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THERMOTROPIC PHASE TRANSITIONS OF PHOSPHATIDYLCHOLINES WITH ODD-NUMBERED *n*-ACYL CHAINS

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Summary

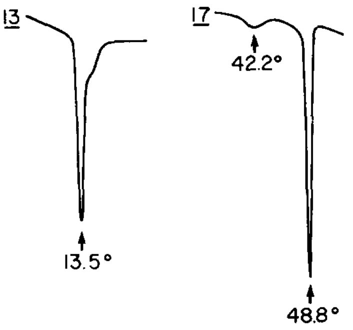
Diacyl phosphatidylcholines with n-C_{13}, -C_{15}, -C_{17} and -C_{19} saturated acyl chains have been synthesized and their phase transitions in the presence of excess water monitored by differential thermal analysis. The C_{15}-, C_{17}- and C_{19}-diacyl species show gel to liquid-crystalline transitions and pretransitions like those of the even-chain phosphatidylcholines. A plot of the main phase transition temperature, T_c, vs. acyl chain length is a smooth curve on which the T_c values of both odd- and even-chain species fall, while a similar plot for the pretransition temperature, T_p, shows significant alternation of T_p between odd- and even-chain species. Consideration of these results in terms of the physical basis of the odd-even alternation of phase transition temperatures in homologous series of paraffinic compounds suggests that the acyl chains of disaturated phosphatidylcholines are tilted with respect to the bilayer normal below T_p but become perpendicular to the bilayer surface above the pretransition temperature.

While phosphatidylcholines are without doubt the most intensively studied phospholipid as a model for the behavior of membrane polar lipids in general, almost all of the studies reported to date with synthetic phosphatidylcholines have focused on the properties of species with n-alkyl or -alkenyl chains of even carbon number. Knowledge of the properties of phosphatidylcholines with other types of acyl chains would be useful to further our understanding both of the functional roles of such alternative types of acyl groups in the membrane lipids of various organisms in which they occur [1] and of the detailed structural and dynamic properties of phospholipids in general. In the present work, we have synthesized some odd-chain phosphatidylcholines and have investigated their phase transitions by differential thermal analysis. Comparison of the transition temperatures of odd- and even-chain diacyl phosphatidylcholines in this study has shed some light on the question of the orientation of the acyl chains in hydrated phosphatidylcholines in the gel state, a matter of some controversy at present [2—6].

Phosphatidylcholines were synthesized by condensation of acyl anhydrides (formed by treating free fatty acids in carbon tetrachloride with dicyclohexylcarbodiimide [7]) with the anhydrous cadmium chloride adduct of α-L-glycerophosphorylcholine using the basic procedure of Patel et al. [8], which employs a mixture of benzene and dimethylsulfoxide as the solvent and 4-pyrrolidinylpyridine as the catalyst. The pure phosphatidylcholines were isolated from the crude products by chromatography on columns of silicic acid or carboxymethyl cellulose [9,10], occasionally followed by preparative thin-layer chromatography and a final precipitation of the product from acetone. The purified lipids were freed of solvents under high vacuum, packed into capillary tubes, and rehydrated with 50% (w/w) distilled water as described by Ladbrooke et al. [11]. Differential thermal analysis was carried out on a Dupont 900 Thermal Analyzer, usually at a temperature scan rate of 3°C per min. Fatty acids (>99.7% purity by gas-liquid chromatography of the methyl esters) were obtained from Nu-Chek Prep (Elysian, MN); α-L-glycerophosphorylcholine (cadmium chloride complex) and synthetic even-chain phosphatidylcholines (free of lysolipids or free fatty acids by thin layer chromatography) were products of Sigma (St. Louis, MO). All chromatographic adsorbents were prewashed with organic solvents to remove any organic contaminants; all solvents were redistilled.

Heating scans for ditridecanoyl and diheptadecanoyl phosphatidylcholines are shown in Fig. 1. As can be seen, the C_{17}-acyl species shows two thermotropic transition endotherms, a small pretransition endotherm followed by a large, sharper endotherm at a slightly higher temperature. This heating behavior was also observed for the C_{15}- and C_{19}-acyl species and is completely comparable with the previously studied thermotropic behavior of the C_{14}-, C_{16}- and C_{18}-acyl species [12]. By contrast, the C_{13} species shows no pretransition endotherm above −5°C but does show a shoulder on the high-temperature side of a sharp endotherm, as was previously observed for the C_{12} species [12]. In Fig. 2, the peak temperatures of the pre- and main transition endotherms are plotted as a function of acyl chain length for the odd-chain phosphatidylcholines and for commercial even-chain phosphatidylcholines analyzed using the same conditions for differential thermal analysis as were used for the odd-chain species. It can be seen that while the T_c values for odd- and even-chain phosphatidylcholines lie on a single smooth curve, the T_p values do not, showing odd-even alternation which is clearly evident when $(T_c - T_p)$ is plotted vs. acyl chain length, as is also shown in Fig. 2.

To ensure that the results shown in Fig. 2, particularly those regarding T_p, are reliable, several tests of the results were carried out. First, to ensure that the T_p values of the odd-chain phosphatidylcholines are not depressed by impurities in the samples, all of the preparations were repurified by preparative thin-layer chromatography and reanalyzed by differential thermal



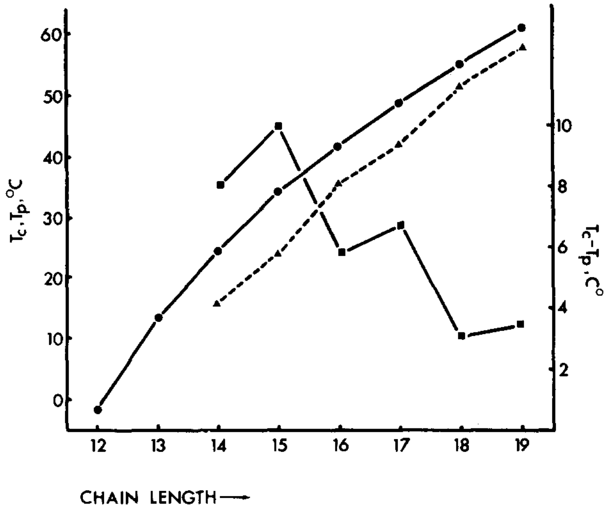


Fig. 1. Differential thermal analytical heating scans for ditridecanoyl and diheptadecanoyl phosphatidylcholines dispersed in 50% (w/w) water. Downward deflections, indicated by arrows, represent endothermic transitions at the indicated temperatures. Samples were heated at a rate of 3°C/min, and the temperature difference between the sample and a glass bead-containing reference cell (vertical axis) was recorded as a function of the sample temperature (horizontal axis). No scale is given for the vertical axis, as the magnitude of the differential temperature does not hold a constant relationship to the energy of the transition from sample to sample and is of little direct physical significance.

Fig. 2. Values of the transition temperature T_c (●), the pretransition temperature T_p (▲) and the difference between these two temperatures (■) determined by differential thermal analysis of phosphatidylcholine samples in 50% (w/w) water, heated at a rate of 3°C/min.

analysis, and the T_c and T_p values of C_{15}- and C_{17}-acyl phosphatidylcholines purified by silicic acid vs. carboxymethylcellulose column chromatography were compared. In all cases, the T_c and T_p values determined for various samples of the same species of phosphatidylcholine agreed to within 1°C (often considerably better than this limit). Secondly, a sample of dipalmitoyl phosphatidylcholine prepared and purified by the methods used for the odd-chain phosphatidylcholines gave T_p and T_c values of 35.8° and 41.5°C, respectively, in excellent agreement with the values for the commercial product and those reported by other workers [12]. Finally, we investigated the dependence of the measured T_p values of even- and odd-chain phosphatidylcholines on the heating rate in our system, as Lentz et al. [13] have recently reported that the pretransition is a slow process which is not in true equilibrium as monitored by scanning calorimetry. We confirmed that T_p is indeed a function of the heating rate used in our differential thermal analyses. However, the T_p values for odd- and even-chain phosphatidylcholines varied in parallel with changes in the heating rate in the range of rates studied (3°C—15°C/min); no differential variation of T_p with heating rate was found for odd vs. even-chain species. Therefore, while the T_p values measured by us are a few degrees above the true equilibrium pretransition temperatures, odd-even alternation appears to be a characteristic of the latter temperature values as well.

Alternation of phase transition temperatures between compounds of odd and even carbon chain lengths is frequently observed in homologous series of paraffinic compounds. Such alternation is invariably associated with an arrangement of the hydrocarbon chains in at least one of the phases involved in the transition such that the major chain axis is not perfectly perpendicular to the plane(s) of the endgroups of the hydrocarbon chains (for a fuller explanation, see Ref. 14). Since T_p but not T_c shows odd-even alternation in the disaturated phosphatidylcholines, it would seem that the acyl chains are tilted with respect to the bilayer normal below T_p but become perpendicular to it above T_p, so that the main transition shows no alternation of T_c values. This proposal is in agreement with the results of some X-ray studies [2,5] but does not agree with the studies of Janiak et al. [4] or the proposal of Larsson [6]. However, Hui [3] has noted that diffraction evidence for tilted acyl chains above T_p can also be interpreted as a rippling of the bilayer surface with the acyl chains everywhere locally normal to the bilayer surface or central plane. Such rippling has been directly demonstrated by freeze-fracture electron microscopy [15]. Our results are most easily interpreted in terms of a rippled bilayer, with acyl chains everywhere locally perpendicular to the central plane of the bilayer, existing between T_p and T_c, for it is the angle between the acyl chain axis and this plane (not the macroscopic plane of the bilayer as a whole) that determines the presence or absence of alternation. Below T_p, the clear odd-even alternation of this parameter suggests that the acyl chains are tilted with respect to the bilayer normal, in agreement with the conclusions of a number of workers who have used diffraction methods as the basis of their studies [2—5].

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