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Thermotropic phase behavior of mixed-chain phosphatidylglycerols: implications for acyl chain packing in fully hydrated bilayers

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Abstract

In this communication we report the first systematic investigation of the thermodynamic properties of fully hydrated mixed-chain phosphatidylglycerols (PG) using high-resolution differential scanning calorimetry (DSC). The crystal structure of dimyristoylphosphatidylglycerol shows an acyl chain conformation that is nearly opposite to that of phosphatidylcholine (PC). In PC, the *sn*-1 chain is straight while the *sn*-2 chain contains a bend; for PG, the *sn*-1 contains a bend while the *sn*-2 chain is in the all-*trans* conformation (R.H. Pearson, I. Pascher, The molecular structure of lecithin dihydrate, Nature, 281 (1978) 499–501; I. Pascher, S. Sundell, K. Harlos, H. Eibl, Conformational and packing properties of membrane lipids: the crystal structure of sodium dimyristoylphosphatidylglycerol, Biochim. Biophys. Acta, 896 (1987) 77–88). If the structure of PG found in the single crystal can be extrapolated to that in the fully hydrated gel-state bilayer, the observed difference in acyl chain conformations implies that modulation of the acyl chain asymmetry will have an opposite effect on the thermotropic phase behavior of PG and PC. For example, it is expected, based on the crystal structures, that C(15):C(13)PG should have a higher main phase transition temperature (T_m) than C(14):C(14)PG, and C(13):C(15)PG should have a lower T_m than C(14):C(14)PG. However, our DSC studies show clearly that the expectation is not borne out by experimental data. Rather, the T_m values of C(15):C(13)PG, C(14):C(14)PG, and C(13):C(15)PG are 18.2°C, 23.1°C, and 24.4°C, respectively. Several other PGs, each with a unique acyl chain composition, have also been studied in this laboratory using high-resolution DSC. It is shown that the acyl chain conformation of fully hydrated PG in general is nearly opposite to that seen in the PG crystal structure. © 1999 Elsevier Science B.V. All rights reserved.

Keywords: Differential scanning calorimetry; Acyl chain asymmetry; Phase transition temperature

1. Introduction

For a number of years our laboratory has investigated the effects of acyl chain composition on the thermodynamic behavior of diacyl phospholipids. Since both phosphatidylcholine (PC) and phosphatidylethanolamine (PE) are quantitatively the most important lipids in mammalian membranes, the bulk of our work has concentrated on elucidating the effect of acyl chain composition, including acyl chain length and unsaturation, on the thermotropic phase behavior of these phospholipids [1]. An interesting structural parameter that is important in understanding the effect of acyl chain length on the thermotropic phase behavior of both PC and PE is the normalized acyl chain asymmetry, designated as $\Delta C/CL$ [1]. The numerator of this ratio, ΔC, is defined as the effective chain length difference in C-C

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bond length units between the two acyl chains of a phospholipid packed in the gel-state bilayer. The denominator of the ratio, CL, is defined as the effective length of the longer acyl chain. For any C(X):C(Y)PC, where the C(X) designates the number of carbon atoms in the *sn*-1 acyl chain and C(Y) designates the number of carbons in the *sn*-2 chain, the ΔC value of the hydrated gel phase can be calculated from the formula: $\Delta C = |X - Y + 1.5|$. If the *sn*-1 chain of PC is longer than the *sn*-2 chain, then CL can be calculated as $CL = X - 1$; if the *sn*-2 chain is longer than the *sn*-1 chain, then $CL = Y - 2.5$. The normalized acyl chain asymmetry can thus be calculated by dividing ΔC by CL. For PCs with $\Delta C/CL < 0.41$, the lipids are found in the gel-state bilayer with a partially interdigitated packing motif, where the terminal methyl group of the *sn*-1 chain of one PC molecule is facing the *sn*-2 chain of a PC in the opposite leaflet of the bilayer [2]. For $\Delta C/CL \geq 0.41$, the acyl chains are packed in the mixed-interdigitated packing motif at $T < T_m$. In this packing motif, the shorter acyl chain of one PC molecule faces the shorter chain of a PC in the opposite leaflet, and the longer chains of both molecules extend fully across the hydrocarbon core of the lipid bilayer. It is thus possible to predict accurately the packing motif of any saturated PC or PE simply by knowing the lengths of the acyl chains of the phospholipid [3]. The behavior of both PC and PE has been well characterized by this laboratory in the past two decades, and in this present investigation we extend these studies to another class of phospholipids, phosphatidylglycerol (PG).

C(X):C(Y)PG is an anionic diacyl phospholipid with a glycerol moiety attached to the phosphate group. Due to the small amount of PG found in most mammalian cells, fewer studies have been done on PG as compared to PC and PE [4]. Although rarely found in significant quantities, PG is nonetheless sine qua non with respect to the function of a number of membrane-associated proteins and the viability of many cells [5]. Due to the presence of both a negative charge and hydroxyl groups on the headgroup of PG, the potential for electrostatic as well as hydrogen-bonding interactions between PG and other membrane components has been the focus of many investigations. For example, PG must be present for the proper functioning of the *Escherichia coli* phosphotransferase system [6]. PG plays an important role in forming the active structure of the phosphotransferase membrane protein complex. Similarly, PG was found to be important in the α-helix formation of the prePhoE signal peptide, a signal sequence that cannot translocate proteins to the *E. coli* outer membrane properly in the absence of PG [7,8]. Recently, more attention has been given to the study of the physical and structural properties of pure PG bilayers. Zhang et al. studied the thermotropic phase behavior of a series of saturated diacyl identical-chain PGs [9]. It was demonstrated that identical-chain PGs have an extremely complex phase behavior, which is expected with anionic phospholipids. After long-term incubation, 1,2-diacyl PGs typically form high-melting crystalline-like polymorphic phases in a chain length-dependent manner; once these crystalline-like phases are melted, they are not observed upon immediate cooling and reheating. The typical gel-to-liquid crystalline phase transition of 1,2-diacyl PGs is remarkably similar to the main phase transition of 1,2-diacyl PCs, with the qualifier that the main phase transition parameters of identical-chain PGs are all less than those of the corresponding PCs [10]. Spectroscopic studies done on pure PG samples indicate that PG bilayers may be more loosely packed than other lipid [11].

In the crystal structure, the naturally occurring stereoisomer of PG has an acyl chain conformation that is opposite to that found in the PC crystal structure [10]. The *sn*-1 acyl chain of PG in the single crystal has a bend at the C(2) carbon, while all the dihedral angles of the *sn*-2 chain are 180°, corresponding to an all-*trans* conformation. This acyl chain structure seen in PG is opposite to the conformation of PC, as shown in Fig. 1 [11]. If it is assumed that the acyl chain conformation of PG in the single crystal persists in the hydrated gel phase, then C(15):C(13)PG would be predicted to have a smaller chain asymmetry than C(14):C(14)PG due to the bend in the *sn*-1 chain. C(15):C(13)PG would then be expected to have a higher T_m than C(14):C(14)PG, since there is a direct correlation between acyl chain asymmetry and T_m [3]. Similarly, C(13):C(15)PG is predicted to have a larger $\Delta C/CL$ and a lower T_m than C(14):C(14)PG. In this investigation, we report the first ever study of the influence of acyl

chain asymmetry on the main phase transition parameters of 19 molecular species of C(*X*):C(*Y*)PG, each having a different acyl chain composition. Using high-resolution differential scanning calorimetry, we demonstrate that the acyl chain conformation of C(*X*):C(*Y*)PG in the hydrated gel phase is deduced to be nearly opposite to the structure found in the single crystal of PG.

2. Materials and methods

2.1. Semisynthesis of C(X):C(Y)PG

Lysophosphatidylcholines were bought from Avanti Polar Lipids (Birmingham, AL), and fatty acids, phospholipase D, and other chemicals were purchased from Sigma (St. Louis, MO). All chemicals were of reagent grade, and all organic solvents were of spectroscopic grade. Initially, C(*X*):C(*Y*)PC was semisynthesized according to the modified procedure of Mena and Djerassi [12] as previously described by this laboratory [13]. The C(*X*):C(*Y*)PC was converted to the corresponding C(*X*):C(*Y*)PG using a procedure similar to that used by Dawson [14]. Briefly, C(*X*):C(*Y*)PG was produced from C(*X*):C(*Y*)PC by the transphosphatidylation reaction as catalyzed by phospholipase D from cabbage in the presence of excess glycerol. A two-phase solvent system of equal amounts of diethyl ether and aqueous buffer (40 mM $CaCl_2$, pH 5.6) was used. Upon completion of the reaction, the ether phase was evaporated, and the lipid was extracted from the aqueous phase using an organic solution of $CHCl_3$:MeOH (5:1). This organic phase was then washed with four different aqueous solutions in this order: (1) 2 M NaCl, (2) 200 mM EDTA, (3) 5 mM HCl, and (4) distilled water. The PG was dried and purified using silica gel 60 Å (mesh size: 230–400) column chromatography. All lipids synthesized were 99% pure as deduced from thin-layer chromatography, which was done on silica gel 60 Å plates (Whatman) using a solvent system of chloroform:methanol:25% NH_4OH (60:30:5).

Table 1
Thermodynamic values associated with the main phase transition of a series of homologous C(*X*):C(*Y*)PCs and C(*X*):C(*Y*)PGs

C(*X*):C(*Y*)PG	Δ*C*/CL	T_m (°C)	Δ*H* (kcal/mol)	Δ*S* (cal/mol/K)
C(12):C(20)PG	N/A*	25.3	5.3	17.8
C(13):C(19)PG	N/A	31.5	6.6	21.7
C(14):C(18)PG	N/A	35.6	8.5	28.5
C(15):C(17)PG	N/A	41.2	14.3	45.5
C(16):C(16)PG	N/A	40.6	10.3	32.8
C(17):C(15)PG	N/A	36.2	9.3	30.1
C(18):C(14)PG	N/A	30.2	6.4	21.1
C(19):C(13)PG	N/A	23.3	4.7	15.9
C(20):C(12)PG	N/A	32.6	15.6	51.0
C(10):C(22)PC	0.538	37.8	11.9	38.3
C(11):C(21)PC	0.460	32.6	9.4	30.7
C(12):C(20)PC	0.371	25.6	5.7	19.1
C(13):C(19)PC	0.271	32.6	7.2	23.5
C(14):C(18)PC	0.161	39.3	7.9	25.3
C(15):C(17)PC	0.035	41.7	10.1	34.9
C(16):C(16)PC	0.100	41.5	8.5	27.0
C(17):C(15)PC	0.219	37.7	7.4	23.8
C(18):C(14)PC	0.324	29.6	5.5	17.2
C(19):C(13)PC	0.417	23.8	5.0	16.8
C(20):C(12)PC	0.500	34.5	11.5	37.4

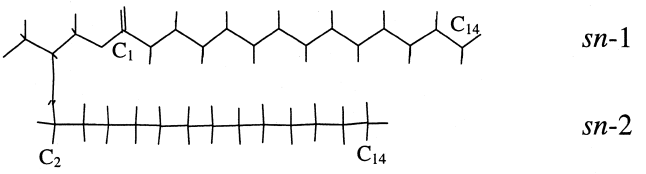
The total number of carbons in the acyl chains of each lipid is 32. The C(*X*):C(*Y*)PC data were previously published by this laboratory [15].

*The acyl chain asymmetry values for C(*X*):C(*Y*)PG cannot be calculated because the ΔC_{ref} of a diacyl PG has not been experimentally determined yet.

2.2. Differential scanning calorimetry

4 mg of C(*X*):C(*Y*)PG was dispersed in 2 ml aqueous buffer (50 mM HEPES, 150 mM NaCl, 1 mM EDTA, pH 7.4) to make a final lipid concentration of 3–5 mM. Samples were stored at 4°C for at least 24 h prior to injection into the calorimeter. The DSC measurements were made using a Microcal MC-2 high-resolution differential scanning calorimeter (Microcal, Northampton, MA). The methods of data capture and analysis are the same as previously described by this laboratory [2,3]. Only the second or third heating scans are reported in this communication in order to avoid any thermal history-dependent transition behavior. The main phase transition temperature (T_m) is defined as the temperature at which the heat capacity reaches a maximum. The enthalpy (ΔH) of the main phase transition is measured by calculating the area under the curve of the transition peak divided by the lipid concentration. The transition entropy (ΔS) is calculated by the relationship of $\Delta S = \Delta H/T_m$. The width of the peak ($\Delta T_{1/2}$) is defined as the width of the transition peak when the heat capacity is at one-half the maximal value.

C(14):C(14)PC



C(14):C(14)PG

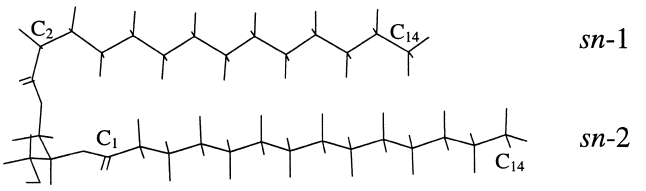


Fig. 1. Molecular graphics representations of the diacyl glyceride moieties of the energy-minimized crystal structures of C(14):C(14)PC and C(14):C(14)PG. Initial atomic coordinates were taken from Pearson and Pascher [11] and Pascher et al. [10], respectively. Structures were minimized using Allinger's MM2 program [24]. Note that the *sn*-1 chain of C(14):C(14)PC is straight while the *sn*-2 chain is bent; in C(14):C(14)PG, the *sn*-1 chain contains the bend while the *sn*-2 chain is straight. Figures were generated using HyperChem 5.0 (Hypercube, Gainesville, FL).

3. Results

In this investigation, we report the first study that has been carried out to elucidate the effect of acyl chain asymmetry on the thermotropic phase behavior of mixed-chain C(*X*):C(*Y*)PGs. Nineteen molecular species comprising two homologous series of C(*X*):C(*Y*)PG were semisynthesized and their thermotropic phase behavior was investigated using high-resolution DSC. Fig. 2 shows the DSC scans obtained with one homologous series of C(*X*):C(*Y*)PGs. In this series, the total number of carbons contained in the acyl chains of each C(*X*):C(*Y*)PG is 32; i.e., each lipid in Fig. 2 has a molecular weight equivalent to that of C(16):C(16)PG. The main phase transition parameters of each of the DSC scans in Fig. 2 are listed in Table 1 along with the transition parameters of the corresponding C(*X*):C(*Y*)PCs, which were previously published from this laboratory [15]. As a side note, it is apparent from the data in Tables 1 and 2 that the overall trend in the entropies in each homologous series is the same as that seen in the transition enthalpy as well as the T_m of the lipids in each series. As such, for each lipid in the two homologous series of mixed-chain PG, the main contribution to T_m is enthalpic, not entropic, due to $T_m = \Delta H/\Delta S$. Consequently, only the transition temperature and transition enthalpy of each lipid will be discussed in detail. In this homologous series of PGs, the lipid with the shortest *sn*-1 chain, C(12):C(20)PG, has a main phase transition temperature of 25.3°C with a ΔH of 5.3 kcal/mol. The transition peak is rather low and broad when compared to the other lipids shown in Fig. 2. C(13):C(19)PG has a T_m of 31.5°C and a transition enthalpy of 6.6 kcal/mol. The peak of C(13):C(19)PG is larger and sharper than that of C(12):C(20)PG. C(14):C(18)PG has a higher transition temperature (35.6°C) and transition enthalpy (8.5 kcal/mol) than C(13):C(19)PG, along with a sharper peak. This trend of larger and sharper peaks, as well as larger transition parameters, is observed as the length of the *sn*-1 chain is lengthened from C(*X*) = 12 to C(*X*) = 15 and the length of the *sn*-2

chain is concomitantly shortened from C(Y) = 20 to C(Y) = 17.

The DSC scan of C(14):C(18)PG has a T_m of 35.6°C and ΔH of 8.5 kcal/mol. C(15):C(17)PG has the highest transition parameters, with T_m = 41.2°C and ΔH = 14.3 kcal/mol. Interestingly, it is predicted, based on the crystal structure of DMPG [10] and previous studies with other phospholipids in this laboratory [3,14,15] that fully hydrated C(15):C(17)PG should have main phase transition parameters that are lower than those of C(16):C(16)PG. However, the observed values of the phase transition parameters of C(15):C(17)PG are all higher than those of C(16):C(16)PG, which has a T_m of 40.6°C and a transition enthalpy of 10.3 kcal/mol. The width of the C(16):C(16)PG transition peak is similar in sharpness to that of C(15):C(17)PG ($\Delta T_{1/2} \approx 0.4$°C), and the transition parameters of C(16):C(16)PG are similar to those previously reported [16,17]. As already mentioned, it was expected, based on the DMPG crystal structure, that the T_m of C(17):C(15)PG should be higher than the T_m of C(16):C(16)PG. However, as seen in Fig. 2 and Table 1, the T_m of C(17):C(15)PG is 36.2°C, more than 4°C below the T_m of C(16):C(16)PG; the corresponding ΔH is also lower: 9.3 kcal/mol for C(17):C(15)PG versus 10.3 kcal/mol for DPPG. As the *sn*-1 acyl chain length further increases, all of the transition parameters for each lipid decrease. For example, C(18):C(14)PG has a T_m of 30.2°C with a ΔH of 6.4 kcal/mol, and C(19):C(13)PG has a T_m of 23.2°C and a ΔH of 4.7 kcal/mol. Once the *sn*-1 acyl chain reaches a length of 20 carbons, the parameters all increase again. C(20):C(12)PG has a main phase transition temperature of 32.6°C and a transition enthalpy of 15.6 kcal/mol.

Fig. 3 shows a summary plot of the T_m data of the homologous series of C(X):C(Y)PGs with molecular weight equivalent to that of C(16):C(16)PG. The main phase transition temperature of the DSC scans from Fig. 2 are plotted as a function of the *sn*-1 chain length of each lipid. The data of the corre-

Table 2
Main phase transition parameters of two homologous series of C(X):C(Y)PGs and C(X):C(Y)PCs

C(X):C(Y)PC	ΔC/CL	T_m (°C)	ΔH (kcal/mol)	ΔS (cal/mol/K)
C(9):C(19)PG	N/A*	17.6	5.8	20.0
C(10):C(18)PG	N/A	10.6	4.3	15.0
C(11):C(17)PG	N/A	11.7	4.4	15.5
C(12):C(16)PG	N/A	21.1	5.9	20.2
C(13):C(15)PG	N/A	24.4	6.8	22.9
C(14):C(14)PG	N/A	23.1	6.0	20.4
C(15):C(13)PG	N/A	18.2	4.2	14.5
C(16):C(12)PG	N/A	9.7	3.9	13.7
C(17):C(11)PG	N/A	10.9	6.5	22.9
C(18):C(10)PG	N/A	16.7	8.9	30.7
C(19):C(9)PG	N/A	13.8	7.9	27.7
C(9):C(19)PC	0.515	19.6	11.3	38.6
C(10):C(18)PC	0.419	11.1	6.2	21.8
C(11):C(17)PC	0.310	13.8	4.9	16.3
C(12):C(16)PC	0.185	21.7	5.7	19.3
C(13):C(15)PC	0.040	25.5	6.0	20.1
C(14):C(14)PC	0.115	24.1	6.0	20.5
C(15):C(13)PC	0.250	18.8	5.3	18.2
C(16):C(12)PC	0.367	11.3	4.5	15.8
C(17):C(11)PC	0.469	12.8	6.9	24.1
C(18):C(10)PC	0.559	18.7	9.0	30.8
C(19):C(9)PC	0.639	13.3	8.3	29.0

The total number of carbons in the acyl chains of each lipid is 28. Data for C(X):C(Y)PCs were previously published by this laboratory [14].

*The acyl chain asymmetry values for C(X):C(Y)PG cannot be calculated because the ΔC_{ref} of a diacyl PG has not been experimentally determined yet.

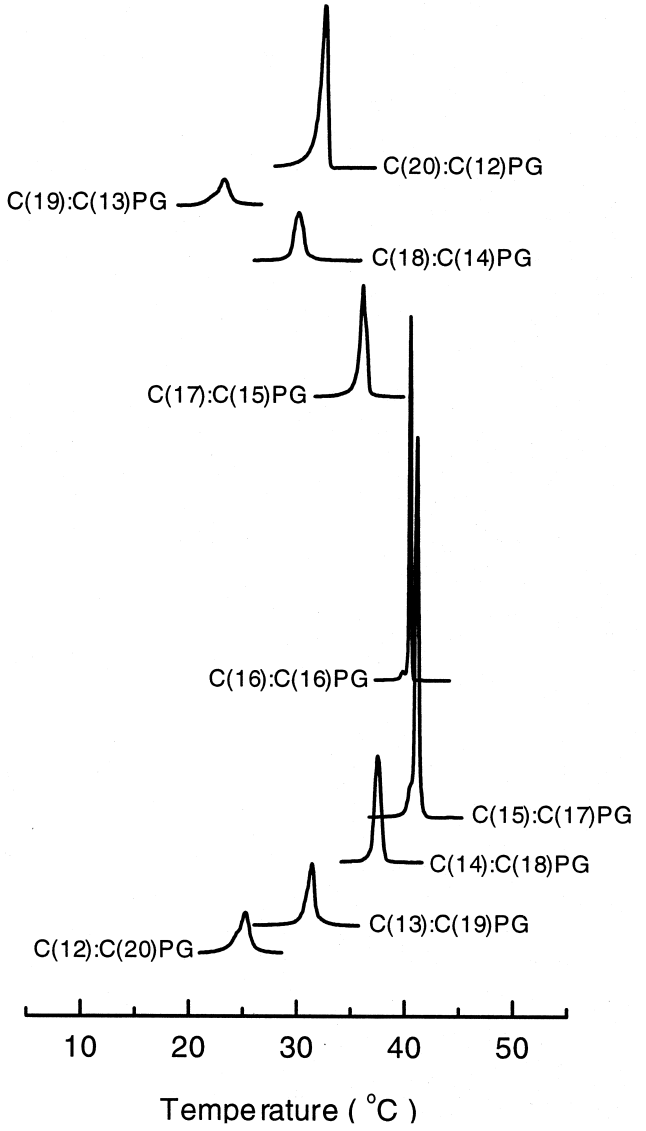


Fig. 2. DSC scans of nine molecular species of C(X):C(Y)PG, each with a molecular weight equivalent to that of C(16):C(16)PG. The third heating scan of each lipid is shown; the nominal scan rate was 15°C/h.

sponding C(X):C(Y)PCs, previously reported by this laboratory [15], are also plotted in Fig. 3 for comparison. It can be seen that for both C(X):C(Y)PG and C(X):C(Y)PC, the lipid with C(X) = 15 has the highest main phase transition temperature. The T_m values of the lipids all decrease as the *sn*-1 chain length increases or decreases away from C(X) = 15. For the C(X):C(Y)PC series, this behavior is explained by the fact that the lipids near C(X) = 15 adopt the partially interdigitated packing motif, where the *sn*-1 chain of one lipid is juxtaposed with the *sn*-2 chain of another lipid from the opposite leaflet, thus forming a transbilayer dimer. As the acyl chain asymmetry of the C(X):C(Y)PC increases, the region of overlap of the acyl chains becomes larger. This increased overlap causes the packing of the hydrocarbon chains to become more and more disrupted, thus decreasing the main phase transition parameters [1]. Only at C(X) = 20 does the plot deviate from the overall trend. Both lipids with C(X) = 20 have higher T_m values than the corresponding lipids at C(X) = 19. It has been shown that this higher melting temperature seen in highly asymmetric phosphatidylcholines and phosphatidylethanolamines is a result of the lipid adopting the mixed-interdigitated packing motif, where the shorter acyl chain of one lipid in a bilayer is juxtaposed with the shorter chain of another lipid from the opposite bilayer leaflet, and the longer acyl chains of both lipids extend fully across the bilayer [3,14,18]. Most interestingly, the trend seen in the T_m values of the C(X):C(Y)PC series is exactly the same trend observed in the transition temperatures of the corresponding C(X):C(Y)PG series. This similarity in the behavior of the transition parameters observed in the two homologous series of PC and PG shown in Figs.

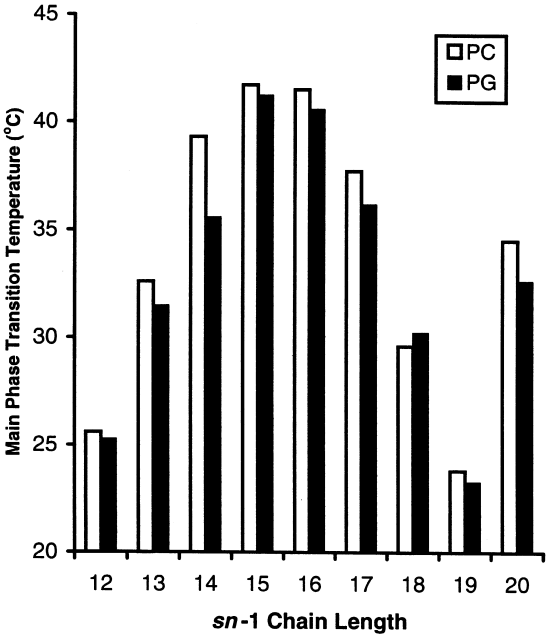


Fig. 3. Plot of T_m versus *sn*-1 chain length of two homologous series of C(X):C(Y)PC and C(X):C(Y)PG. The total number of carbons in the acyl chains of each lipid is 32.

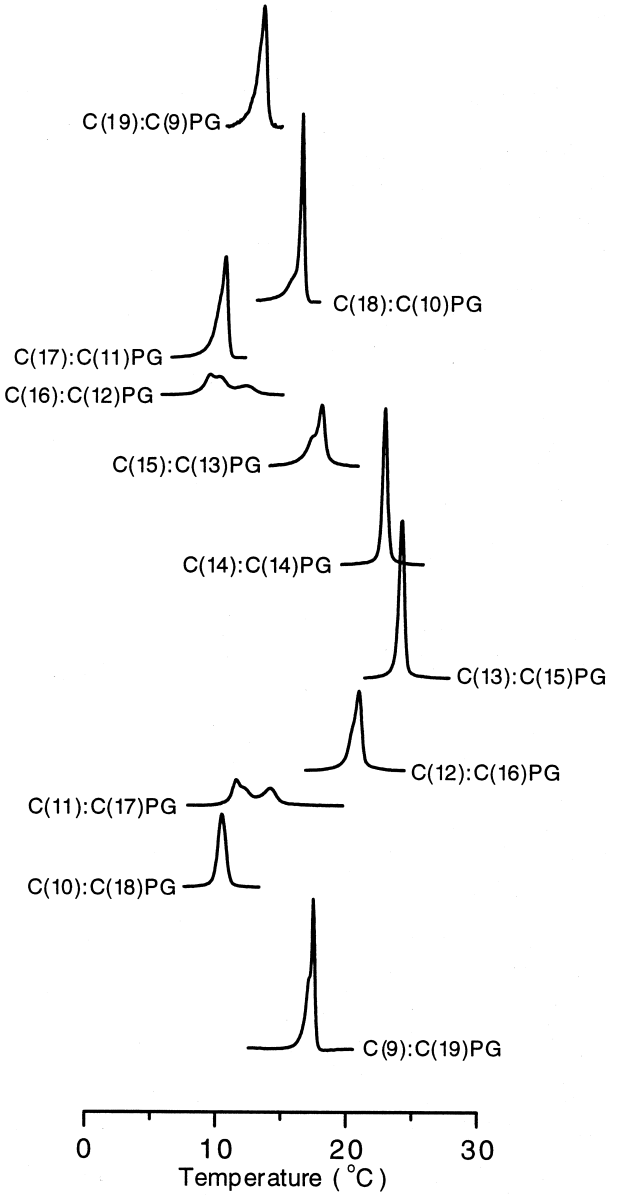


Fig. 4. DSC scans of 13 molecular species of C(X):C(Y)PG, each with a molecular weight equivalent to that of C(14):C(14)PG.

2 and 3 is also observed for the homologous series where the total number of carbons in the acyl chains is 28 (Fig. 5).

Shown in Fig. 4 are the DSC scans of the homologous series of C(X):C(Y)PGs with molecular weight equivalent to that of C(14):C(14)PG. The transition parameters associated with each PG shown in Fig. 4 are listed in Table 2 along with the corresponding C(X):C(Y)PCs that were previously published by this laboratory [14]. The lipid with the shortest *sn*-1 acyl chain, C(9):C(19)PG, has a T_m of 17.6°C and a ΔH of 5.8 kcal/mol. The transition peak of C(9):C(19)PG has a $\Delta T_{1/2}$ of 0.6°C. The lipid with the next shortest *sn*-1 acyl chain, C(10):C(18)PG, has a smaller, broader peak with lower transition parameters ($T_m = 10.6$°C and $\Delta H = 4.3$ kcal/mol) than C(9):C(19)PG. However, this seemingly downward trend in the transition parameters is reversed for the subsequent PGs, where the transition parameters all increase as the *sn*-1 acyl chain increases from C(X) = 10 to C(X) = 13.

For example, C(11):C(17)PG has a melting temperature of 11.7°C and a transition enthalpy of 4.4 kcal/mol, which are higher than the corresponding values for C(10):C(18)PG. The transition peak of C(11):C(17)PG shows two overlapped peaks of nearly equal height. This pattern is very similar to the main phase transition peak observed for C(11):C(17)PC. For C(11):C(17)PC, two peaks are observed in the heating thermogram due to the overlap of the pretransition and the main phase transition peaks [19]. C(12):C(16)PG melts at 21.1°C with $\Delta H = 5.9$ kcal/mol. Among the lipids within this homologous series, C(13):C(15)PG has the highest

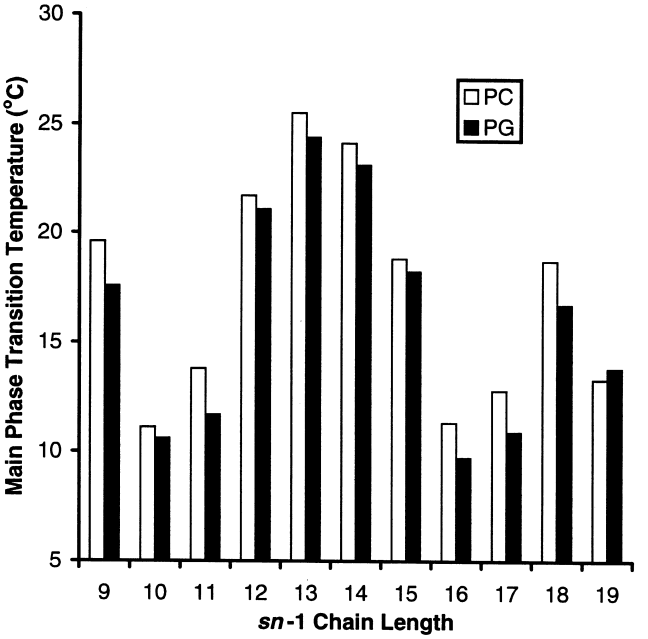


Fig. 5. Plot of T_m versus *sn*-1 chain length of two homologous series of C(X):C(Y)PC and C(X):C(Y)PG. The total number of carbons in the acyl chains of each lipid is 28.

main phase transition temperature, with T_m = 24.4°C and ΔH = 6.8 kcal/mol. As the *sn*-1 acyl chain length is further increased, all of the main phase transition parameters decrease. For example, C(14): C(14)PG has a T_m of 23.1°C with a ΔH of 6.0 kcal/mol. C(15):C(13)PG, which was expected to have a higher T_m than DMPG, has a melting temperature of 18.2°C and a transition enthalpy of 4.2 kcal/mol. This downward trend is seen from C(X) = 13 to C(X) = 16. C(16):C(12)PG has the lowest melting temperature among the lipids studied in this investigation, with a T_m of 9.7°C and a ΔH of 3.9 kcal/mol.

Once the number of carbons in the *sn*-1 chain reaches above C(X) = 16 in this homologous series, the transition parameters are observed to increase with increasing *sn*-1 chain length. C(17):C(11)PC melts from the gel phase to the liquid crystalline phase at 10.9°C, and the transition enthalpy is 6.5 kcal/mol; the peak of the main phase transition of C(17):C(11)PG is much sharper than C(16): C(12)PG. C(18):C(10)PG has an even sharper main phase transition at T_m = 16.7°C with ΔH = 8.9 kcal/mol. C(19):C(9)PG does not follow this trend, instead having a T_m of 13.8°C and a ΔH of 7.9 kcal/mol. This trend in the melting temperatures seen in this homologous series of C(X):C(Y)PG, as well as the other homologous C(X):C(Y)PG series described above, is exactly the same trend that is observed in the corresponding two C(X):C(Y)PC homologous series. The implications of the similarity in the trends observed in the main phase transition parameters of C(X):C(Y)PG and C(X):C(Y)PC will be discussed in some detail in Section 4.

4. Discussion

Previous studies in this laboratory have delineated the correlation between the normalized acyl chain asymmetry and the trend in the main phase transition parameters observed in saturated and unsaturated diacyl phospholipids. Most of these studies have focused on PC and PE, the two most abundant phospholipids in mammalian membranes [4]. In the present investigation, we have extended these studies to another phospholipid group: the phosphatidylglycerols. Using high-resolution DSC, we have characterized the main phase transition parameters of two homologous series of C(X):C(Y)PG. The results presented herein are rather surprising, considering the predictions that were made based on the acyl chain conformation of the single crystal structure of DMPG. We will begin our analysis by outlining the predictions made based on the crystal structure, and then we will demonstrate how the results of our experiments with fully hydrated mixed-chain PGs indicate that the acyl chain conformation of the hydrated gel phase of C(X):C(Y)PG is nearly opposite to that observed in the single crystal.

The high-resolution DSC results reported herein show that fully hydrated C(15):C(17)PG has a higher T_m than C(16):C(16)PG, and C(17):C(15)PG has a lower T_m than C(16):C(16)PG. Similarly, C(13): C(15)PG has a higher T_m than C(14):C(14)PG, and C(15):C(13)PG has a lower T_m than C(14):C(14)PG. The prediction was that in a homologous series C(X+1):C(X−1)PG would have a higher T_m than C(X):C(X)PG, and C(X−1):C(X+1)PG would have a lower T_m than C(X):C(X)PG. However, it is shown calorimetrically that C(X−1):C(X+1)PG has a *higher* T_m than C(X):C(X)PG, and C(X+1):C(X−1)PG has a *lower* T_m than C(X):C(X)PG. These observations cannot be explained by any structural model where the *sn*-1 chain contains a bend and the *sn*-2 chain is completely straight. If C(X−1):C(X+1)PG has higher phase transition parameters than C(X):C(X)PG, then C(X−1):C(X+1)PG must have a lower acyl chain asymmetry than C(X):C(X)PG. The only way that this can be true is if the gel phase conformation of the acyl chains of PG is such that the *sn*-2 chain is effectively shorter than the *sn*-1 chain. If there was an effective shortening of the *sn*-2 chain, then results similar to the thermotropic phase behavior of PC would be expected, and indeed this is what is observed for PG.

In both homologous PG series reported in this investigation, C(X−1):C(X+1)PG has a higher T_m than C(X):C(X)PG, just as C(X−1):C(X+1)PC has a higher T_m than C(X):C(X)PC, as listed in Tables 1 and 2 [14,15]. As the numerical chain length inequivalence – i.e., the quantity $|X-Y|$ – is further increased, the phase transition parameters all decrease. This is expected since, regardless of the conformation of the acyl chains, a large numerical inequivalence in the acyl chains will cause a larger chain asymmetry,

leading to greater terminal methyl overlap and acyl chain destabilization. As the asymmetry becomes very large, the partially interdigitated packing motif becomes more unstable than the mixed-interdigitated packing motif, so the lipid will adopt the latter conformation. Thus, the transition parameters of C(20):C(12)PG are higher than those of C(19):C(13)PG; the T_m of C(17):C(11)PG is higher than that of C(16):C(12)PG; and the T_m of C(9):C(19)PG is higher than that of C(10):C(18)PG. As the chain inequivalence is further increased, the transition parameters all increase accordingly, since the mixed-interdigitated packing motif is stabilized by the increased van der Waals contacts in more asymmetric phospholipids [3,14].

Several aspects of PG behavior that have been previously reported point to some possible reasons why the acyl chain conformation of C(X):C(Y)PG in the fully hydrated gel phase is nearly opposite to the chain conformation seen in the single crystal structure. When incubated at low temperatures for long periods of time, diacyl PG tends to form a high-melting stable gel phase [20]. The melting temperature and the transition enthalpy of this unusual phase transition are much higher than those of the normal gel-to-liquid crystalline phase transition. Using various spectroscopic methods, it was determined that this high-melting gel phase contains interlipid hydrogen bonds, something that has not been reported for the normal fully hydrated gel phase [9,20]. Moreover, the headgroup region as well as the hydrocarbon core are packed more tightly. This stable gel phase has also been reported to be less hydrated than the normal gel phase [9], indicating that it has more crystal-like properties than does the fully hydrated gel phase. Furthermore, in the fully hydrated gel phase, it has been observed that the headgroup structure and motional properties of PG and PC are very similar [21,22]. Indirect evidence seems to indicate that PC and PG behave similarly, and this present investigation demonstrates for the first time that the acyl chain conformation of C(X):C(Y)PG is similar in nature to that of C(X):C(Y)PC. This represents a rare situation where the molecular conformation observed in the single crystal is discernibly different from that of the fully hydrated structure.

4.1. Estimation of the acyl chain asymmetry of C(X):C(Y)PG

Although advanced spectroscopic methods must be used in the future to determine the exact value of ΔC for C(X):C(Y)PG, an estimation of ΔC can be made from the DSC data presented in this investigation. It is known that for C(X):C(Y)PC, the equation for the unnormalized acyl chain asymmetry is $\Delta C = |(X-1)-(Y-1-\Delta C_{ref})| = |X-Y+\Delta C_{ref}|$, where $\Delta C_{ref} = 1.5$ [3]. This equation was derived from the fact that the *sn*-1 chain of gel phase PC is in the all-*trans* conformation and the *sn*-2 chain contains a bend at the C(2) position [1]. The bend in the *sn*-2 chain shortens the effective length of this acyl chain, thus creating an inherent chain length asymmetry even in identical-chain PC. The chain asymmetry of C(16):C(16)PC, as determined from neutron diffraction, is about 1.5 C-C bond lengths.

If the crystal structure of PG is assumed to be similar to the hydrated gel phase structure [10], then the *sn*-1 chain is expected to be bent, and the *sn*-2 chain would be straight. In this case, the *sn*-1 chain, in C-C bond lengths, is effectively less than the total number of carbon atoms in the acyl chain. Thus, the appropriate chain length for the *sn*-1 chain would be $X-1-\Delta C_{ref}$ and the length for the *sn*-2 chain would simply be Y, the number of carbons in the chains. The equation for the expected C(X):C(Y)PG gel phase would be $\Delta C = |(X-1-\Delta C_{ref})-(Y-1)| = |X-Y-\Delta C_{ref}|$. Using this equation, and assuming that $0 < \Delta C_{ref} < X$ or Y, it would be expected that the unnormalized asymmetry of C(17):C(15)PG would be less than the value for C(16):C(16)PG. The T_m of C(17):C(15)PG would be predicted to be greater than that of C(16):C(16)PG, and the T_m of C(15):C(17)PG would be lower than that of C(16):C(16)PG. However, the DSC results presented in this present investigation clearly demonstrate that the T_m of C(17):C(15)PG is actually less than the T_m of C(16):C(16)PG. Indeed, the T_m of C(15):C(17)PG is higher than that of C(16):C(16)PG. It can be deduced from these results that the actual formula for calculating the chain asymmetry of C(X):C(Y)PG is $\Delta C = |X-Y+\Delta C_{ref}|$, which is in fact the same formula for C(X):C(Y)PC. Furthermore, using the DSC data presented in this

investigation, it is possible to estimate the value of ΔC_{ref} for C(X):C(Y)PG.

The value of ΔC_{ref} can be estimated from the trend seen in the T_m values of either series of homologous C(X):C(Y)PG. For example, it can be seen from the asymmetry equation that:

$$\Delta C^{C(14):C(18)PG} = |14-18+\Delta C_{ref}| = |-4+\Delta C_{ref}|$$

$$\Delta C^{C(15):C(17)PG} = |15-17+\Delta C_{ref}| = |-2+\Delta C_{ref}|$$

$$\Delta C^{C(16):C(16)PG} = |16-16+\Delta C_{ref}| = |\Delta C_{ref}|$$

$$\Delta C^{C(17):C(15)PG} = |17-15+\Delta C_{ref}| = |2+\Delta C_{ref}|.$$

The T_m values for C(14):C(18)PG, C(15):C(17)PG, C(16):C(16)PG, and C(17):C(15)PG are 37.6°C, 41.2°C, 40.6°C, and 36.2°C, respectively. Since it is expected that the acyl chain asymmetry of a phospholipid is inversely proportional to its T_m, the following relations can be deduced from the above data:

$$T_m^{C(15):C(17)PG} > T_m^{C(16):C(16)PG} > T_m^{C(14):C(18)PG} > T_m^{C(17):C(15)PG}$$

$$\Delta C^{C(15):C(17)PG} < \Delta C^{C(16):C(16)PG} < \Delta C^{C(14):C(18)PG} < \Delta C^{C(17):C(15)PG}$$

$$|-2+\Delta C_{ref}| < |\Delta C_{ref}| < |-4+\Delta C_{ref}| < |2+\Delta C_{ref}|$$

$$|-2+\Delta C_{ref}| < |\Delta C_{ref}| \quad (1)$$

$$|\Delta C_{ref}| < |-4+\Delta C_{ref}| \quad (2)$$

$$1 < \Delta C_{ref} < 2$$

In order for both Eqa. 1 and 2 to be true, ΔC_{ref} must be in the range of $\Delta C_{ref} \in \{1,2\}$. The range of permissible values for the acyl chain asymmetry of identical chain C(X):C(X)PG is between 1 and 2 C-C bond lengths. It is most interesting to note that the ΔC_{ref} for C(X):C(Y)PC is 1.5, as measured by neutron diffraction [23].

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