Cholesterol Interactions with Tetracosenoic Acid Phospholipids in Model Cell Membranes: Role of the Double-Bond Position†

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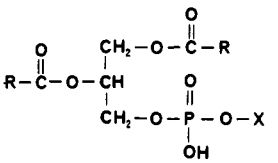
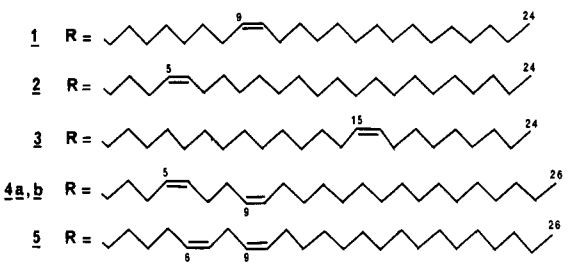
ABSTRACT: The synthesis and thermotropic properties of 1,2-di-(9*Z*)-9-tetracosenoylphosphatidylcholine [Δ^9-PC(24:1,24:1), **1**], 1,2-di-(5*Z*)-5-tetracosenoylphosphatidylcholine [Δ^5-PC(24:1,24:1), **2**], and 1,2-di-(15*Z*)-15-tetracosenoylphosphatidylcholine [Δ^{15}-PC(24:1,24:1), **3**] are reported. Liposomes prepared from these phospholipids differ from those of the natural sponge phospholipids, 1,2-di-(5*Z*,9*Z*)-5,9-hexacosadienoylphosphatidylcholine (**4a**) and the corresponding ethanolamine (**4b**), both of which virtually exclude cholesterol from their bilayers. The behavior of **1** and **2** is similar to that of 1,2-di-(6*Z*,9*Z*)-6,9-hexacosadienoylphosphatidylcholine (**5**), which exhibits a partial molecular interaction with cholesterol. In the case of **3**, cholesterol appears to interact with the saturated acyl chain regions of this phospholipid in a manner similar to that of its interaction with DPPC acyl chains. This study delineates the effect of the double-bond location in long fatty acyl chains of phospholipids on their interactions with cholesterol.

The lipid composition of sponges, the most primitive animals, is significantly different from those of other organisms. Generally, major phospholipids of living organisms possess fatty acyl groups with 16–18 carbon atoms (Cullis & Hope, 1985). It is also known that the two fatty acyl groups connected to the glycerol backbone of one molecular species are usually different (Patton et al., 1982; de Haas et al., 1986). Animals biosynthesize and possess more saturated fatty acids than plants. The majority of naturally occurring unsaturated fatty acids have one double bond at C-9, additional double bonds are usually found between the C-9 double bond and the ω-carbon of the chain (Stryer, 1988; Rawn, 1988). On the other hand, the major fatty acids of sponges are in general very long, bearing 24–30 carbon atoms (Litchfield et al., 1980; Ayanoglu et al., 1985). The double-bond pattern is also peculiar, the major sponge phospholipids displaying $\Delta^{5,9}$ di-unsaturation (Litchfield et al., 1980; Ayanoglu et al., 1985), or Δ^5 mono-unsaturation (Dasgupta et al., 1984). Most strikingly, recent HPLC[1] and fast atom bombardment (FAB) mass spectral studies (Dasgupta et al., 1986, 1987) established that the major phospholipids have identical fatty acids on both C-1 and C-2 of the glycerol backbone.

Sponges also contain a large variety of novel sterols, other than cholesterol [Djerassi, 1984; Ikekawa (1985) and review references cited therein]. Studies carried out in our laboratories indicate that these unusual phospholipids and sterols are cell membrane constituents in sponges such as *Axinella verrucosa* (Ayanoglu et al., 1988) and *Reniera* sp. and *Pseudaxinyssa* sp. (Lawson et al., 1988a,b,c). For these reasons we have initiated a systematic study of the thermotropic properties of these phospholipids and their possible molecular interactions with other membrane constituents, primarily sterols and conventional phospholipids. In the process we observed that the common major sponge phospholipids, 1,2-di-(5*Z*,9*Z*)-5,9-hexacosadienoylphosphatidylcholine (**4a**) and -phosphatidylethanolamine (**4b**), virtually exclude cholesterol and marine sterols from their bilayers (Ayanoglu et al., 1986).





In order to understand the possible role of the very long chain length and the $\Delta^{5,9}$ double-bond pattern, we have synthesized various analogues of 1,2-di-(5*Z*,9*Z*)-5,9-hexacosadienoylphosphatidylcholine. Cholesterol was readily integrated into the bilayers of shorter chain analogues of (**4a**), namely, $\Delta^{5,9}$-PC(18:2,18:2), $\Delta^{5,9}$-PC(22:2,22:2), and $\Delta^{5,9}$-PC-(24:2,24:2), underscoring the significance of the C_{26} chain length (Ayanoglu et al., 1988).

When the behavior of synthetic 1,2-di-(6*Z*,9*Z*)-6,9-hexacosadienoylphosphatidylcholine (**5**) with cholesterol was examined, it was observed that a partial interaction between this unusual phospholipid and cholesterol took place in model membranes (Li et al., 1988). These results led us to conclude that while the chain length is the prevalent factor in deter-

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[1] Abbreviations: DPPC, dipalmitoylphosphatidylcholine; SOPC, stearoyloleoylphosphatidylcholine; HPLC, high-performance liquid chromatography; DCI, desorption chemical ionization; EI, electron impact.

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mining the interactions of these phospholipids with sterols, the location of the double bonds may also have considerable influence (Li et al., 1988). To examine the latter possibility, we have studied the interaction of cholesterol with three synthetic 24:1 phosphatidylcholines, in which the double bond is located at different positions along the acyl chains. These lipids are 1,2-di-(9*Z*)-9-tetracosenoylphosphatidylcholine (**1**), 1,2-di-(5*Z*)-5-tetracosenoylphosphatidylcholine (**2**), and 1,2-di-(15*Z*)-15-tetracosenoylphosphatidylcholine (**3**).

Materials and Methods

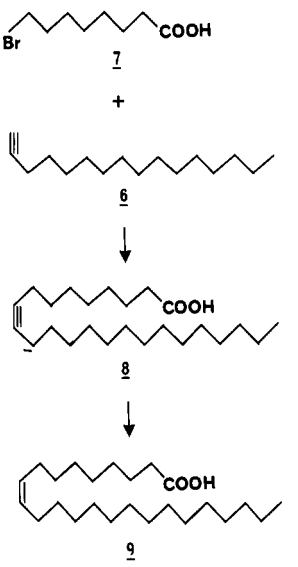
Differential scanning calorimetry measurements were carried out with a Perkin-Elmer DSC-2 calorimeter as described previously (Ayanoglu et al., 1988). The samples were scanned at a rate of 5 °C/min at a sensitivity setting of 0.2, 0.5, or 1 mcal/s, depending on the availability of the sample. The liposome samples were prepared by rotoevaporation and evaporation under high vacuum and/or freeze-drying of pure or mixed solutions in chloroform, followed by hydration of the dry films above their transition temperatures in a buffer solution containing 5 mM 2-[[tris(hydroxymethyl)methyl]-amino]ethanesulfonic acid (TES), pH 7.4, and 100 mM NaCl under argon and vortexing. The liposomes were centrifuged in an Eppendorf centrifuge to form concentrated pellets. The samples were then transferred immediately to aluminum calorimeter pans and sealed hermetically under pressure. The enthalpy values were determined by measuring the area under the transition endotherm, indium being used as a standard, and determining the phospholipid concentration in the pans by a colorimetric assay for inorganic phosphate (Bartlett, 1959).

^{1}H NMR spectra were recorded on a Nicolet NT 300WB (300 MHz) spectrometer, employing $CDCl_3$ as solvent and tetramethylsilane as internal reference. Mass spectra were obtained on a Hewlett-Packard HP 5995 gas chromatograph/mass spectrometer or a Ribermag R-10-10C mass spectrometer.

Column chromatography was performed on Davisil 62 silica gel from Davison Chemical Co. 1-Hexadecyne and 5-hexyn-1-ol were obtained from Wiley Organics, Coshocton, OH. Δ^{15}-PC(24:1,24:1) (**3**) was purchased from Avanti Polar Lipids, Birmingham, AL. Its purity was first checked by TLC, and the purity of the acyl groups was confirmed by GC analysis of the methyl esters which were prepared by transesterification in HCl/MeOH.

9-Tetracosynoic Acid (**8**). 1-Hexadecyne (**6**) (1.0 g; 4.50 mM) in dry hexamethyl phosphoramide (6.0 mL) was cooled to 0–5 °C, under a nitrogen atmosphere. A 2.5 M solution of *n*-butyllithium in hexane (4.0 mL; 10 mM) was then added dropwise over 10 min. The resulting reddish brown mixture was left stirring at 0–5 °C for 20 min and then cooled to −35 °C and treated in one portion with 8-bromooctanoic acid (**7**) (1.0 g; 4.50 mM) (Aldrich Chemical Co., Milwaukee, WI) in dry hexamethyl phosphoramide (4 mL). The resulting mixture was left stirring at −35 to 22 °C for 2 h.

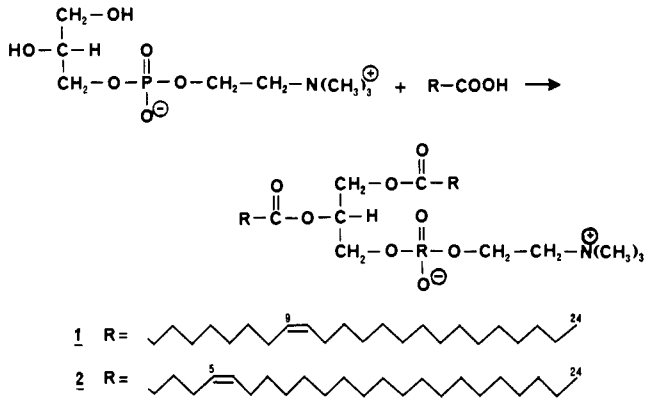
The light gray mixture was diluted with water (100 mL) and 2 M H_2SO_4 (20 mL). The product was extracted with ether (3 × 50 mL); the combined ethereal extracts were washed with water (2 × 20 mL), dried ($MgSO_4$), and filtered. The filtrate was evaporated to dryness, leaving a yellow crystalline solid. This was recrystallized from hexane to give the desired acid (0.74 g; 45%) as a white crystalline solid (mp 69–71 °C): R_f (hexane/diethyl ether, 1:1) 0.43; ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.875 (3 H, t, J = 6.8 Hz, terminal CH_3), 1.25–1.37 (30 H, m, aliphatic protons), 1.454 (2 H, two overlapping triplets, 12-CH_2), 1.632 (2 H, t, J = 7.2 Hz, 3-CH_2), 2.131 (4 H, t, J = 6.3 Hz, 8,11-CH_2), 2.344 (2 H, t, J = 7.5 Hz, 2-CH_2); mass spectrum, DCI (−), m/z (relative intensity) 365 (M + 1, 11.5), 364 (M, 31.1), 363 (100), 306 (2.5), 277 (10.7).



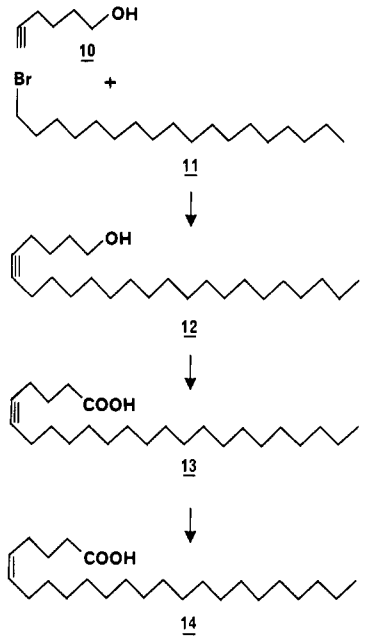
9-Tetracosenoic Acid (**9**). 9-Tetracosynoic acid (**8**) (0.40 g; 1.10 mM) in ethyl acetate (20 mL) was hydrogenated over a Lindlar catalyst [palladium on calcium carbonate, poisoned with lead (0.40 g), and further poisoned by the addition of quinoline (100 mg)]. The mixture was stirred at 25 °C for 17 h, whereupon the catalyst was filtered and washed with ether. The filtrate was washed with 2 M sulfuric acid (10 mL), followed by water (4 × 10 mL). The organic layer was dried ($MgSO_4$), filtered, and evaporated, leaving the desired acid (0.37 g; 92%) as a white crystalline solid (mp 40–41 °C) (the product may be recrystallized from hexane if necessary): R_f (hexane/diethyl ether, 1:1) 0.38; ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.878 (3 H, t, J = 6.8 Hz, terminal CH_3), 1.25–1.31 (32 H, m, aliphatic protons), 1.643 (2 H, two overlapping triplets, 3-CH_2), 1.98–2.02 (4 H, m, allylic protons), 2.349 (2 H, t, J = 7.5 Hz, 2-CH_2), 5.336 (2 H, m, olefinic protons); mass spectrum, EI, m/z (relative intensity) 366 (M^+ 10.3), 349 (29.0), 348 (97.1), 264 (10.0), 111 (45.2), 83 (55.4), 71 (86.0), 70 (34.5), 69 (81.9), 67 (3.2), 57 (82.9), 55 (100); mass spectrum, DCI (+), 384 (M + 18, 100).

1,2-Di-(9Z)-9-tetracosenoylphosphatidylcholine (**1**). 9-Tetracosenoic acid (**9**) (0.090 g; 0.26 mM), (dimethylamino)pyridine (15 mg; 0.12 mM), and the cadmium complex of *sn*-glycerophosphocholine (Sigma Chemical Co., St. Louis, MO) (22 mg; 0.050 mM) in dry, distilled chloroform (3 mL) were treated with dicyclohexylcarbodiimide (0.20 g; 0.97 M). The resulting mixture was left stirring at room temperature for 45 h. The dicyclohexylurea was filtered off and washed with a mixture of chloroform/methanol (1:1) (20 mL). The filtrate was adsorbed onto a column of silica gel, and a solution of chloroform/methanol (100:25) was passed through the column until the (dimethylamino)pyridine and other relatively nonpolar compounds ceased to come through. The column was then eluted with a mixture of chloroform/methanol/water (100:25:4) and the desired phospholipid (22 mg, 45%) collected as a white amorphous solid: R_f ($CDCl_3$/MeOH/H_2O, 65:35:4) 0.29; ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.878 (6 H, t, J = 6.8 Hz, terminal CH_3), 1.25–1.29 (64 H, m, aliphatic protons), 1.581 (4 H, br m, CHCCO), 1.99–2.01 (8 H, m, allylic protons), 2.291 (4 H, q, J = 7.5 Hz, α-methylene protons), 3.397 [9 H, s, $N(CH_3)_3$], 3.912 (2 H, m, CH_2N), 4.005 (2 H, br t, J = 7.0 Hz, CH_2OP), 4.01–4.39 (2 H, 8 lines,

AB part of an ANX system, CH_2OCO), 4.399 (2 H, br m, PO_3CH_2), 5.210 (1 H, br m, CHOCO), 5.336 (4 H, m, olefinic protons).



5-Tetracosyn-1-ol (**12**). 5-Hexyn-1-ol (**10**) (0.50 g; 5.0 mmol) in dry hexamethylphosphoramide (3 mL) was cooled under a nitrogen atmosphere to 0 °C. A 1.6 M solution of *n*-butyllithium in hexane (6.5 mL; 10 mmol) was then added dropwise over 10 min. The resulting light reddish brown mixture was left stirring for 15 min and then treated with a solution of 1-bromooctadecane (**11**) (1.67 g; 5.0 mmol) in dry hexamethyl phosphoramide (3 mL) dropwise over 15 min. Stirring was continued at 0–25 °C overnight (16 h).



The reaction mixture was diluted with water (50 mL) and the product extracted with diethyl ether (3 × 30 mL); the combined ethereal extracts were washed with water (3 × 20 mL), dried with $MgSO_4$, and filtered. The filtrate was evaporated to dryness, leaving an orange oil. This was passed down a column of silica gel (43–60 μm) with diethyl ether/hexane (1:1) as eluent. The alcohol (1.21 g; 68%) was obtained as a white crystalline solid (mp 55–56 °C): R_f (hexane/diethyl ether, 1:1) 0.37; ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.877 (3 H, t, J = 6.6 Hz, terminal CH_3), 1.251 (30 H, m, aliphatic CH_2), 1.41–1.73 (6 H, complex m, C-2, C-3, C-8), 2.10–2.24 (4 H, complex m, allylic protons), 3.675 (3 H, t, J = 6.3 Hz, 1-CH_3); mass spectrum, DCI (+), m/z (relative intensity) 368 (M + 18, 14.7), 355 (5.8), 354 (15.3), 351 (6.6), 296 (13.2), 121 (4.7), 115 (7.9), 114 (100), 112 (10.5), 111 (7.9).

5-Tetracosynoic Acid (**13**). 5-Tetracosy-1-ol (**12**) (0.60 g; 1.71 mM) in acetone (30 mL) was cooled in ice to 0–5 °C and treated dropwise with a solution of Jones reagent (2.5 mL). The resulting reddish solution was then left stirring at room temperature overnight.

The reddish green mixture was diluted with water (200 mL), and the acid was extracted with ether (3 × 150 mL). The combined extracts were washed with water (5 × 70 mL), dried ($MgSO_4$), and filtered. The filtrate was evaporated to dryness, leaving a pale yellow crystalline solid (0.53 g). This product was recrystallized from hexane to give the desired acid as a white crystalline solid (0.42 g, 68%; mp 66–68 °C). It is better to purify the product by recrystallization, since considerable deterioration will occur if it is passed down a column of silica gel (43–60 μM) with hexane/ether (1:1) as eluent. R_f (hexane/diethyl ether, 1:1) 0.41; ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.876 (3 H, t, J = 6.6 Hz, terminal CH_3), 1.249–1.467 (32 H, m, aliphatic protons), 1.806 (2 H, quintet, J = 7.2 Hz, 3-CH_2), 2.125–2.240 (4 H, m, 4,7-CH_2), 2.483 (2 H, t, J = 7.4 Hz, 2-CH_2); mass spectrum, DCI (+), m/z (relative intensity) 382 (M + 18 100), 354 (9.7), 340 (3.6), 266 (3.1), 255 (21.7), 182 (9.7), 151 (11.1), 150 (11.0), 149 (11.2), 137 (16.7), 130 (55.6), 126 (37.5), 122 (16.7), 123 (16.6), 124 (16.5), 114 (21.6), 108 (21.1), 100 (19.4).

5-Tetracosenoic Acid (**14**). 5-Tetracosynoic acid (**13**) (53 mg; 0.146 mM) was hydrogenated in the same manner as **8**, yielding an off-white crystalline solid (mp 42–44 °C). This was recrystallized from hexane, giving the desired alkene (36 mg; 68%) as a white crystalline solid: R_f (hexane/diethyl ether, 1:1) 0.44; ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.877 (3 H, t, J = 6.6 Hz, terminal CH_3), 1.251–1.287 (32 H, m, aliphatic protons), 1.698 (2 H, quintet, J = 7.2 Hz, 3-CH_2), 1.97–2.14 (4 H, m, 4,5-CH_2), 2.362 (2 H, t, J = 7.5 Hz, 2-CH_2), 5.28–5.47 (2 H, m, olefinic protons); mass spectrum, DCI (+), m/z (relative intensity) 384 (M + 18, 100), 348 (8.2), 300 (3.3), 130 (13.1), 110 (8.2), 109 (6.6), 98 (11.5), 97 (15.6), 96 (6.6), 95 (18.0), 94 (13.1), 81 (7.4), 69 (11.5), 58 (11.9), 55 (26.2), 54 (11.5).

1,2-Di-(5Z)-5-tetracosenoylphosphatidylcholine (**2**). 5-Tetracosenoic acid (**14**) (0.12 g; 0.33 mM) was transformed in the same manner as **8** into the desired phospholipid (31 mg; 49%) collected as a white amorphous solid: R_f (CHCl/MeOH/H_2O, 65:35:4) 0.31; ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.875 (6 H, t, J = 6.8 Zh, terminal CH_3), 1.249–1.315 (64 H, m, aliphatic protons), 1.651 (4 H, br m, CHCCO), 1.96–2.11 (8 H, m, allylic protons), 2.302 (4 H, q, J = 7.5 Hz, α-methylene protons), 3.366 [9 H, s, $N(CH_3)_3$], 3.822 (2 H, m, CH_2N), 3.985 (2 H, br t, J = 7.0 Hz, CH_2OP), 4.11–4.43 (2 H, 8 lines, m, AB part of an ANX system, CH_2OCO), 4.360 (2 H, br m, PO_3CH_2), 5.22–5.45 (5 H, complex m, CHOCO, olefinic protons).

Results and Discussion

Synthesis of Tetracosenoylphosphatidylcholines. Fatty acids with a cis double bond can be prepared via the Wittig reaction. However, stereochemical control is not absolute under these circumstances, and the resulting desired unsaturated acid is usually contaminated with its trans isomer, which requires purification by HPLC. A more straightforward way of preparing such acids in stereochemically pure form involves the selective reduction of an appropriate alkyne precursor. For our purposes, these molecules were obtained by coupling a bromide with the appropriate acetylene. Since stereoselective control of alkene generation in the partial hydrogenation of the corresponding alkyne could be provided by Lindlar catalysis, this method was employed for the synthesis of the hitherto unknown (9Z)-9-tetracosenoic (**9**) and (5Z)-5-tetracosenoic (**14**) acids. Their chemical structures and purities

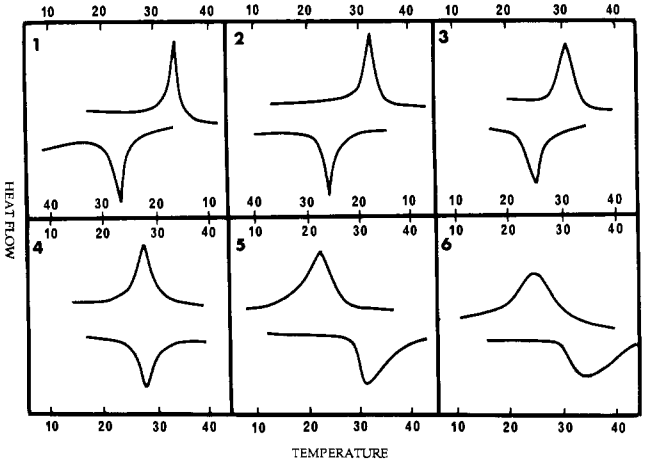


FIGURE 1: Thermotropic behavior of 1,2-di-(9Z)-9-tetracosenoylphosphatidylcholine (**1**): (1) pure phospholipid; (2–6) in the presence of 10, 20, 30, 40, and 50 mol % cholesterol, respectively. Upper scans in boxes represent heating; lower scans represent cooling.

Table I: Transition Temperatures (T_m) and Widths at Half Peak Height of Various Phosphatidylcholines

phospholipid	T_m (°C) heating	T_m (°C) cooling	W (°C) heating	W (°C) cooling
Δ^9-PC(24:1,24:1)	34	25	4	4.5
Δ^5-PC(24:1,24:1)	59	51	3	2.5
Δ^{15}-PC(24:1,24:1)	30	22.5, 19.5	2	5
$\Delta^{5,9}$-PC(24:1,24:1)[a]	31	27	1.7	

[a] Data from Ayanoglu et al. (1988).

Table II: Enthalpy of Transition for Various Phosphatidylcholines as a Function of Cholesterol Mole Percent

cholesterol mol %	ΔH (kcal/mol) DPPC[a]	SOPC	1	2	3
0	8.7	6.5	13.4	15.0	14.7
10	7.0	5.2	15.2	14.1	12.6
20	4.6	4.7	10.0	14.5	7.3
30	2.5	3.8	8.6	14.4	3.5
40	0	0		13.9	0
50	0	0	7.8	13.4	0

[a] Data from Estep et al. (1978).

were confirmed by ^{1}H NMR, mass spectral, and chromatographic analyses.

Thermotropic Properties of Δ^9-PC(24:1,24:1) and Its Mixtures with Cholesterol. The midpoint transition endotherm (T_m) of fully hydrated 1,2-di-(9Z)-9-tetracosenoyl-PC (**1**), the phospholipid with a double bond at the most conventional position, was found at 34 °C on heating (Figure 1 and Table I). This value is 8 °C lower than that of $\Delta^{5,9}$-PC(26:2,26:2) (Ayanoglu et al., 1988). The cooling scans exhibited transition endotherms lower than those of the heating scans, which is expected at the relatively fast scan rate of 5 °C/min. The enthalpy of transition for this lipid was 13.4 kcal/mL (Table II), which would be expected on the basis of an extrapolation from the ΔH of saturated PC's [6.7 kcal/mol for DMPC, 8.7 kcal/mol for DPPC, and 10.7 kcal/mol for DSPC (Estep et al., 1978), extrapolating to 16.7 kcal/mol for ditetracosanoyl-PC] and the reduction in ΔH due to a double bond C-9 (about 7.6 kcal/mol for DOPC vs 10.7 kcal/mol for DSPC). The estimate from this extrapolation is 16.7–3.1 = 13.6 kcal/mol, which compares favorably with the experimental value.

The differential scanning calorimetry heating and cooling thermograms for mixtures of cholesterol (0, 10, 20, 30, 40, and 50 mol %) with Δ^9-PC(24:1,24:1) (**1**) are illustrated in Figure 1. In the presence of increasing mole ratios of cholesterol, the transition peaks of **1** were broadened, but even in the presence of 50 mol % cholesterol, a distinct transition peak was observed. The two peaks observed in the cooling scan at 50 mol % cholesterol suggest the formation of two types of phospholipid/cholesterol complexes with distinct temperatures for transformation into the gel phase. In conventional phospholipid membranes, even the presence of just 30 mol % cholesterol makes the transition very broad and almost undetectable (Ladbrooke et al., 1968; Estep et al., 1981; Small, 1985; Li et al., 1988). These observations are indicative of a different type and partial lack of interaction between **1** and cholesterol. Increased quantities of cholesterol reduced the transition temperatures as can be observed in Figure 1. This was also seen when the thermotropic behavior of $\Delta^{6,9}$-PC-(26:2,26:2) was investigated with different proportions of cholesterol (Li et al., 1988), similar to the result reported for some unsaturated phospholipids (Davis & Keough, 1983; Finean & Hutchinson, 1988). The enthalpy of transition of **1** decreased as a function of cholesterol, after an increase at 10 mol %. However, even at 50 mol % cholesterol the enthalpy was 7.8 ± 0.5 kcal/mol (Table II).

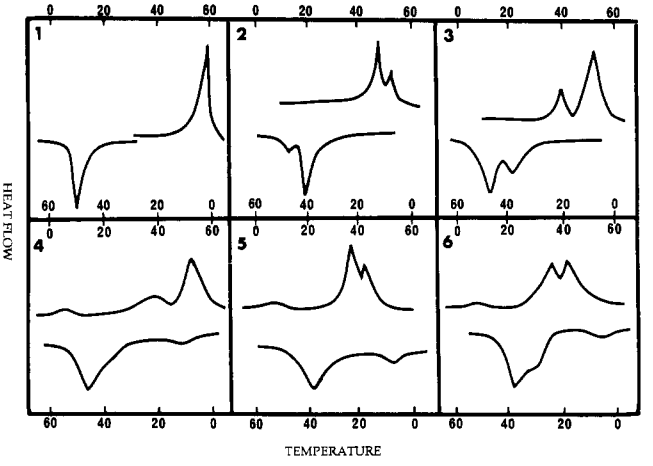


FIGURE 2: Thermotropic behavior of 1,2-di-(5Z)-5-tetracosenoylphosphatidylcholine (**2**): (1) pure phospholipid; (2–6) in the presence of 10, 20, 30, 40, and 50 mol % cholesterol, respectively. Upper scans in boxes represent heating; lower scans represent cooling.

Thermotropic Properties of Δ^5-PC(24:1,24:1) and Its Mixtures with Cholesterol. Liposomes composed of pure 1,2-di-(5Z)-5-tetracosenoylphosphatidylcholine (**2**) gave a transition endotherm at 59 °C on heating and at 51 °C on cooling (Figure 2 and Table I). This is 25 °C higher than that of **1**, consistent with previous studies, in which it was found that a double bond closer to the middle of the fatty acyl chains results in transition endotherms at lower temperatures (Barton & Gunstone, 1975). The double bond at C-9 probably provides a greater kink for the acyl chains than a double bond at C-5, accounting for the higher temperature of the transition endotherm **1** compared to that of **2**. The unusual difference between the transition temperatures may be attributed to the very long chains (18 saturated carbon atoms after the double bond at C-5). The enthalpy of transition of Δ^5-PC(24:1,24:1) is higher than that of Δ^9-PC(24:1,24:1) by about 1.5 kcal/mol (Table II), again consistent with the interpretation given above regarding the kinking in the acyl chains.

Beginning with the 90 mol % **2** and 10 mol % cholesterol mixture, two transition endotherms were observed (Figure 2), a small one at 53 °C and a large one at 48 °C. The mixture containing 20 mol % cholesterol gave two transition endotherms both on heating and on cooling. On heating, the smaller endotherm was fluctuating between 38 and 40 °C on

different runs, while the larger endotherm peak was observed at 51 °C without significant changes. Although the presence of 30 mol % cholesterol in conventional phospholipid bilayers broadens the transition peak almost to the base line, this was not the case with **2**—as it was not with our other very long chain phospholipids, **1**, **4a**, and **5**—not even in the presence of 50 mol % cholesterol (Figure 2). In the presence of 30 mol % cholesterol, a small peak at 16–17 °C was observed together with two large peaks at 40 and 53 °C, the latter being the largest. On cooling there was one large peak around 46 °C (with a small shoulder around 35 °C) and a small peak at 12–13 °C. In the presence of 40 and 50 mol % cholesterol, again several transition peaks were present. The intensities of the peaks fluctuated from one run to another. Such peculiar behavior is most likely associated with the location of the double bond of the 5-tetracosenoic acyl chains since the double bond is unusually close to the glycerol backbone and the rest of the hydrophobic chain is very long. Apparently, the interaction of cholesterol does not result in a stable behavior. Huang (1977) proposed that the two angular methyl groups of cholesterol are accommodated by the hydrophobic pocket formed between the carbonyl groups of the acyl chain and the trans–gauche kink adjacent to the Δ^9 double bond. Thus, a Δ^5 double bond would create a hydrophobic pocket too short to accommodate the cholesterol nucleus.

In one of our earlier studies (Ayanoglu et al., 1986), where we investigated the interaction of cholesterol with $\Delta^{5,9}$-PC-(26:2,26:2) in model membranes, we observed a physical separation, an exclusion of cholesterol from the liquid phase during the formation of the liposomes. This separation took place even at temperatures 20 °C above the phase transition of the phospholipid. An accumulation of aggregates at the air/water interface was observed. In the present study, no such aggregation was seen with any of the phospholipids. The formation of an almost pure cholesterol phase is seen in mixtures of some phospholipids with cholesterol (Bach, 1984; Wachtel & Bach, 1987; Bach & Wachtel 1989). In these studies, at low concentrations of cholesterol, only one peak is observed in DSC thermograms, corresponding to the phospholipid–cholesterol mixture. At a ratio of about 2:1 phospholipid:cholesterol, a peak around 38 °C appears, due to the transition of the almost pure cholesterol phase. When the mole fraction of cholesterol is increased however, more cholesterol is incorporated into the mixture. In our case, especially with Δ^5-PC, the cholesterol may be partially phase separated, since peak slightly below 40 °C is seen in the presence of 20 mol % or more cholesterol. However, the enthalpy value hardly changes from its original value (15 kcal/mol) in any of the mixtures. Examining the areas under the peak, it is unlikely the peak below 40 °C would be pure cholesterol, because the enthalpy of transition of pure cholesterol in aqueous medium is much lower (0.8 kcal/mol) (Bach, 1984). Nevertheless, the peak may reflect a cholesterol-rich phase.

Thermotropic Properties of Δ^{15}-PC(24:1,24:1) and Its Mixtures with Cholesterol. Since mixtures of both **1** and **2** with cholesterol displayed unusual thermotropic properties, we also examined the behavior of a commercially available double-bond analogue, Δ^{15}-PC(24:1,24:1) (**3**). Fully hydrated bilayers of **3** had an endothermic transition at 30 °C upon heating and two exothermic transitions at 22.5 and 19.5 °C upon cooling (Figure 3 and Table I). The addition of cholesterol reduced both the T_m and the enthalpy of transition (Figure 3 and Table II). The enthalpy was reduced to a much greater extent than in the case of Δ^9- or Δ^5-PC(24:1,24:1). For example, even at 20 mol %, ΔH for Δ^{15}-PC(24:1,24:1) is half

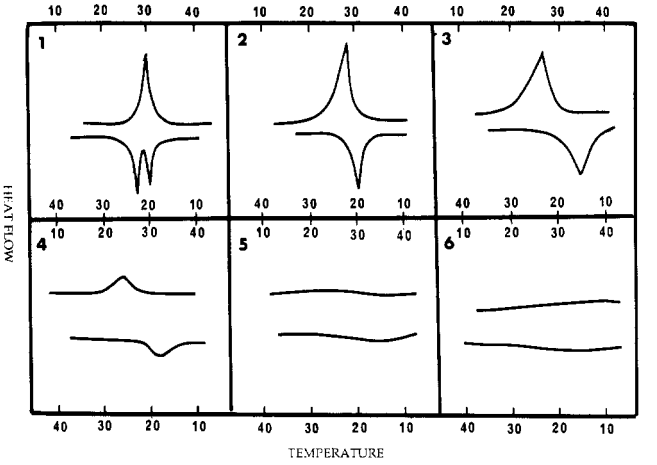


FIGURE 3: Thermotropic behavior of 1,2-di-(15*Z*)-15-tetracosenoylphosphatidylcholine (**3**): (1) pure phospholipid; (2–6) in the presence of 10, 20, 30, 40, and 50 mol % cholesterol, respectively. Upper scans in boxes represent heating; lower scans represent cooling.

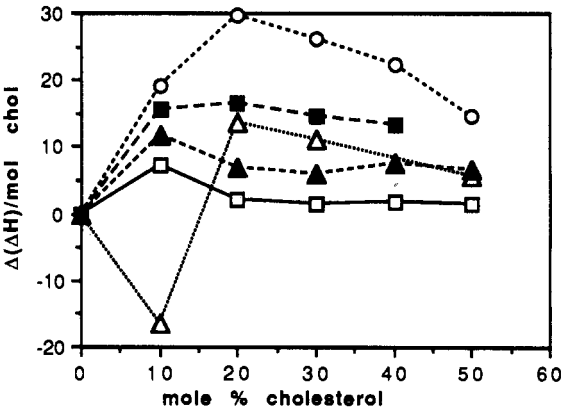


FIGURE 4: Change in the absolute value of ΔH for various phospholipids as a function of the mole percent of cholesterol in model membranes: (□) Δ^5-PC(24:1,24:1); (△) Δ^9-PC(24:1,24:1); (○) Δ^{15}-PC(24:1,24:1); (▲) SOPC; (■) DPPC.

the value for its Δ^5 counterpart, which has an enthalpy comparable to that of Δ^{15}-PC(24:1,24:1) in the absence of cholesterol (Table II). The enthalpy of Δ^{15}-PC was reduced by about 50% in the presence of 20 mol % cholesterol while that of DPPC was reduced by about 47%. This reduction was around 25% for Δ^9-PC and only about 3% for Δ^5-PC (Table II). At higher mole fractions of cholesterol the enthalpy of Δ^{15}-PC was reduced further and eventually reached nil. This observation sets this lipid apart from the Δ^9 and Δ^5 species with the same acyl chain length. The similarity of the effect of cholesterol on Δ^{15}-PC and DPPC, i.e., the reduction of the enthalpy and abolition of the transition endotherm above 30 mol %, suggests that cholesterol interacts with the saturated acyl chain regions of Δ^{15}-PC in a manner similar to that of its interaction with DPPC acyl chains.

The occurrence of the gel–liquid crystalline transition at 30 °C is somewhat surprising, since the transition temperature of cis unsaturated dioctadecenoic phosphatidylcholine having double bonds at varying positions along the acyl chain decreases as the double bond moves from Δ^2 and Δ^9 and then increases above Δ^{10}. Indeed for 15-dioctadecenoic PC, the T_m is higher than that of 5-dioctadecenoic PC (Barton & Gunstone, 1975).

To compare the effect of cholesterol on the thermotropic properties of the three tetracosenoic acid PC's, we have plotted the change in the absolute value of ΔH (Figure 4) and the change in ΔH per mole fraction cholesterol (Figure 5) as a

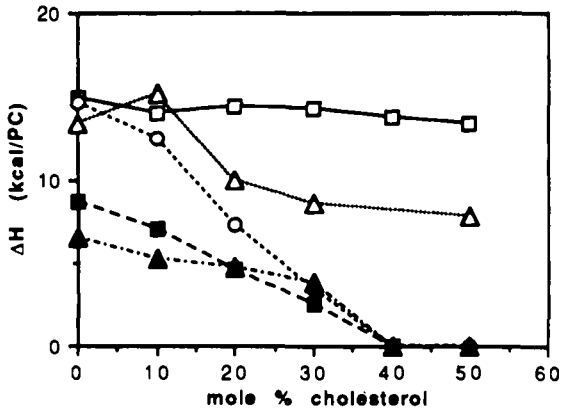


FIGURE 5: Change in ΔH for various phospholipids per mole cholesterol as a function of the mole percent of cholesterol in model membranes: (□) Δ^5-PC(24:1,24:1); (△) Δ^9-PC(24:1,24:1); (○) Δ^{15}-PC(24:1,24:1); (▲) SOPC; (■) DPPC.

function of the mole percent cholesterol in the membrane.

As indicated earlier (Ayanoglu et al., 1988), synthetic phospholipids such as PC(18:2,18:2), PC(22:2,22:2), and PC(24:2,24:2) with the $\Delta^{5,9}$ unsaturation pattern readily incorporated cholesterol into their bilayers, while the naturally occurring phospholipids PC(26:2,26:2) and PE(26:2,26:2) with the same unsaturation pattern did not (Ayanoglu et al., 1986). The most recently synthesized phospholipids in our laboratory, PC(24:1,24:1) with double bonds at Δ^9 and Δ^5 (**1** and **2**), exhibit only partial interactions with cholesterol, a behavior similar to that of PC(26:2,26:2) with $\Delta^{6,9}$ unsaturation (**5**), another synthetic sponge phospholipid analogue prepared in our laboratory. In considering all the calorimetric data, it appears that naturally occurring sponge phospholipids **4a** and **4b** may be functioning in the absence (or with small amounts) of sterols in some of the cell membranes of these organisms. Recent work in our laboratory seems to confirm this suggestion, since such phospholipids with C_{26} and C_{28} acyl groups are concentrated in membrane fractions of cells different from those containing sterols (Zimmerman et al., unpublished results). For example, choanocytes, which are located in epithelia and produce water current through the sponge canal system, are rich in phospholipids with very long fatty acyl chains. Their structure also suggests the entrapment of small particles or colloidal substances due to their collars and flagellates. These cells may require unique phospholipids that may not have to interact with sterols, because of their specific function. Hydrophobic membrane proteins may be taking part in such interactions. Research is underway to investigate the possible involvement of such other components.

The presently observed prominent differences associated with the location of double bonds in our phospholipids and their interaction with cholesterol may be applicable to other natural cell membranes.

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