

THERMOTROPIC PHASE BEHAVIOUR AND MISCIBILITY PROPERTIES OF PHOSPHATIDYLCHOLINES CONTAINING BRANCHED FATTY ACID CHAINS

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Phosphatidylcholines with branched fatty acid chains were investigated by differential scanning calorimetry, X-ray diffraction, and ESR-spectroscopy. Depending on both the length of the branches in 2-position of the acyl chains and the position of the methyl branches in the acyl chains, the main-transition temperatures and enthalpies pass through a minimum. The systematic change of the phase transition parameters is connected with a modified structural polymorphism.

In the gel phase the miscibility properties depend on the phase structures of the components. For one system a phase diagram with a critical point has been found.

Branched fatty acids widely occur as components of the lipids of micro-organisms, plants, and animals. Despite their widespread occurrence in nature only little attention has been paid to study the influence of branching on biophysical and biochemical properties of the lipids.

To continue our works on the phase behaviour and miscibility properties of phospholipid-water model-systems in dependence on the chemical structure of the phospholipids [1–5], the thermotropic phase behaviour of partially synthesized 1,2-diacyl-*sn*-glycero-phosphocholines with different series of alkyl branched fatty acid residues was investigated.

Materials and methods

Branched 1,2-diacyl-*sn*-glycero-phosphocholines were synthesized according to published procedures [3]. The abbreviation di(x C $_m$ – n :0)PC is used where x indicates the position of the branches in the main chain, n the total length of the fatty acid and m the number of C-atoms of the branches. The fatty acid spin label 14-SASL (14-(4',4'-dimethyloxazolidinyl-N-oxy)-stearic acid) was obtained from Aldrich Chemical Company, USA.

The calorimetric measurements were performed using a Perkin-Elmer DSC-2 instrument which was standardized and operated as described elsewhere [1].

X-ray diffraction was carried out with a powder diffractometer HZG 4 (VEB Präzisionsmechanik Freiberg) in a transmission technique [6].

ESR spectra were recorded by a JEOL JES-PE-1X spectrometer using the 100 kHz modulation technique. The molar ratio of phospholipid and spin probe was at least 100:1. The order parameter was calculated from the ESR spectra using the outer and inner splitting [7].

The phospholipids and their mixtures in chosen proportions were dissolved in chloroform, dried in vacuum ($1.33 \cdot 10^{-2}$ Pa) and dispersed in water at 50% (w/w) by vortexing for 1-2 min at temperatures above the transition temperature of the higher-melting component.

Results

Phase behaviour of phospholipid-water dispersions

The behaviour of phospholipids in the presence of water is quite complex because various hydrated phases occur in the excess-water samples depending on the temperature. All phospholipids show the so called main transition (index m) with a chain melting. Above the main transition liquid-crystalline phases with fluid hydrocarbon chains (index α) occur. Below the main transition different gel phases with rigid chains (index β) were observed.

In Fig. 1 the main-transition temperatures and enthalpies of homologous di($x\text{C}_1-16:0$)PC/water systems are plotted as a function of the position x of the methyl branches. It can be seen that the main-transition enthalpies and temperatures decrease with increasing x . For the homologues with methyl side chains in the middle part of the main chains ($x = 8, 9$ and 10) no transition was found in the temperature range from 140 K to 360 K, except the melting and freezing of free water. For $x = 14$ and 15 an increase of the main-transition temperature with increasing x was observed [8-10].

The structural behaviour of the di($2\text{C}_1-16:0$)PC and di($8\text{C}_1-16:0$)PC was studied by X-ray measurements. The repeat distance of about 5.05 nm in the di($2\text{C}_1-16:0$)PC indicates a gel phase with interdigitated chains. Interdigitation produces a large increase in the order parameter of a fatty acid spin labeled near the terminal methyl (14-SASL) [11]. Indeed, the measured high order parameter is maintained up to the main transition where the highly oriented anisotropic motion is abruptly transformed into an isotropic type of motion characteristic of 14-SASL in the liquid-crystalline phase of lipids. Therefore, in PCs with methyl branches in the position

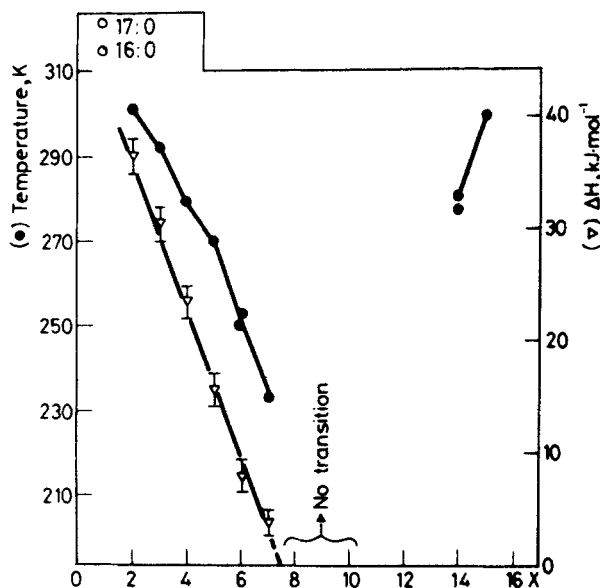


Fig. 1 Dependence of the main-transition temperatures (●) and enthalpies (Δ) of di($x\text{C}_1-16:0$) PC/50% (w/w) water dispersions on the position x of the methyl branches in the acyl chains. 16:0 hexadecanoyl (palmitoyl) acyl chain, 17:0 heptadecanoyl acyl chain.

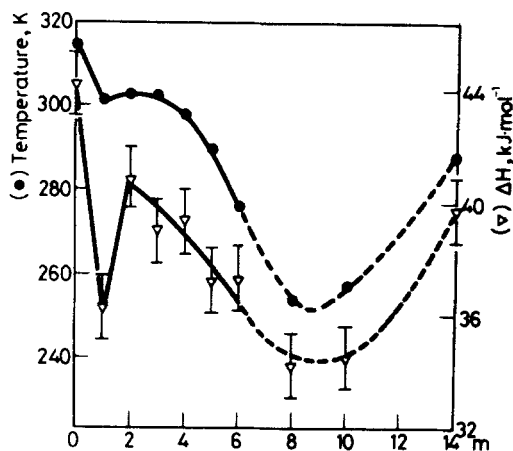


Fig. 2 Main-transition temperatures (●) and the sum of pre- and main-transition enthalpies (Δ) of di($2\text{C}_m-16:0$) PC/50% (w/w) water dispersions as a function of the chain length m of the branches.

$x < 8$ a gel phase with interdigitated chains can be assumed. For the di(8C₁ - -16:0)PC only a lamellar phase with molten chains could be observed.

The characteristic change of the main-transition temperatures and the enthalpy values in dependence on the side chain length m (Fig. 2) is connected with a modified polymorphism in "short-chain branched" and "long-chain branched" systems. In phases with molten chains a change from lamellar (L) to inverse hexagonal (H) structures was observed if the chain length of the branches increases. From the molecule shape of the compounds with $m > 4$, which looks like a wedge, the existence of an inverse hexagonal high-temperature phase can be deduced [11].

The main transition temperatures pass through a minimum if the terminal methyl groups of the side chains centered near the middle part of the main chains ($m \sim 9$).

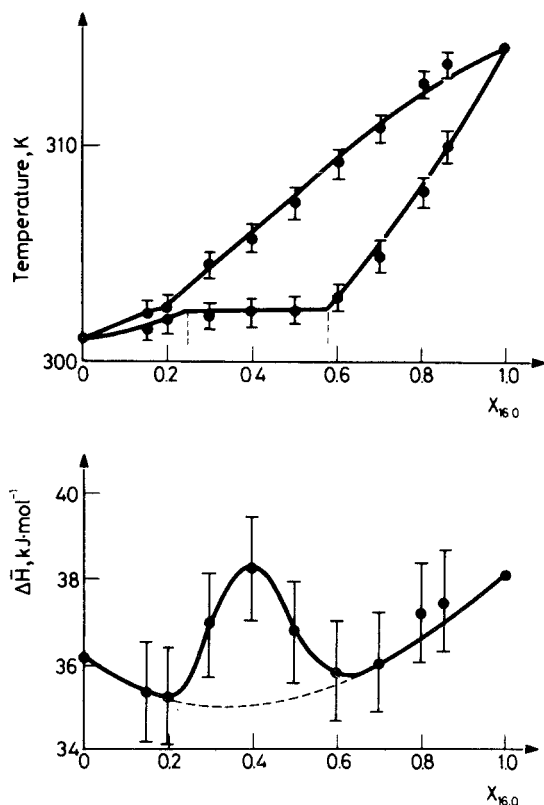


Fig. 3 Phase diagram constructed for mixing of di(16:0) PC with di(2C₁-16:0)PC using corrected onset temperatures for heating (solidus) and cooling (liquidus) scans and transition enthalpies of the mixtures.

Miscibility properties of pseudobinary phospholipid mixtures

The phase diagrams, fig. 3, 4 were constructed from calorimetric heating and cooling scans of various mixtures obtained at the usual scanning rate of 1.25 deg per min.

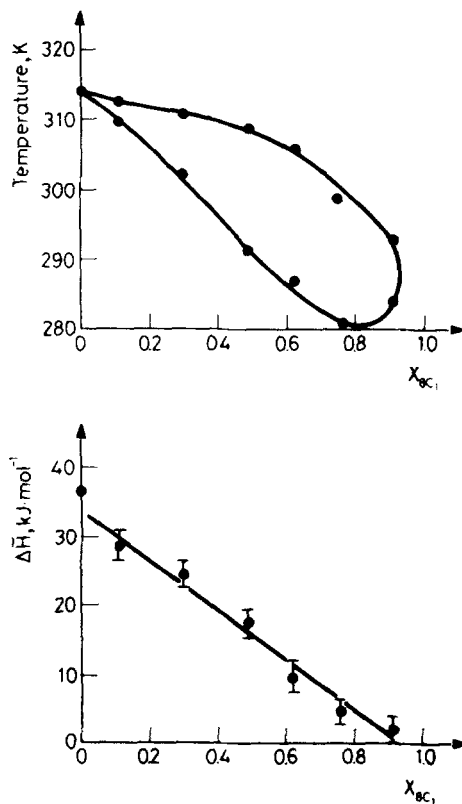


Fig. 4 Phase diagram for di(16:0)PC/di(8C₁-16:0)PC and transition enthalpies of the mixtures.

The phase diagram of the aqueous dispersion of di(2C₁-16:0)PC/di(16:0)PC (Fig. 3) shows that a miscibility gap in the gel phase appears. In the concentration region from 25 to 60 mol% of di(16:0)PC the mixture shows two transition peaks and outside of this region only one homogeneous peak. The corrected onset temperature of the first sharp peak in the region of the miscibility gap gave a horizontal three-phase line which was quite above the main transition temperature of di(2C₁-16:0)PC. In the region of the gel

phase miscibility gap the transition enthalpies obtained in the present study show positive deviation from the enthalpy-mole fraction curve outside of this region. In the liquid-crystalline L_α -phase both components are miscible over the whole range.

For the phase diagram of the aqueous dispersion of di(8C₁-16:0)PC/di(16:0)PC, (Fig. 4) derived from both ESR and differential scanning calorimetry data, a "drop-like" shape was drawn, which is supported by the linear decrease of the transition enthalpy with increasing concentration of di(8C₁-16:0)PC. In the liquid-crystalline L_α -phase both components are fully miscible. On the right side of the phase diagram a critical point exists. With increasing concentration of di(8C₁-16:0) PC the disorder of the gel phase increases, so that at the critical concentration the structures of the gel phase and the liquid-crystalline L_α -phase become identically. However, the transition peaks were very broad and because of the small transition enthalpy values very flat, so that another interpretation based on the existence of a small miscibility gap between the gel and the liquid-crystalline phase cannot be excluded.

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Zusammenfassung – Phosphatidylcholine mit verzweigt-kettigen Fettsäureresten wurden mittels DSC, Röntgenbeugung und ESR-Spektroskopie untersucht. In Abhängigkeit sowohl von der Länge der Seitenkette in 2-Stellung der Acylkette als auch von der Stellung einer Methylverzweigung in der Acylkette gehen die Temperaturen und Enthalpien der Hauptumwandlung durch ein Minimum. Die systematische Änderung der Umwandlungsparameter ist mit einem modifizierten Strukturpolymorphismus verknüpft.

In der Gelpase hängen die Mischbarkeitseigenschaften von der Phasenstruktur der Komponenten ab. Für ein System wurde ein Phasendiagramm mit einem kritischen Punkt gefunden.

РЕЗЮМЕ — С помощью ДСК, рентгено-структурного анализа и ЭПР спектроскопии исследованы фосфатидилхолины с разветвленными цепями жирных кислот. В зависимости от длины разветвления в 2-положении ацильных цепей и положения метильных ветвей в ацильных цепях, главные температуры переходов и их энтальпии проходят через минимум. Систематическое изменение параметров фазовых переходов связано с видоизмененным структурным полиморфизмом. В гелевой фазе характеристики смешиваемости зависят от фазовых структур компонентов. Для одной системы была найдена фазовая диаграмма с критической точкой.