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Interdigitated hydrocarbon chains in C20 and C22 phosphatidylcholines induced by hydrostatic pressure

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Abstract

Saturated diacyl-phosphatidylcholines (PCs) can exist in an unusual gel phase in which the acyl chains from opposing monolayers interdigitate. This phase is induced by a variety of small amphiphilic molecules such as ethanol and by hydrostatic pressure. Pressure–temperature (P–T) phase diagrams previously established conditions for interdigitation of dipalmitoyl-PC (C16) and distearoyl-PC (C18). Measurements have now been made for P–T phase diagrams up to 1 kbar pressure for diarachidoyl-PC (C20) and dibehenoyl-PC (C22) using the small-angle neutron scattering facilities at NIST. The minimum pressure for interdigitation was found to be about 0.6 kbar for diarachidoyl-PC and slightly less for dibehenoyl-PC. Together with previous results for distearoyl-PC and dipalmitoyl-PC, these results establish that the minimum pressures for interdigitation, which occur at about 60 C except for dibehenoyl-PC, decrease monotonically with acyl chain length. The data are fit reasonably well by an exponential. Also, the curved phase boundaries for interdigitation show that entropy changes associated with interdigitation change sign with increasing temperature at about 60 C. © 1998 Elsevier Science B.V. All rights reserved.

Keywords: Biological lipids; Membranes; Phase transitions; Hydrostatic pressure

Diacyl-phospatidylcholines are membrane lipids which contain saturated symmetrical or asymmetrical hydrocarbon chains. These lipids normally form smectic bilayer structures in the presence of water, but since the 1980s it has been recognized that these lipids can also exist in an unusual gel phase in which the acyl chains from opposing monolayers interdigitate. The interdigitated phase is induced by a variety of small, amphiphilic molecules such as ethanol [1–4], ethylene glycol, benzyl alcohol and tetracaine [5,6]. It is also formed by other lipids. This phase was originally found for commerical DPPG (1,2-dipalmitoyl-phosphatidylglycerol) which contained choline as impurity [7] and acetylcholine was found to induce interdigitation as well. The antibiotic peptidolipid Polymyxin B, which causes rapid permeability changes in cytoplasmic membranes of bacteria, protozoa and yeast, has a high and specific affinity for acidic phospholipids such as DPPG and induces interdigitation in this lipid [8].

The interdigitated phase in phosphatidylcholines is also produced by hydrostatic pressures of

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1–2 kbar for DPPC and DSPC (1,2-dipalmitoylphospatidylcholine and 1,2-distearoylphosphatidylcholine) and the temperature–pressure phase diagrams of these lipids were mapped up to 2 kbar [9]. DPPC and DSPC differ only in acyl chain length (C16 and C18, respectively), but this has a substantial influence on the formation of the interdigitated phase, which begins at a minimum pressure of 0.9 kbar for DSPC and 1.6 kbar for DPPC (both at about 60 C). The interdigitated phase was not found for DMPC (C14) up to 2 kbar but it is likely to occur above 2 kbar. When used together, pressure and ethanol induce interdigitation cooperatively, so less ethanol is needed as pressure increases, but in a nonlinear manner [10].

Formation of the interdigitated phase as a function of acyl chain length in phosphatidylcholines was further investigated at NIST using the automated hydrostatic pressure facility and the 30 m SANS instrument. The temperature–pressure phase diagrams of DAPC (1,2-diarachidoyl-phosphatidylcholine) and DBPC (1,2-dibehenoylphosphatidylcholine), which have acyl chains of 20 and 22 carbon atoms, respectively, were mapped up to 1 kbar pressure and from 40 C to 90 C. Fig. 1 shows the phase diagram established for DBPC (C22). The samples were multilamellar vesicles in D_2O at 2–3% lipid by weight. Mild sonication with a probe produced suitably dispersed samples. Different structural phases (lamellar gel phase, rippled gel phase, interdigitated gel phase, and lamellar liquid crystalline phase) were identified by characteristic low-angle diffraction in the Q range of 0.02–0.15 $Å^{-1}$ [11]. The interdigitated phase was found at a minimum pressure of 0.6 kbar for DAPC (again at about 60 C and slightly less for DBPC. With the minimum pressures of 0.9 kbar for DSPC and 1.6 kbar for DPPC, the new results for DAPC and DBPC show that the minimum pressure for interdigitation changes systematically with acyl chain length and is fit well by an exponential, as shown in Fig. 2. The fit to the data indicates that the interdigitated phase for DMPC (C14) should occur at 2.6–2.7 kbar and 60 C.

Formation of the interdigitated phase by hydrostatic pressure is largely driven by the closer packing of acyl chains which is otherwise difficult to achieve because of the large cross-sectional areas of

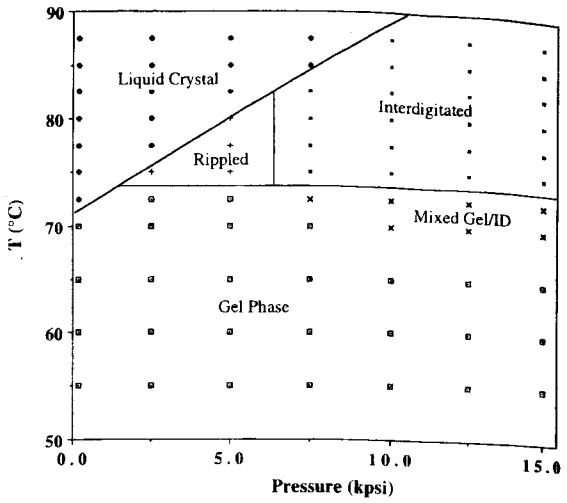


Fig. 1. Pressure–temperature phase diagram for dibehenoylphosphatidylcholine (C22) as established by small-angle neutron scattering from samples of multilamellar vesicles in D2O. Points are plotted at P–T values of measurements. The lines were added to indicate approximate phase boundaries.

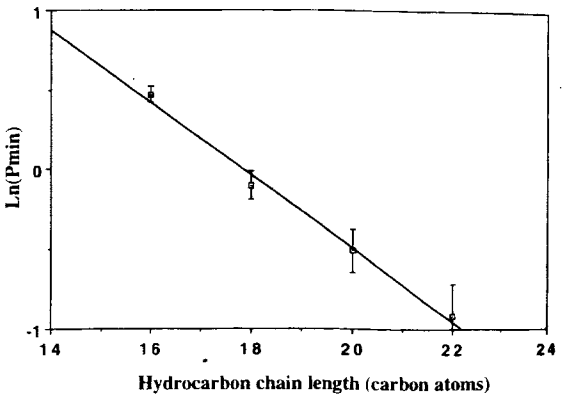
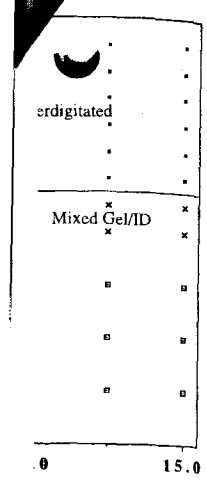


Fig. 2. The minimum pressure for interdigitation as a function of acyl chain lengths for phosphatidylcholines is reasonably well fit by an exponential. The fit predicts interdigitation for C14-PC at about 2.6 kbar (60 C).

the polar head groups. However, the curved phase boundary for interdigitation demonstrates complex properties for this packing. From the Clausius–Clapeyron equation, it is concluded that the volume and entropy changes associated with interdigitation are not constant with temperature and pressure, as is the case for the transitions to



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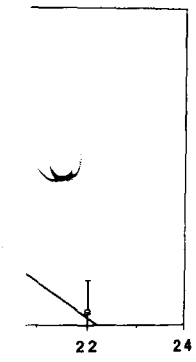

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a liquid crystalline phase at higher temperatures (linear phase boundary). In particular, there is a change of sign for the entropy change at about 60°C with all four of the phosphatidylcholines studied. These results should help to clarify the molecular and thermodynamic properties involved with interdigitation.

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