Biochimica et Biophysica Acta 902 (1987) 1–10
Elsevier

BBA 73613

The influence of unsaturation on the phase transition temperatures of a series of heteroacid phosphatidylcholines containing twenty-carbon chains

K.M.W. Keough, B. Giffin and N. Kariel

Department of Biochemistry, Memorial University of Newfoundland, St. John's, Newfoundland A1B 3X9 (Canada)

(Received 19 January 1987)
(Revised manuscript received 22 April 1987)

Key words: Phosphatidylcholine, mixed-acid; Model membrane; Liposome; Polyunsaturated lipid; Phase transition temperature

A series of heteroacid *sn*-1,2 diacyl phosphatidylcholines (PC) with twenty-carbon fatty acyl chains has been synthesized. Each PC contained eicosanoate (20:0) in the *sn*-1 position and one of a group of eicosaenoic acids with increasing numbers of *cis* double bonds in the *sn*-2 position. The double bonds were at positions Δ11 (20:1), Δ11,14 (20:2), Δ11,14,17 (20:3), or Δ5,8,11,14 (20:4). The disaturated PC containing two eicosanoate chains was also studied. Aqueous dispersions of these PC were analyzed by differential scanning calorimetry, and data for the gel to liquid-crystalline transitions (given as PC: T_c (°C), T_{max} (°C), ΔH(kcal/mol)) were as follows – 20:0-20:0 PC: 66.8, 68.4, 15; 20:0-20:1 PC: 19.8, 22.2, 8; 20:0-20:2 PC: −4.3, 1.8, 5; 20:0-20:3 PC: 1.2, 4.4, 7; 20:0-20:4 PC: −10.7, −6.8, 3. Double bonds in excess of two per chain did not substantially change the transition temperatures of these heteroacid PC. There was a small effect of the location of the multiple double bonds on the transition temperature. The data is consistent with the model that the transition temperatures are determined by a balance between a decrease in the packing density in the gel and a decrease in the rotational freedom of the chains in the liquid crystal, both caused by the double bonds ((1983) Biochemistry 22, 1466–1473).

Abbreviations: DSC, differential scanning calorimetry; $T_{G\text{-}LC}$, gel to liquid-crystalline transition temperature; T_c, the temperature of the gel to liquid-crystalline transition measured calorimetrically as the point of intersection of the baseline and the tangent to the leading edge of the transition; T_{max}, the temperature at which the maximum heat flows into or out of a sample undergoing a transition; PC, 1,2-diacyl-*sn*-glycero-3-phosphocholine; 20:0-20:*x* PC, a PC containing eicosanoate (arachidate) at the *sn*-1 position and one of a series of twenty carbon chains with varying numbers of double bonds at the *sn*-2 position; 20:0-20:0 PC, 1,2-dieicosanoyl-*sn*-glycerol-3-phosphocholine; 20:0-20:1 PC, 1-eicosanoyl-2-*cis*-eicosa-11-enoyl-*sn*-glycero-3-phosphocholine; 20:0-20:2 PC, 1-eicosanoyl-2-*cis,cis*-eicosa-11,14-dienoyl-*sn*-glycero-3-phosphocholine; 20:0-20:3 PC, 1-eicosanoyl-2-*cis,cis,cis*-eicosa-11,14,17-trienoyl-*sn*-glycero-3-phosphocholine; 20:0-20:4 PC, 1-eicosanoyl-2-*cis,cis,cis,cis*-eicosa-5,8,11,14-tetraenoyl-*sn*-glycero-3-phosphocholine.

Correspondence: K.M.W. Keough, Department of Biochemistry, Memorial University of Newfoundland, St. John's, Newfoundland, Canada, A1B 3X9.

Introduction

The phospholipids of many biological membranes, especially those from plants and animals, contain substantial amounts of fatty acids in which there are two or more double bonds per chain. While polyunsaturated chains are present in all phospholipid classes, in a number of membranes some classes have more of these acids than do others. Phosphatidylinositol and its phosphorylated derivatives are remarkable in that these lipids from almost all mammalian sources have quite high contents of arachidonic acid. In terms of total mass, however, phosphatidylcholines and phosphatidylethanolamines account for most of the polyunsaturated lipids in many membranes.

Until recently conventional wisdom would have it that the polyunsaturated lipids would be expected to have very low phase transition tempera-

tures. Altering the amount of polyenoic chains in the lipids of membranes might be expected to influence lipid chain motion and related viscosity at any given temperature. Unsaturation indices, which give equal weight to each double bond, have been used as guides to the assessment of the physical state of membrane lipids. Recent work on the properties of the transitions of heteroacid (mixed-acid) lipids with more than one double bond suggests that such inferences should be drawn with care. These studies have shown that progressive increases in the numbers of double bonds per chain of heteroacid lipids did not lead to progressive proportional decreases in $T_{G\text{-}LC}$ or in the order and motion of the lipid chain [1–3]. Thus the unsaturation index may be misleading as an indicator of the physical state of a membrane especially in cases where large amounts of polyunsaturated chains are present.

Previous studies on the physical properties of model membranes made of heteroacid lipids with polyenoic chains have included comparisons among lipids with chains of two different lengths in addition to different extents of unsaturation, both of which can influence the physical properties. To avoid the complicating effects of differing chain lengths, we have synthesized and measured $T_{G\text{-}LC}$ for a series of PC which contain arachidonic acid (20 : 0) in the *sn*-1 position and a series of 20-carbon chains with interesting numbers of double bonds in the *sn*-2 position.

Materials and methods

Materials

Dieicosanoyl (diarachidoyl) PC (20 : 0-20 : 0 PC) was purchased from Avanti Polar Lipids (Birmingham, AL) and Sigma Chemical Co. (St. Louis, MO). After transmethylation, only one peak with the retention time of methyl arachidate on an SP2330 column (Supelco, Inc., Bellefonte, PA) was observed for each lot [4]. The anhydrides of the four twenty-carbon fatty acids with *cis* double bonds at Δ11, Δ11,14, Δ11,14,18, and Δ5,8,11,14 were obtained from Nu Chek Prep (Elysian, MN). Each anhydride gave only one peak of the appropriate retention time on analysis of the methyl esters by gas-liquid chromatography.

Hydroquinone and 4-dimethylaminopyridine were bought from Sigma, and 4-pyrrolidinopyridine from Fluka AG, Chem. Fabrik (Hauppauge, NY). These materials were recrystallized before use (hydroquinone from chloroform; dimethylaminopyridine from chloroform/diethyl ether (1:1, v/v); pyrrolidinopyridine from petroleum ether, bp. 35–60 °C).

Crotalus adamanteus venom was obtained from the Miami Serpentarium, Miami, FL.

Routine chemicals were obtained from Sigma or Fisher Scientific (Dartmouth, NS). Solvents were obtained from Fisher Scientific and BDH Chemicals (Dartmouth, NS), except for 95% ethanol which came from GSW Consolidated Alcohols (Toronto).

Chemicals and solvents were ACS reagent grade quality or better. Ethanol and ACS grade methanol, chloroform and acetone were distilled before use. 'Pesticide grade' hexane, 99.9 mol% methanol and 99 mol% chloroform (Fisher) were used as received. Water was deionized and distilled twice in glass, the second time from dilute potassium permanganate solution.

Methods

In order to minimize autoxidation, manipulations that involved polyunsaturated lipids were performed as much as possible in reduced lighting conditions and in solvents which had been bubbled with O_2-free N_2.

Thin-layer chromatography of PC was carried out routinely on silica gel G using chloroform/methanol/water (65 : 25 : 4, v/v). Phosphate analyses were done by a modification of previous procedures as described by Keough and Kariel [4].

Phospholipid synthesis

Deacylation. Deacylation of 20 : 0-20 : 0 PC in diethyl ether/chloroform/methanol (18 : 1 : 1, v/v) was done following the timing of additions of *C. adamanteus* venom described by Keough and Davis [5], but 48 μg venom/mg lipid was used at zero time and after 10 min, and 24 μg/mg at 30 and 60 min. After 90 min of incubation, if deacylation was not complete as judged by thin-layer chromatography, the solvents were evaporated on a rotary evaporator, and the residue was washed a few times with diethyl ether.

The material was redissolved in the original solvent, an additional aliquot of venom suspension (48 μg venom per mg lipid) was added and shaking continued for another 30 min. This procedure usually resulted in only lysoPC being detectable on thin layer plates. In later syntheses it was found that performing the drying and washing steps and readdition of enzyme after 60 min of incubation generally led to complete deacylation in a total of 90 min. The deacylation products were washed several times with diethyl ether to remove some free fatty acids.

LysoPC purification. In early syntheses the lysoPC formed by the sequence described above was reacylated directly. For some of these syntheses, however, the final products gave fatty acid compositions consistent with the presence of a fair amount (up to 22%) of 20 : 0-20 : 0 PC. This might have resulted from the incomplete removal of 20 : 0 fatty acids, and their subsequent reincorporation into the PC through mixed anhydrides. Also in some syntheses the reacylation efficiency was poor, and what may have been traces of 20 : 0-20 : 0 PC remaining after deacylation became significant portions of the total PC after reacylation. In later syntheses we routinely purified the lyso PC formed in the deacylation step.

The residue from the deacylation was suspended in chloroform/methanol (2 : 1, v/v) at 30 mg lipid per ml, and the suspension was centrifuged for 10 min in a bench centrifuge to remove insoluble material. The products in the supernatant were dried, and redissolved in a small volume of chloroform/methanol/water (65 : 25 : 2.5, v/v), and subjected to flash chromatography [6] on a silica gel (Baker Silica Gel for Flash Chromatography, Canlab, Dartmouth, NS) column that had been conditioned with chloroform. The ratio of lipid to gel was 1 : 100 (w/w). The column was eluted with 25 bed volumes of chloroform/methanol/water (65 : 25 : 2.5, v/v) at a pressure of 3–5 psig (20–34 kP) under N_2. Eluant was collected in fractions of half a bed volume, and monitored by phosphorus analysis and thin-layer chromatography. LysoPC eluted between 10 and 20 bed volumes.

Reacylation. The lysoPC was dried overnight in vacuo over P_2O_5, and it was dissolved at 20 mg/ml in dry, ethanol-free chloroform (shaken several times with P_2O_5 and distilled over P_2O_5 immediately before use). Anhydride (2.5 mol per mol lysoPC), catalyst (one mol per mol lysoPC), and antioxidant (0.05 mg per mg lysoPC) were added. The flask was flushed with O_2-free N_2, stoppered, and covered with aluminum foil. Most reacylations were carried out with continuous stirring in the dark for at least 30 h at room temperature in the presence of 4-dimethylaminopyridine as the catalyst [7]. For two syntheses (20 : 0-20 : 1 PC (2) and 20 : 0-20 : 3 PC (2)) 4-pyrrolidinopyridine was used as the catalyst [8,9], and the reactions were allowed to proceed with occasional shaking at 37 °C for 24 h.

The extent of acylation was monitored by thin-layer chromatography. After acylation the material was subjected to chromatography on Rexyn I-300 [7]. The column eluant was converted to the two phase system of Folch et al. [10] and the lower phase collected as described by Davis and Keough [11].

Column chromatography. Two procedures were used to purify the heteroacid PC. Some preparations were chromatographed on Whatman CM-52 cellulose in a manner similar to that described by Comfurius and Zwaal [12] at a loading factor of 2 mg of lipid per ml of column bed volume. The column was eluted with 10 bed volumes of chloroform, three bed volumes of chloroform/methanol (99 : 1, v/v), five bed volumes of chloroform/methanol (98 : 2, v/v), four to eight bed volumes of chloroform/methanol (96 : 4, v/v), and five to ten bed volumes of chloroform/methanol (95 : 5, v/v). PC eluted in the last two solvents.

The second method of purification was flash chromatography under the same conditions as described above except that the application and eluted solvent was chloroform/methanol/water (65 : 25 : 1.5, v/v). PC eluted between five and fifteen bed volumes.

Column eluants were monitored by phosphorus analysis and thin-layer chromatography.

Plate purification. Almost all the syntheses resulted in the production of some 1,3-diacyl-2-phosphoryl isomer in addition to the expected 1,2-diacyl PC. The materials used for calorimetric analyses were further purified by thin-layer chromatography on either silica gel G or H. Plates were cleaned by running them in chloroform/

methanol (1 : 1, v/v) followed by methanol before activating them. The plates were chromatographed in chloroform/methanol/water (65 : 25 : 4, v/v) and dried for a few minutes. The PC regions were identified by spraying with water, and the appropriate regions of gel collected.

The gel was treated in one of two ways: either extraction twice with chloroform/methanol (1 : 1,v/v) and once with methanol followed by solvent removal under reduced pressure; or extraction three times with chloroform/methanol/water (1 : 2 : 0.8, v/v) [13]. In the latter case the combined extracts were adjusted to chloroform/methanol/water (2 : 2 : 1.8, v/v) and the mixture was shaken. The resulting lower phase was collected and taken to dryness. The extracted lipids were dissolved in chloroform and the solution vacuum-filtered through a series of Millipore filters (HA 0.45 m plus GS 0.22 m plus VM 0.05 m) to remove gel fines.

Acetone precipitation. Following Millipore filtration the filtrate was concentrated to 0.5 to 1.0 ml under O_2-free N_2. 10 ml of redistilled acetone was added and the lipid stored at $-20\,^{\circ}C$ overnight. The precipitate was obtained by centrifugation of the acetone suspensions for three to four minutes in a bench-top centrifuge in which the metal tube shields had been precooled in liquid N_2 to prevent warming and redissolution of the lipid during centrifugation. The acetone precipitate was dried in vacuo in the presence of P_2O_5.

Differential scanning calorimetry

Samples of lipid in chloroform were transferred to small conical vials. The solvent was removed under O_2-free N_2 and the lipid film dried in vacuum overnight in the dark in the presence of P_2O_5. Water to the extent of twice the weight of lipid was added, and the material dispersed by vortexing above the expected $T_{G\text{-}LC}$.

Differential scanning calorimetry was carried out in a Perkin-Elmer DSC-2 using scanning rates of 5 Cdeg/min and full scale sensitivities from 0.2 to 0.5 mcal/s. All samples were carried through the ice-water transition to ensure the presence of excess water. All lipid transitions were observed at least twice in each direction to ensure their reproducibility. Areas of endotherms and exotherms were obtained using a Zeiss MOP 3.

Lipid analyses

After DSC analysis, sample containers were opened and the contents extracted several times with chloroform/methanol (1 : 1, v/v) to give a final volume of 10 ml. The extracts were subjected to phosphorus, thin-layer chromatographic, fatty acid, and ultraviolet spectral analyses [4,14]. In addition they were tested for isomeric purity by positional analysis of the fatty acids.

Positional analysis. Some materials from the DSC extracts were dissolved in diethyl ether/methanol (20 : 1, v/v) and subjected to digestion by *C. adamanteus* venom as described above. LysoPC was isolated by thin-layer chromatography in chloroform/methanol/water (65 : 25 : 4, v/v), and free fatty acids by chromatography in hexane/diethyl ether/glacial acetic acid (90 : 10 : 1, v/v). After identification of appropriate regions of gel under ultraviolet light subsequent to spraying the plate with 0.2% 2′,7′-dichlorofluorescein in 95% ethanol, they were subjected to transmethylation and the fatty acid methyl esters were analyzed [4,15].

Results

Synthetic products

The yield of pure lysoPC from 20 : 0-20 : 0 PC was usually in the range of 75%. Not all yields were measured after reacylation, but, for those that were, a yield of 30–70% of column-purified PC was obtained (relative to original lysoPC). Yields of 20 : 0-20 : *x* PC obtained after column chromatography were in the range of 4–50% based upon starting 20 : 0-20 : 0 PC. Yields from thin-layer chromatographic purification were not determined.

Variable amounts of the 1,3-diacyl-*sn*-glycero-2-phosphocholine isomers of the desired 1,2-diacyl PC products occurred in these preparations. The occurrence of fairly large amounts of the 1,3-diacyl compounds was a more frequent finding with these preparations that has been our experience in the synthesis of other heteroacid PC [3,5,11,16,17]. Why there were relatively large extents of phosphoryl group migration during the synthesis of some of these lipids has not been established. Some migration may have occurred during the reacylation. Also, after some deacylations with *C.*

TABLE I

ANALYTICAL DATA ON 20:0-20:x PC AFTER CALORIMETRIC ANALYSIS

Lipid *sn*-1-20:0-2-20:x PC	Sample [a]	Ultraviolet spectra		Thin-layer chromatography (% of recovered phosphorus)				Fatty acid (mol%)		Positional analysis		
		A^{230}_{mol}	A^{270}_{mol}	% main PC [b] (*sn*-3 PC)	% other PC [b] (*sn*-2 PC)	% lyso-PC	% other [c]	20:0	20:x	20:0-20:0 PC (%) [d]	*sn*-1-20:0-2-20:x PC (%)	*sn*-1-20:x-2-20:0 PC (%)
20:0-20:0 PC	(1)	293	92					100	0	100		
20:0-20:1 PC	(1)	2185	840	98.2	1.8	0	0	n.d. [e]	n.d.	n.d.	100	0
	(2)	1333	297	95.8	tr	3.9	0	50	50	0	94	6
20:0-20:2 PC	(1)	9388	2857	94.1	5.9	0	0	n.d.	n.d.	n.d.	n.d.	n.d.
	(2)	3450	741	91.9	2.6	4.2	1.3	58	42	16	75	9
20:0-20:3 PC	(1)	2382	853	98.1	0.9	0.8	0.2	53	47	6	92	2
	(2)	985	287	91.8	3.4	4.8	0	53	47	6	60	34
20:0-20:4 PC	(1)	2038	570	98.2	1.8	0	0	61	39	22	76	2
	(2)	5312	625	n.d.	n.d.	n.d.	n.d.	61	39	22	78	0
	(3)	1114	163	94.7	0	5.3	0	50	50	0	83	17

[a] Each sample was from a separate synthetic preparation.
[b] 'Main' PC was the predominant phosphoryl isomer of the sample. 'Other' PC was the other phosphoryl isomer.
[c] 'Other' spots on thin-layer chromatograph correspond to the areas where oxidative products migrated [14,18].
[d] Estimate based upon combination of data from positional and fatty acid analyses.
[e] Not determined.

adamanteus venum a compound migrating just ahead of 1-acyl PC, the expected product, on thin-layer plates was observed. This faster-moving compound may have been the 2-phosphoryl lysoPC isomer. The amount of phosphoryl group migration which occurred did not appear to be associated with any specific condition such as the lot of enzyme, the source of the 20:0-20:0 PC, or other obvious factors associated with the deacylation. If detectable (by visual inspection) amounts of the 2-phosphoryl isomer of lysoPC were observed, the samples were not used for reacylation. The data in Table I give the percentage of the phosphoryl group isomers in each PC product. Often it was not possible to effect complete separation of the 1,2- and 1,3-PC even with plate purification without such substantial losses in yield as to make the overall syntheses prohibitively expensive.

Other analytical data on the various samples used for DSC are also given in Table I. Most of the lipids had undergone a limited amount of oxidation by the end of the synthesis, purification and DSC analysis. Based upon our previous studies [3,14] we anticipate that the calorimetric scans of these lipids have not been especially influenced by oxidation products. Both the 1,2-diacyl PC and the 1,3-diacyl PC of the first synthesis with 20:2 (20:0-20:2 PC (1)) gave ultraviolet spectra showing a moderate amount of oxidation. The T_{max} of 20:0-20:2 PC (1) was a fair bit higher than that for 20:0-20:2 PC (2), and its transition width was also larger. Both these properties are consistent with the effects of an intermediate amount of oxidation having occurred [14].

Fatty acid analyses indicated that most of the products contained some molar excess of 20:0. While a small amount of the polyunsaturated chains may be lost during transmethylation and subsequent work up, repeated estimates of the compositions of these lipids and of stearoyl-linoleoyl PC under a number of transmethylation conditions suggested that the loss of polyunsaturated acids is small (usually $< 3\%$) under the conditions employed. We therefore have taken the conservative view that the excess arachidate is not an artifact and comes from the samples. Since the fatty acid compositions of the samples in solution and the PC spots from thin-layer plates were essentially the same, we assume that the excess of arachidate represents the presence of 20:0-20:0

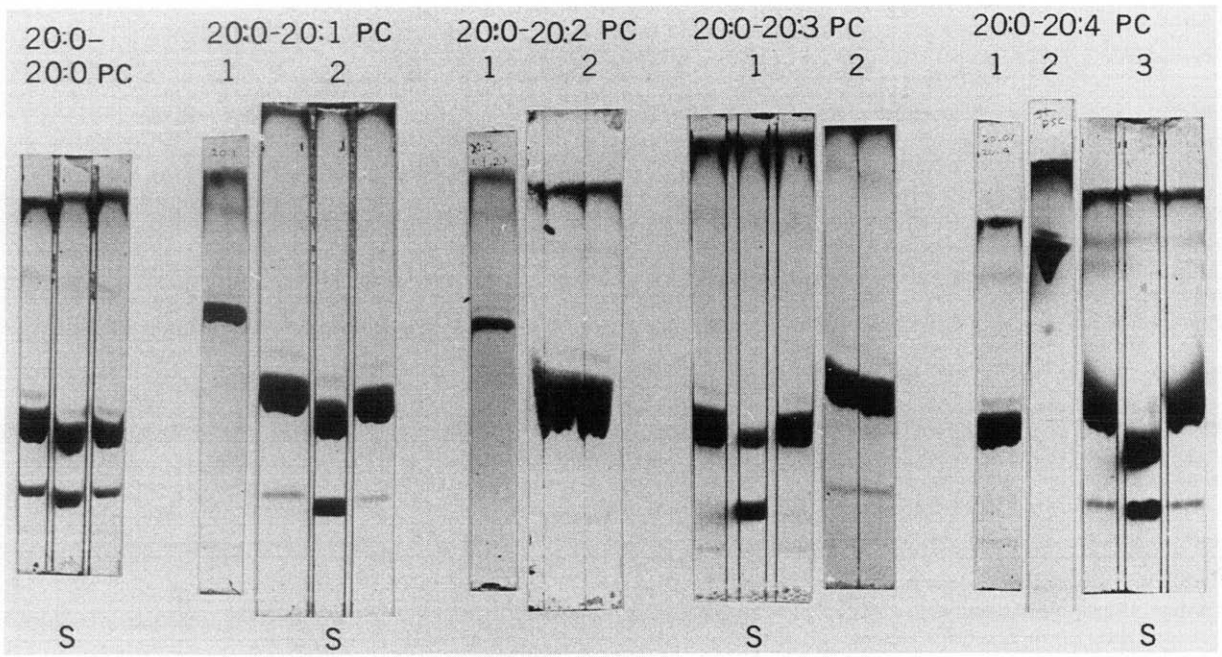

Fig. 1. Thin-layer chromatograms of the various PC. This figure is a composite of lanes from chromatograms run at different times under slightly different conditions of humidity and plate activation. The major spot of all samples had the mobility of PC. Lanes marked with an 'S' contained a mixture of saturated PC and lysoPC. Unsaturated PC runs slightly faster than saturated PC in this system. The faint bands just in front of the major PC spot are the 1,3-diacyl isomers. The labelling corresponds to that used in the tables.

PC, not contamination by free arachidate.

Thin-layer chromatography of the DSC samples (Fig. 1) showed that some conversion to oxidative products [14,18] and to lysoPC had occurred. In our experience, many samples of phospholipids, even saturated ones, show small amounts of lysoPC after DSC analysis.

Differential scanning calorimetry

Fig. 2 shows traces of DSC transitions for representatives of the series of 20 : 0-20 : x PC, and Table II gives the detailed calorimetric data on the transitions. With the exception of the 20 : 0-20 : 2 PC samples, all the lipids gave endothermic transitions with fairly narrow widths at half maximum excess heat capacity ($\Delta T_{1/2}$).

The difference in $T_{G\text{-}LC}$ or T_{max} between 20 : 0-20 : 0 PC and 20 : 0-20 : 1 PC was about 44 Cdeg. A much smaller difference of about 16 Cdeg existed between the $T_{G\text{-}LC}$ of 20 : 0-20 : 1 PC and 20 : 0-20 : 2 PC. The introduction of the third double bond at Δ17 in 20 : 0-20 : 3 PC produced not a

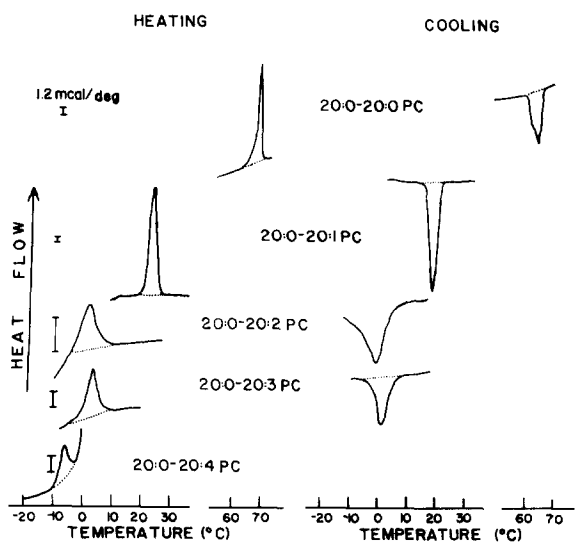

Fig. 2. Endotherms of exotherms of the gel to liquid-crystalline transitions of a series of 1,2-diacyl PC with eicosanoate in the *sn*-1 position and one of a series of unsaturated chains with increasing numbers of double bonds in the *sn*-2 position.

TABLE II

CALORIMETRIC DATA ON A SERIES OF 20:0-20:*x* PC

Lipid *sn*-1-20:0-2-20:*x* PC	Sample [a]	Heating scans				Cooling scans			
		T_c (°C)	T_{max} (°C)	$\Delta T_{1/2}$ (Cdeg)	ΔH (kcal·mol^{-1})	T_c (°C)	T_{max} (°C)	$\Delta T_{1/2}$ (Cdeg)	ΔH (kcal·mol^{-1})
20:0-20:0 PC	(1)	66.8	68.4	1.3	15	67.1	65.5	3.2	14
20:0-20:1 PC	(1)	20.4	21.4	2.2	6	21.3	19.5	1.6	5
	(2)	19.2	23.0	3.0	9	22.1	18.8	3.0	9
	$\bar{x}$	19.8	22.2		8	21.7	19.2		7
20:0-20:2 PC	(1)	−4.2	6.6	12.5	6	15.9	4.2	9.4	6
	(2) [b]	−4.3	1.8	6.0	5	4.4	−1.0	7.1	5 [c]
20:0-20:3 PC	(1)	−1.4	2.8	4.3	7	4.4	0.8	3.9	7
	(2)	3.8	6.0	2.9	7	7.2	4.2	3.0	7
	$\bar{x}$	1.2	4.4		7	5.8	2.5		7
20:0-20:4 PC	(1)	−9.5	−6.1	3.4	4	Not observed [d]			
	(2)	−8.5	−6.4	2.5	3	– [d]	−10.0	–	–
	(3)	−11.8	−7.4	4.3	3	– [d]	−12.3	–	–
	$\bar{x}$ [e]	−10.7	−6.8	3.8	3				

[a] Each sample came from a separate chemical synthesis. The values given represent averages for each sample taken from two to four separate repetitive scans except for 20:0-20:2 PC (1) for which data from only one transition is recorded.

[b] Based on analytical data (Table I), these samples are considered the more reliable of the 20:0-20:2 PC products.

[c] Cooling enthalpy may be slightly low beause supercooled water froze near the end of the exotherms.

[d] Transitions not observed, or only partly observed, because of masking by the freezing of supercooled water.

[e] Average taken from samples 1 and 3, these being considered the more reliable ones based on the analytical data in Table I.

decrease, but a slight increase in $T_{G\text{-}LC}$ over that of the dienoic PC. The presence of arachidonic acid (20:4) in the *sn*-2 position gave a lipid with the lowest $T_{G\text{-}LC}$ of the series, it being about 10 Cdeg lower than that of 20:0-20:3 PC. The pattern of these changes is most readily seen in Fig. 3.

It was undesirable to use substances such as ethylene glycol or glycerol to remove the ice-water transition since these might influence the properties of the lipid transition (see, for example, Refs. 16, 19, 20). For some transitions which would normally be masked by the ice-water melt we have taken advantage of the ability to supercool aqueous dispersions in the DSC-2. Some endotherms which occurred below 0 °C could be observed in supercooled liquid water (those for 20:0-20:2 PC and 20:0-20:3 PC). This meant, however, that these transitions started very near the initial transients that occur on the DSC-2 as temperature programming is started. This means that conventional baselines in some cases could not be established before transitions (see e.g. Fig. 2, 20:0-20:2 PC). We have in such cases extrapolated the final baseline back as is shown. The transition of 20:0-20:4 PC occurred at a temperature which was on the rising slope of the ice-water transition. Supercooling was not useful for observing 20:0-20:4 PC transition. Establishing exact baselines for these transitions was also more difficult than if the transitions had occurred in a temperature region where there was less change in the baseline. Nevertheless, moving the baseline in reasonable ways in these transitions suggested that baseline selection was not likely to cause any greater error in the measured enthalpy changes then might undetermined factors such as dispersion states (see, for example, Ref. 21). We have investigated at least two separate synthetic products of each heteroacid PC. Where no substantial analytical difference was detected between the products, we believe the averages are good measures of the parameters of their transitions. When there was good reason on the basis of analytical data, we have selected some rather than all of the data as being most representative.

The enthalpy changes associated with the gel to liquid-crystalline transitions for this series of lipids

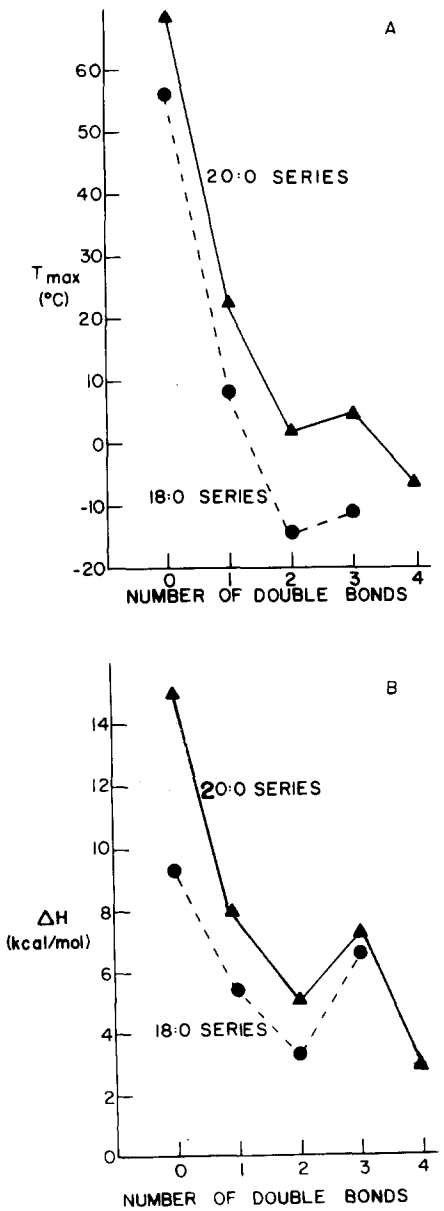

Fig. 3. T_{max} and ΔH versus number of double bonds in the *sn*-2 chain for a series of PC with twenty-carbon chains (this work) and a series with eighteen-carbon chains [3].

followed a pattern which was similar to that of the transition temperatures (Table II and Fig. 3).

In the syntheses of 20 : 0-20 : 2 PC and 20 : 0-20 : 3 PC there was sufficient production of the 1,3-diacyl 'contaminant' for us to attempt to obtain a small amount of material for calorimetric analysis. Preliminary analyses suggested that the values for T_{max} of the 1,3-isomers were near those for the corresponding 1,2-isomers.

Discussion

The influence of the number of double bonds in one chain of a heteroacid PC on the transition temperatures of two series of lipids can be seen in Fig. 3, where data from this work and previous investigations [3,16] are presented. The $T_{G\text{-}LC}$ and ΔH for both series of PC followed similar patterns with respect to the number of double bonds in the unsaturated chain. The introduction of the first double bond near the middle of the chain in the *sn*-2 position ($\Delta 9$ in the 18-carbon chain (C18) and $\Delta 11$ in the 20-carbon chain (C20)) caused a substantial reduction in $T_{G\text{-}LC}$. A large drop in $T_{G\text{-}LC}$ caused by a double bond in the center of a chain is a well-known effect [22,23].

The effects of additional double bonds in the *sn*-2 chain were not as great as those that might be predicted from the results on lipids with monoenoic chains, even when the influence of the positions along the chain of the additional double bonds is taken into account [23]. The introduction of the second double bond at $\Delta 12$ in C18 and $\Delta 14$ in C20 produced a further reduction of $T_{G\text{-}LC}$ of approx. 16 Cdeg beyond the $T_{G\text{-}LC}$ of the corresponding monoenoic PC. Based on the results of Barton and Gunstone [23] for the $T_{G\text{-}LC}$ of heteroacid PC with C18 chains, a reduction of about 36 Cdeg in $T_{G\text{-}LC}$ compared to the disaturated PC could be expected for a double bond six carbons from the terminal methyl group. If the influence of each double bond were independent of the others and the effects were additive, the $T_{G\text{-}LC}$ of 20 : 0-20 : 2 PC and 1-stearoyl-2-linoleoyl PC would be about 80–85 Cdeg below the $T_{G\text{-}LC}$ of the corresponding PC with two saturated chains. This would represent a reduction of 45–50 Cdeg for the double bond at $n-9$ plus about 36 Cdeg for the one at $n-6$. The actual reductions in $T_{G\text{-}LC}$ are of the order of 60 Cdeg.

A third double bond introduced in the position $n-3$ of either series of PC, not only did not cause a further reduction in $T_{G\text{-}LC}$, but resulted in slightly higher values than those for the corresponding dienoic lipids. $T_{G\text{-}LC}$ of 20 : 0-20 : 4 PC, were it determined by independent and additive effects of

double bonds at the appropriate positions in the *sn*-2 chain [23], would be about 120 Cdeg below that of 20 : 0-20 : 0 PC. It is, however, only about 75 Cdeg lower, consistent with the idea that factors in addition to a cumulative disruption of packing in the gel state determine the $T_{G\text{-}LC}$ of polyenoic lipids. There is some correspondence between the influence of double bonds on $T_{G\text{-}LC}$ and on the properties of heteroacid PC in monolayers. Demel et al. [24] found that the areas per molecule for a series of unsaturated heteroacid PC were increased over that of saturated PC. The change in molecular area caused by a second double bond in the unsaturated chain was less than that caused by only one double bond. Subsequent additions of more double bonds per chain had only small influences on the area per molecule.

$T_{G\text{-}LC}$ of 20 : 0-20 : 4 PC is lower than that of 20 : 0-20 : 3 PC. The double bonds in 20 : 0-20 : 3 PC are between the center and the terminal methyl group of the *sn*-2 chain, while those of 20 : 0-20 : 4 PC are distributed about the center of the chain. Based upon the results of Barton and Gunstone [23] the set of double bonds in 20 : 4 would be expected to be more disruptive of packing in the gel than those of 20 : 3 chain. Thus, in these polyunsaturated lipids there remains an influence of double bond position on the $T_{G\text{-}LC}$. Evans and Tinoco [25] observed that the areas per molecule of a series of 20 : 0-20 : 3 PC in monolayers were influenced by the positions of the double bonds in the *sn*-2 chains. Molecular area was largest when the bonds were closest to the head group.

The enthalpy changes associated with the transitions of these heteroacid PC follow a pattern similar to that displayed by the $T_{G\text{-}LC}$ values. The observed enthalpy change for 20 : 0-20 : 0 PC is anomolously high [4,17] in comparison to ΔH for the transitions of other saturated PC (for a summary, see for example, Ref. 26). Perhaps this is due to the presence of an unresolved transition corresponding to the pretransition of PC with shorter saturated chains. The entropy changes associated with the transitions, when expressed per mole of carbon in the chains of the PC, also followed the biphasic pattern shown by the $T_{G\text{-}LC}$ and ΔH; that is, they decreased in the transitions of PC containing up to two double bonds in the chain, increased for the transitions of the trienoic PC, and dropped again for PC with four double bonds. The entropy change per mole of carbon atoms in the fatty acid chains for the two lipids with three double bonds in their *sn*-2 chains were even slightly higher (0.7 e.u. per mole C for 18 : 0-18 : 3 PC and 0.7 e.u. per mole C for 20 : 0-20 : 3 PC) than those for the corresponding monoenoic PC (0.6 and 0.5 e.u. per mole C, respectively). This suggests that the trienoic PC might have slightly greater order or packing efficiency in the gel state than the monoenoic PC.

The preliminary data on the 1,3-diacyl isomers of the PC indicates that these isomers have nearly equal or slightly lower $T_{G\text{-}LC}$ values than do their 1,2-diacyl counterparts. The 1,3-diacyl isomers of saturated PC had $T_{G\text{-}LC}$ values which were 3–4 Cdeg below those of their corresponding 1,2-diacyl isomers [27–30]. It is suggested that the chains of 1,3-dipalmitoyl PC are interdigitated in the gel state [29]. Perhaps chain interdigitation occurs in the gel state of bilayers of the 1,3-diacyl isomers of the polyenoic PC also.

The thermodynamic data on the transitions of these polyenoic lipids support the proposal that $T_{G\text{-}LC}$ is influenced by a balance of forces between the area-expanding influence of the double bonds in the gel state and the decreased rotational freedom of the chains in the liquid-crystalline state [3,31].

Acknowledgements

We thank Dr. G.F. Bennett for the use of the Zeiss MOP-3 used to integrate the areas of endotherms and exotherms. This work was supported by the Medical Research Council of Canada (MT-6936). B.G. was the recipient of a Natural Sciences and Engineering Research Council of Canada Undergraduate Student Research Award, and subsequently a Medical Research Council of Canada Summer Undergraduate Research Scholarship.

References

1 Deese, A.J., Dratz, E.A., Dahlquist, F.W. and Paddy, M.R. (1981) Biochemistry 20, 6420–6427
2 Stubbs, C.D., Kouyama, T., Kinosita, K., Jr. and Ikegami, A. (1981) Biochemistry 20, 4257–4262

3 Coolbear, K.P., Berde, C.B. and Keough, K.M.W. (1983) Biochemistry 22, 1466–1473
4 Keough, K.M.W. and Kariel, N. (1987) Biochim. Biophys. Acta 902, 11–18
5 Keough, K.M.W. and Davis, P.J. (1979) Biochemistry 18, 1453–1459
6 Still, W.C., Khan, M. and Mitra, A. (1978) J. Org. Chem. 43, 2923–2925
7 Gupta, C.M., Radhakrishnan, R. and Khorana, H.G. (1977) Proc. Natl. Acad. Sci. USA 74, 4315–4319
8 Patel, K.M., Morrisett, J.D. and Sparrow, J.T. (1979) J. Lipid Res. 20, 674–677
9 Mason, J.T., Broccoli, A.V. and Huang, C.-H. (1981) Anal. Biochem. 113, 96–101
10 Folch, J., Lees, M. and Sloane-Stanley, G.H. (1957) J. Biol. Chem. 226, 497–509
11 Davis, P.J. and Keough, K.M.W. (1983) Biochemistry 22, 6334–6340
12 Comfurius, P. and Zwaal, R.F.A. (1977) Biochim. Biophys. Acta 488, 36–42
13 Bligh, E.G. and Dyer, W.J. (1959) Can. J. Biochem. Physiol. 37, 911–917
14 Coolbear, K.P. and Keough, K.M.W. (1983) Biochim. Biophys. Acta 732, 531–540
15 Thompson, W. (1969) Biochim. Biophys. Acta 187, 150–153
16 Davis, P.J., Fleming, B.D., Coolbear, K.P. and Keough, K.M.W. (1981) Biochemistry 20, 3633–3666
17 Davis, P.J. and Keough, K.M.W. (1984) Biochim. Biophys. Acta 778, 305–310
18 Shaw, J.M. and Thompson, T.E. (1981) Biochemistry 21, 920–927
19 Van Echteld, C.J.A., De Kruijff, B. and De Gier, J. (1980) Biochim. Biophys. Acta 595, 71–81
20 Dekker, C.J., Geurts van Kessel, W.S.M., Klomp, J.P.G., Pieters, J. and De Kruijff, B. (1983) Chem. Phys. Lipids 33, 93–106
21 Chen, S.C. and Sturtevant, J.M. (1981) Biochemistry 20, 713–718
22 Phillips, M.C., Williams, R.M. and Chapman, D. (1969) Chem. Phys. Lipids 3, 234–244
23 Barton, P.G. and Gunstone, F.D. (1975) J. Biol. Chem. 250, 4470–4476
24 Demel, R.A., Geurts van Kessel, W.S.M. and Van Deenen L.L.M. (1972) Biochim. Biophys. Acta 266, 26–40
25 Evans, R.W. and Tinoco, J. (1978) Chem. Phys. Lipids 22, 207–220
26 Keough, K.M.W. and Davis, P.J. (1984) in Membrane Fluidity (Biomembranes Vol. 12) (Kates, M. and Manson, L.A., eds.), pp. 55–97, Plenum Press, New York
27 Seelig, J., Dijkman, R. and De Haas, G.H. (1980) Biochemistry 19, 2215–2219
28 Stümpel, J., Eibl, H. and Nicksch, A. (1983) Biochim. Biophys. Acta 727, 246–254
29 Serrallach, E.N., Dijkman, R., De Haas, H.G. and Shipley, G.G. (1983) J. Mol. Biol. 170, 155–174
30 Chowdhry, B.Z. and Dalziel, A.W. (1985) Biochemistry 24, 4109–4117
31 Berde, C.B., Andersen, H.C. and Hudson, B.S. (1980) Biochemistry 19, 4279–4293