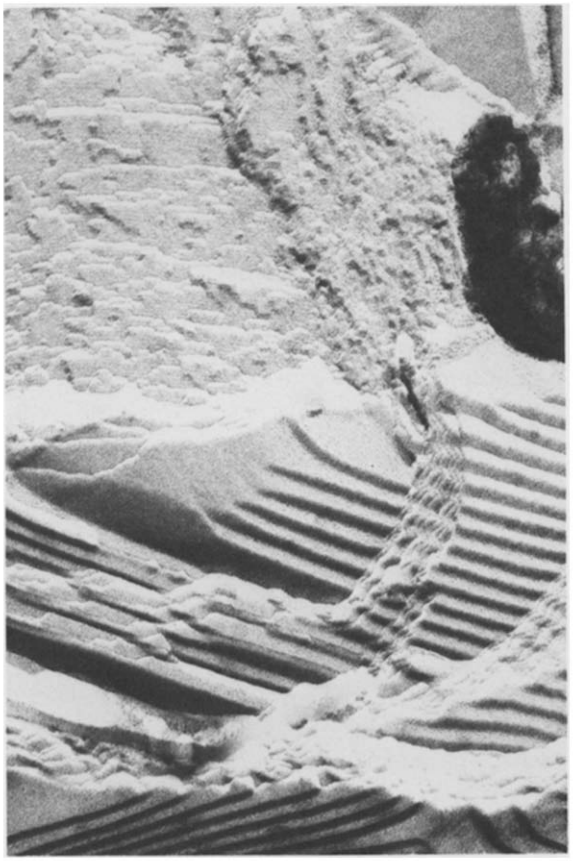

Fig. 9. Freeze-fracture electron microscopy of an aqueous dispersion of a mixture of 16 : 0/16 : 0-thionphosphatidylcholine/cardiolipin (5 : 1) in the presence of $Ca^{2+}$ ($Ca^{2+}$/cardiolipin = 2 : 1, $|Ca^{2+}|_{total}$ = 0.04 M). Quench temperature 25°C. Final magnification 95000×.

organized in a gel state bilayer (compare Fig. 4) and a small isotropic peak, whereas the cardiolipin $-Ca^{2+}$ complex is organized in a hexagonal $H_{II}$ phase (Fig. 8D). This structural phase separation can also be observed by freeze-fracturing where next to areas of hexagonal $H_{II}$ phase the banded patterns typical of gel state long chain saturated phosphatidylcholines [25] are observed (Fig. 9). Increasing the temperature above the gel → liquid crystalline phase transition of the 16:0/16:0-thionphosphatidylcholine again has an effect on the structure of this lipid mixture: both lipids are now mainly organized in bilayers but a significant amount of each lipid is present in a structure which allows the molecules to undergo rapid isotropic motion (Fig. 8). This fraction increases with increasing temperature, demonstrating that both the bilayer and non-bilayer lipid are present in this isotropic structure.

## Concluding remarks

The aim of this study was to synthesize phospholipid analogues which would closely resemble natural phospholipids but whose $^{31}$P-NMR resonance would be completely separated from that of a natural phospholipid and whose $^{31}$P-NMR lineshape would be sensitive to the macroscopic organization of the lipid in water. The thionphospholipids selected for this purpose are relatively easy to synthesize in satisfactory yield. They have similar physico-chemical and structural properties as their natural analogues and the chemical shift of their $^{31}$P resonance is approx. 55 ppm downfield from that of typical phospholipids allowing observation of the signal from both lipids in mixed systems. Most importantly, the sensitivity of the $^{31}$P-NMR spectrum to the structure of the lipids is similar to that of the natural phospholipids thus making it possible to discriminate between lipids organized in extended bilayers, cylindrical structures such as the hexagonal $H_{II}$ phase and structures in which the lipids can undergo isotropic motion.

A drawback of the thionphospholipids is that due to the relatively small value of the effective chemical shift anisotropy of the $^{31}$P-NMR spectrum of lipids organized in cylindrical structures it will be difficult to distinguish the hexagonal phase from that of broad isotropic $^{31}$P-NMR signals.

Incorporation of these lipids in model and biological membranes can be expected to give useful information on the structural preferences of particular lipids in complex systems.

In this study we used the thionphospholipids to get a deeper insight in some areas of the polymorphism of phospholipids in mixed model membranes. Bilayer → non-bilayer transitions can be modulated by many biological relevant parameters such as temperature, lipid composition, pH, divalent cations and both intrinsic and extrinsic membrane proteins and polypeptides [2]. The present study demonstrates that in addition gel → liquid crystalline phase transitions can trigger bilayer → non-bilayer transitions of lipids in mixed systems. Furthermore, it is demonstrated that typical bilayer forming lipids can be incorporated into non-bilayer structures such as the hexagonal $H_{II}$ phase. This is an important finding in view of the functional aspects of non-bilayer lipid structures. For instance, it has been speculated [15] and demonstrated [26,27] that inverted micelles can mediate the transbilayer movement of both bilayer and non-bilayer lipids in mixed systems, which suggests the presence of the bilayer forming lipid in transiently formed non-bilayer structures.

These preliminary applications of the use of those phospholipids in the study of lipid polymorphism are promising and in the future may lead to an understanding of the structural characteristics of non-bilayer lipids in biological membranes under different physiological conditions.

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