Differential scanning calorimetry study of the influence of phospholipid analogs with a carbonyl-terminated *sn*-2 chain on the interdigitated phases formed by 1-stearoyl-2-capryl-*sn*-glycero-3-phosphatidylcholine (C18:C10-PC)

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Abstract Synthetic glycerophosphocholines with highly asymmetric chain lengths form interdigitated bilayers in the gel phase. In nature, phospholipids with one hydrocarbon chain approximately twice as long as the other can arise from the autooxidation of unsaturated linkages in the acyl chain, and thus the oxidation products would contain a carbonyl group at the chain terminus. In this study, we have investigated the thermotropic behavior of bilayers prepared from mixtures of the well-studied, mixed-chain phospholipid, 1-stearoyl-2-capryl-*sn*-glycero-3-phosphocholine (C18:C10-PC, **1**), with synthetic 1-stearoyl-2-acyl-*sn*-glycero-3-phosphocholines in which the *sn*-2 chain is approximately one-half the length of the *sn*-1 chain and contains a C=O group near the ω terminus. Phase diagrams of binary mixtures of **1** with a chain-terminal ketone-PC analog (**2**) or with a chain-terminal ester-PC analog (**3**) in excess water exhibited gel-phase immiscibility over a wide compositional range, but miscibility in the liquid-crystalline phase. However, **1** was completely miscible with C18:C10:1Δ^{10}-PC (compound **4**), which bears a chain-terminal carbon-carbon double bond, in both the gel and liquid-crystalline phases. The calorimetric data suggest that phosphatidylcholines (PC) with carbonyl-terminated chains, which can be produced by autooxidation of naturally abundant 1-saturated-2-unsaturated phospholipids such as 1-stearoyl-2-oleoyl-PC, may not form the normal triple-chain mixed interdigitated structure characteristic of hydrocarbon-terminated PCs in gel-phase bilayers.—**Ali, S., and R. Bittman.** Differential scanning calorimetry study of the influence of phospholipid analogs with a carbonyl-terminated *sn*-2 chain on the interdigitated phases formed by 1-stearoyl-2-capryl- *sn*-glycero-3-phosphatidylcholine (C18:C10-PC). *J. Lipid Res.* 1996. **37:** 2305–2309.

Supplementary key words thermotropic behavior of bilayers • differential scanning calorimetry • autooxidation

The influence of inequivalence in the length of the two fatty acyl chains of saturated phospholipids on the thermodynamic parameters of the phase transition of lipid bilayers has been investigated in detail (1–4). When one hydrocarbon chain is much longer than the other, the longer chain, having no packing counterpart in the shorter hydrocarbon chain of the bilayer gel phase, may extend beyond its own monolayer and protrude into the shorter chains of the opposing monolayer in order to maximize van der Waals contacts. A mixed interdigitated phase is formed below T_m in which the longer chain spans the entire bilayer and the end of the shorter chain meets the end of the short chain of the opposing phosphatidylcholine (PC) in the center of the bilayer (5, 6). It has been suggested that glycerophospholipids with one hydrocarbon chain approximately twice as long as the other can arise in nature from autooxidation of unsaturated linkages (7). However, the glycerophospholipids with highly asymmetric acyl chains that have been used in spontaneous and short-chain alcohol-induced interdigitation studies (8–10) all have saturated and monounsaturated hydrocarbon chains in the *sn*-1 and *sn*-2 positions. Keto-containing lipids have attracted attention as spectroscopic probes of micelle structure and depth of water penetration (11–13). The present report extends the applications of this type of lipid to calorimetric studies of the

Abbreviations: DSC, differential scanning calorimetry; PC, phosphatidylcholine; T_m, gel to liquid-crystalline phase transition temperature.

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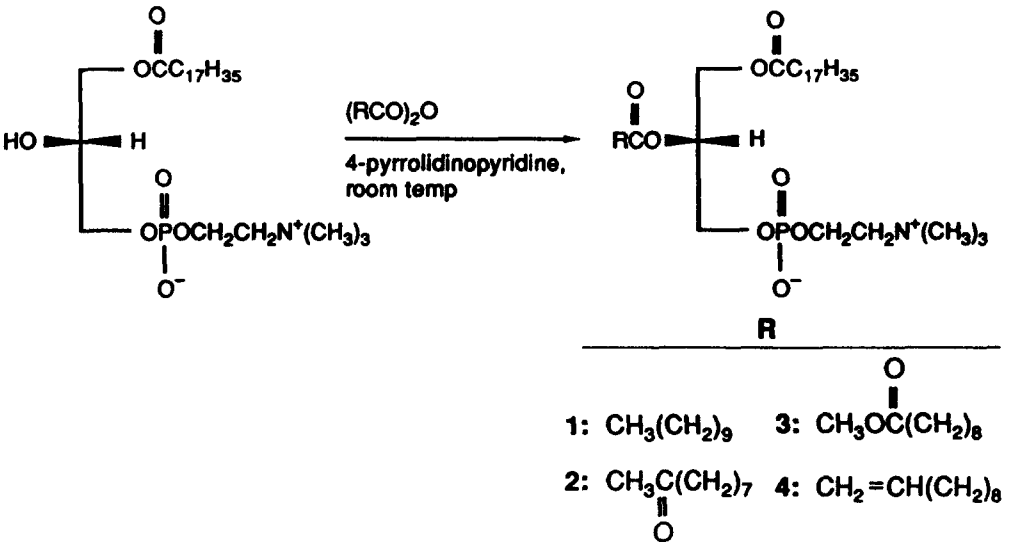

Scheme 1. Preparation of lipids 1–4 from 1-stearoyllyso-PC.

mixing behavior of phosphatidylcholines in interdigitated bilayers.

The products of autooxidation are lipids that terminate in an aldehyde group, e.g., 1-palmitoyl-2-nonal-*sn*-glycero-3-phosphocholine would be formed from 1-palmitoyl-2-oleoyl-*sn*-glycero-3-phosphocholine. In order to examine the hypothesis that a phospholipid bearing a *sn*-2 ω-aldehyde group would pack in a manner analogous to that found for 1-stearoyl-2-capryl-*sn*-glycero-3-phosphocholine (C18:C10-PC, **1**) and other mixed-chain hydrocarbon-terminated phospholipids, we synthesized PCs bearing a stearoyl chain and a short acyl chain with a carbonyl function near the ω terminus. As an aldehyde is susceptible to rapid oxidation and hydration, we synthesized two stable analogs of an aldehyde-terminated PC, i.e., ketone-PC **2** and ester-PC **3**. These phospholipids were prepared by acylation of 1-stearoyl-2-lyso-*sn*-glycero-3-phosphocholine with the anhydrides of 9-oxodecanoic acid and sebacic acid monomethyl ester at room temperature by using 4-pyrrolidinopyridine as catalyst (14). The influence of a C=O function on the packing behavior of PCs with a marked disparity in their acyl-chain lengths was tested by differential scanning calorimetry (DSC). The DSC evidence suggests that a C=O group, which would be generated in glycerophospholipids after autooxidation, interferes with the ability of the extensively studied interdigitatable phospholipid C18:C10-PC (**1**) to pack in a gel-phase interdigitated bilayer.

MATERIALS AND METHODS

Chemicals

1-Stearoyllyso-PC was purchased from Avanti Polar Lipids (Alabaster, AL). 4-Pyrrolidinopyridine was obtained from Aldrich and was recrystallized from chloroform–diethyl ether 1:1. 9-Oxodecanoic acid anhydride and the anhydride of sebacic acid monomethyl ester were prepared from the corresponding carboxylic acids by using dicyclohexylcarbodiimide in carbon tetrachloride as described previously (15). C18:C10-PC (**1**) was prepared by acylation of 1-stearoyllyso-PC as described previously (14). Hydrocarbon-stabilized chloroform was distilled from P_2O_5 and stored over 4A molecular sieves.

Flash chromatography was performed on silica gel 60 (230–400 ASTM mesh; E. Merck, obtained from Aldrich). Analytical TLC was carried out on 0.25-mm silica gel GF glass plates purchased from Analtech (Newark, DE). Phospholipids were detected by spraying the TLC plates with molybdate solution. Elemental analyses were performed by Desert Analytics (Tucson, AZ).

Scheme 1 outlines the acylation of 1-stearoyllyso-PC with fatty acid anhydrides at room temperature in the presence of 4-pyrrolidinopyridine, giving PCs **1–4.**

Synthesis of C18:C(10:ω-CH_3CO)-PC (2) (Scheme 1)

To a suspension of 150 mg (0.28 mmol) of 1-stearoyllyso-PC in 5 ml of dry, ethanol-free chloroform under

nitrogen atmosphere at room temperature were added 600 mg (1.45 mmol) of 9-oxodecanoic acid anhydride and 51 mg (0.34 mmol) of 4-pyrrolidinopyridine. After the mixture had stirred for 6 h, the solvent was removed under vacuum and the residue was purified by flash chromatography. Elution was with chloroform, then with chloroform–methanol 9:1, and finally with chloroform–methanol 1:1. The PC **2** was further purified by preparative TLC (1000-μm plates, Analtech); elution was with chloroform–methanol–water 60:30:4. The product was lyophilized with benzene, yielding 24 mg (40%) of **2** as a white glue; R_f 0.32 ($CHCl_3/CH_3OH/H_2O$ 60:30:4). ^1H NMR (300 MHz, $CDCl_3$) δ 5.22–5.19 (m, $CH_2\underline{C}H\underline{C}H_2$, 1H), 4.52–4.47 (m, $C\underline{H}_2O_2C_{17}H_{35}$, 2H), 4.35–3.90 (m, $C\underline{H}_2OPO(O^-)OCH_2CH_2N^+Me_3$, 6H), 3.41 (s, $N(CH_3)_3$, 9H), 2.42–2.37 (t, J = 6.8 Hz, $CH_3COC\underline{H}_2$, 2H), 2.32–2.25 (m, $COCH_2$, 4H), 2.10 (s, $C\underline{H}_3COCH_2$, 3H), 1.58–1.14 (br m, $(CH_2)_{19}$, 38H), 0.86–0.83 (t, J = 5.0 Hz, ω-CH_3, 3H). IR ($CHCl_3$) 3405, 2907, 2848, 1737, 1719 (sh), 1678, 1467, 1249, 1173, 1096, 1055, 979 cm^{-1}. Anal. calcd for $C_{36}H_{70}O_9NP \cdot 0.5H_2O$: C, 61.68; H, 10.21; N, 1.99. Found: C, 61.61; H, 10.37; N, 2.24.

Synthesis of C18:C(11:ω-CH_3O_2C)-PC (3)

To a suspension of 100 mg (0.18 mmol) of 1-stearoyllyso-PC in 5 ml of dry, ethanol-free chloroform under nitrogen atmosphere at room temperature were added 500 mg (1.8 mmol) of methyl hydrogen sebacic acid anhydride and 41 mg (0.28 mmol) of 4-pyrrolidinopyridine. After the reaction mixture had stirred for 6 h, the solvent was removed, and the residue was purified by flash chromatography, preparative TLC, and lyophilization by the same procedure used to prepare **2.** There was obtained 80 mg (50%) of **3** as a white glue; R_f 0.34 ($CHCl_3/CH_3OH/H_2O$ 60:30:4). ^1H NMR (200 MHz, $CDCl_3$) δ 5.17–5.15 (m, $CH_2C\underline{H}CH_2$, 1H), 4.36–4.31 (m, $C\underline{H}_2O_2C_{17}H_{35}$, 2H), 4.12–3.83 (m, $C\underline{H}_2OPO(O^-)OC\underline{H}_2C\underline{H}_2N^+Me_3$, 6H), 3.62 (s, CH_3, 3H), 3.32 (s, $N(CH_3)_3$, 9H), 2.30–2.21, $COC\underline{H}_2$, 6H), 1.57–1.16 (br m, $(C\underline{H}_2)_{21}$, 42H), 0.87–0.81 (t, J = 6.4 Hz, ω-CH_3, 3H). IR (neat) 3401, 2919, 2851, 1742, 1688, 1680, 1468, 1250, 1171, 1098, 1058, 978 cm^{-1}. Anal. calcd for $C_{37}H_{72}O_{10}NP \cdot H_2O$: C, 60.06; H, 10.08; N, 1.89. Found: C, 60.28; H, 10.00; N, 2.22.

Synthesis of alkene-terminated C18:0/C11:1(Δ^{10})-PC (4)

For the acylation of 1-stearoyllyso-PC with 10-undecenylic anhydride, see Ali and Bittman (14).

Differential scanning calorimetry

DSC measurements were carried out on a Hart Scientific Model 707 calorimeter (Provo, UT) equipped with a Dell 386 computer. Liposome suspensions were prepared by mixing the desired amounts of the PCs in chloroform, evaporating the solvent under nitrogen, and lyophilization of the lipid(s) from spectral-grade benzene; the resulting white powder was suspended (for mixtures of **1** with **2** or **3,** ~7 mg of total PC/ml; for pure PC as in Fig. 1A, 2.5–3.0 mg/ml) in sodium phosphate buffer, pH 7.4, containing 50 mM NaCl and 1 mM EDTA. The aqueous suspension was vortexed and subjected to 4–5 cycles of heating (35°C) and cooling (0°C), followed by storage for 2 days at 0°C to ensure complete mixing and equilibration of the PCs in the bilayers before collection of the DSC data. Liposomes (0.6 ml) were loaded into 1-ml capacity ampoules at room temperature, and data were accumulated in the ascending mode at a scan rate of 15°C/h, beginning at −3°C for **3** and −8°C for **2.**

Phase diagrams

The onset and completion temperatures were determined from the point of first deviation from the base line; these values were obtained by zooming of the onset and completion portions of the thermograms using the KaleidaGraph program on a Macintosh computer. The values of the thermal onset temperatures (T_0) and the thermal completion temperatures (T_c) of the main phase transition were used to construct the temperature-composition phase diagram; T_0 and T_c values are corrected for the finite width of the phase transitions of the pure components as described elsewhere (16).

RESULTS AND DISCUSSION

DSC thermograms of bilayers formed from the individual lipids (**Fig. 1A**) displayed the following values of T_m and ΔH: **1,** 20.4°C, 8.7 kcal mol^{-1}; **2,** 0.0°C, 6.2 kcal mol^{-1}; **3,** 7.5°C, 7.1 kcal mol^{-1}. Thermograms obtained from mixtures of **1** and **3** (Fig. 1B) and of **1** and **2** (Fig. 1C) are broader and more asymmetric than the endotherms of the pure lipids.

Phase diagrams were constructed for these mixtures from the thermograms by using published procedures (16). **Figure 2A,B** shows that **2** and **3** perturb the gel-phase packing behavior of **1,** with a more pronounced perturbation in mixtures prepared with ester-PC **3** than in those prepared with keto-PC **2.** The irregular solidus lines are indicative of solid-phase immiscibility induced by incorporation of 10–50 mol % of **2** into **1** at ≤0°C or of 10–60 mol % of **3** into **1** at ≤7°C. However, the upper boundary line constructed by connecting the completion temperatures follows a smooth curve, indic-

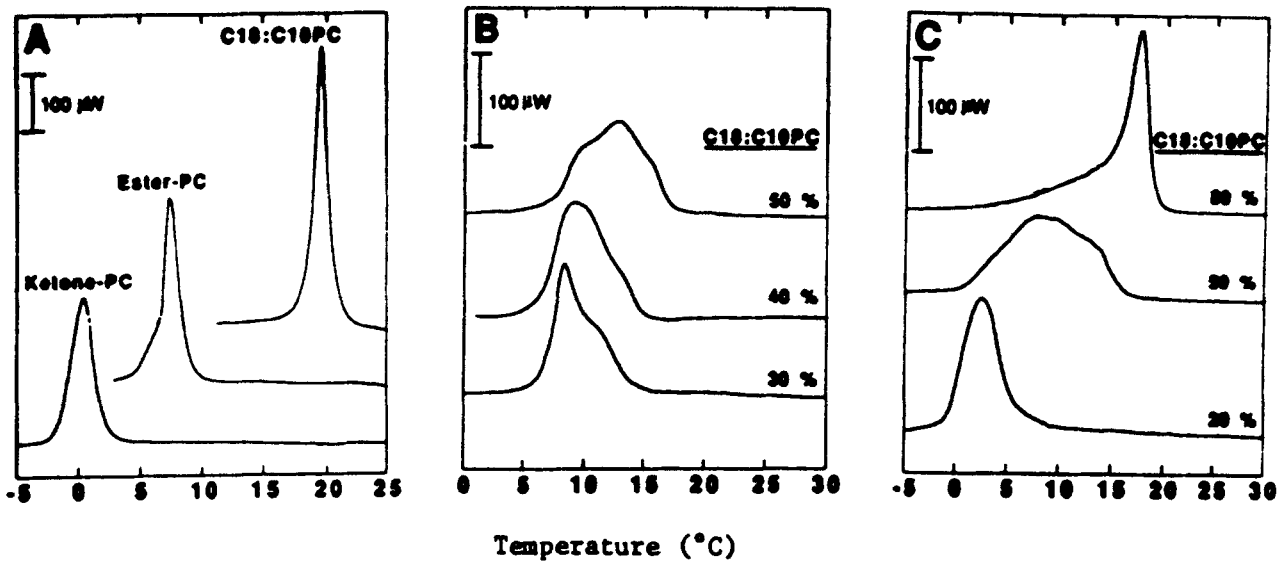

Fig. 1. DSC thermograms of liposomes from (A) pure GPCs: C18:C10-PC (**1**), ketone-PC (**2**), and ester-PC (**3**), and from binary mixtures (B and C) of **1** and carbonyl-terminated PCs **2** and **3**. Representative DSC heating thermograms are shown in B and C for fully hydrated samples of various mol % of **1** in **3** (B) and in **2** (C). In (A), the endotherms are not normalized to the mass of the PC placed in the ampoule.

ative of near ideal mixing behavior for the liquid–crystalline phase.

Above T_m, asymmetric acyl chain PC molecules form a normal bilayer with two acyl chains per lipid head group, but exhibit partial interdigitation, in which the longer acyl chain extends partially into the opposing leaflet, i.e., the short chain of one PC molecule meets end-to-end with the long chain of the opposing PC molecule (6). The conclusion that binary mixtures of **2** or **3** with **1** exhibit complete miscibility above the phase transition temperature suggests that the C=O group does not cause significant disruption of the partially interdigitated packing arrangement. This is consistent with the conclusion that the packing of bilayers formed from **1** above T_m is highly dynamic (17) and disordered (18), although motional restrictions exist (19).

The acyl chains of **1** are nearly fully extended in the highly ordered mixed interdigitated gel-phase bilayers. The partial immiscibility in the gel state induced by **2** and **3**, as indicated by the horizontal segment of the

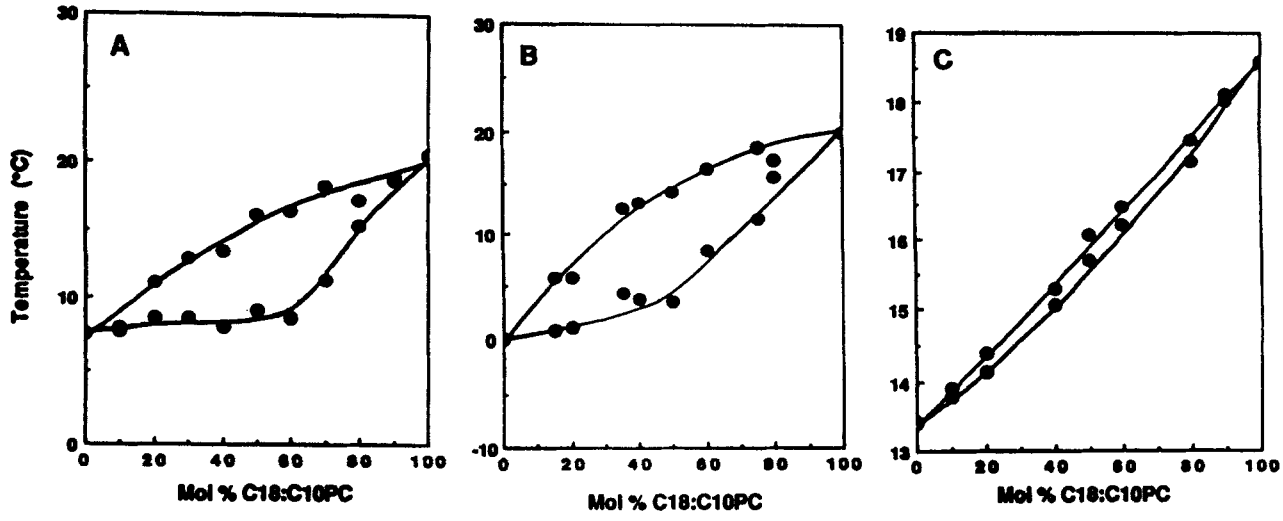

Fig. 2. Temperature–composition phase diagrams of mixtures of **1** with (A) **3**, (B) **2**, and (C) **4**. The solid circles indicate the experimental data (onset and completion temperatures of the DSC transitions). Solid lines define the solidus and liquidus curves after correction for the finite width of the phase transitions of the pure components. For DSC data for **4**, see Ali et al. (20).

solidus curve in Figs. 2A,B, could arise from the disruption of the optimal chain packing because of the dipolar effects of the C=O function or because of steric effects associated with the bulk of the oxygen substituent. As the alkene-terminated PC **4** did not perturb the mixing properties of **1** in the gel phase (Fig. 2C) (20), it is unlikely that the presence of a pi bond can account for the gel-phase immiscibility noted in Figs. 2A,B.

In conclusion, we have shown by using DSC measurements that analogs of aldehyde-terminated PCs that could be generated by autooxidation perturb the gel-state mixing behavior of bilayers formed by **1**, and that electronic or steric effects play a dominant role in accounting for the formation of separate domains of wide compositional ranges of **1** and **3** and of **1** and **2** in the gel phase. However, mixtures of these lipids are miscible in the liquid–crystalline phase, indicating that a carbonyl group near the end of the *sn*-2 chain does not disrupt packing in this state.

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