Surface areas of 1-palmitoyl phosphatidylcholines and their interactions with cholesterol

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1-Palmitoyl phosphatidylcholines (1-palmitoyl PCs), in which the 2-position was occupied respectively by C$_{10}$:0, C$_{12}$:0, C$_{14}$:0, C$_{14}$:1, n-7, C$_{16}$:0, C$_{18}$:1, n-7, C$_{18}$:0, C$_{18}$:1, n-9, C$_{18}$:2, n-6, C$_{18}$:2, n-9, C$_{18}$:3, n-9, C$_{18}$:3, n-6, C$_{18}$:3(10, 12), C$_{20}$:0, C$_{20}$:1, n-9, C$_{22}$:2, n-6, C$_{22}$:3, n-3, C$_{22}$:4, n-6, C$_{22}$:5, n-6 or C$_{22}$:8, n-3 fatty acids, were studied as monolayer films at the air/water interface. Results for molecular area indicated that the areas of the PC (phosphatidylcholine) did not continuously decrease as the length of one chain increased. For series of saturated, monoenoic and dienoic 1-palmitoyl PCs the smallest molecular area was occupied by the PC containing a 20-carbon acid at the 2-position. In the 18-carbon series, introduction of the first and third cis double bonds caused a large increase in molecular area, but in the 22-carbon series the first and second cis double bonds produced large increases in molecular area. Molecules containing three or more cis double bonds varied little in molecular area, regardless of chain length (18–22 carbon atoms). The influence of a trans double bond was intermediate between that of a saturated and a cis double bond. The 18- and 22-carbon series of PCs were studied in mixed monolayers with cholesterol and desmosterol. Condensation of molecular areas occurred in all sterol PC mixed films, and similar results were obtained with cholesterol and desmosterol. Condensation of PC containing a cis or trans double bond within 10 carbon atoms of the carboxyl group initially increased with increasing surface pressure. Condensation of the other PCs decreased as surface pressure increased. All cis- or trans-unsaturated PCs condensed maximally in mixtures of approximately equimolar ratios with sterols, but saturated PCs condensed to the greatest extent in mixtures that contained about 30 mol % sterol.

INTRODUCTION

Individual fatty acids exert a major influence on the physical properties of membrane phospholipids and may also satisfy specific needs of enzymes requiring fatty-acid-containing substrates (Bloch, 1983; Lands et al., 1982; Silbert, 1975). In vertebrate glycerophospholipids, the major unsaturated fatty acids have chain lengths of 18, 20 or 22 carbon atoms and are usually located at the sn-2 position of glycerol, with a saturated fatty acid, chiefly palmitic acid, at the sn-1 position (White, 1973; Montfoort et al., 1971).

Sterols are also major components of membranes, and they interact with membrane phospholipids (Demel & de Kruyff, 1976; Bloch, 1983; Yeagle, 1985). Cholesterol is the most common sterol in animal membranes. Its immediate biosynthetic precursor, desmosterol, occurs in only trace quantities in most tissues, but it is an important component in spermatozoa (Bleau & van den Heuvel, 1974), developing brain (Fumagalli et al., 1964; Dennick et al., 1974) and accumulates during myotonia (Kuhn et al., 1968; Seiler & Kuhn, 1971; Fiehn et al., 1975).

We have previously studied the influence of double-bond position and number on the pressure–area curves of 1-palmitoyl PCs containing 20-carbon acyl groups and the condensation of these PCs with cholesterol (Evans & Tinoco, 1978). The introduction of one double bond greatly increased molecular area, but a second double bond caused little additional increase. A third double bond (C$_{20}$:3, n-9, C$_{20}$:3, n-6 or C$_{20}$:3, n-3) produced another large increase in molecular area, but a fourth or fifth did not.

During the present monolayer studies, we measured the pressure–area curves of 1-palmitoyl PCs containing 18- or 22-carbon acyl chains as well as the condensation of these PCs in mixed monolayers with cholesterol or desmosterol. The 18-carbon acids studied contained up to three double bonds and included cumbic acid (C$_{18}$:3(10, 12), n-9) (Houtsmuller, 1981). Unlike C$_{18}$:3, n-3 and C$_{18}$:3, n-6, cumbic acid is not elongated and subsequently cyclized (Holman et al., 1980). It can be hydroxylated, however, and topical application of the 13-hydroxy isomers can ameliorate the early dermatitis associated with EFA-deficient rats (Elliott et al., 1985). The study of cumbic acid may therefore help to distinguish the various roles of EFA as substrates for lipooxygenases and cyclo-oxygenase and as structural components of membranes.

The 22-carbon acids investigated contained up to six double bonds and included C$_{22}$:3, n-6 and C$_{22}$:4, n-3. Both of these acids are widely distributed in phospholipids, and their biological individuality has been clearly shown by dietary studies (Tinoco et al., 1978; Weiner & Sprecher, 1984).

Finally, we measured the pressure–area curves of 1-palmitoyl PCs containing shorter-chain fatty acids (C$_{10}$:0, C$_{12}$:0, C$_{14}$:0, C$_{14}$:1, n-7, C$_{16}$:0 and C$_{16}$:1, n-7). Under

Abbreviations used: PC(s), phosphatidylcholine(s); EFA, essential fatty acids.

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conditions of unsaturated-fatty-acid starvation, the levels of shorter-chain saturated fatty acids increase in yeast mutants unable to synthesize 16- or 18-carbon unsaturated fatty acids (Proudlock et al., 1971). Palmitoleic acid is widely distributed in animals, plants and micro-organisms (Sprecher, 1977).

EXPERIMENTAL

Analytical techniques

Conditions for g.l.c. of methyl esters and free sterols, and the procedure for phosphorus determination, were reported previously (Evans & Tinoco, 1978).

Preparation of lipids

Cholesterol (Nutritional Biochemicals Corp., Cleveland, OH, U.S.A.), recrystallized twice from 100% ethanol and from light petroleum (b.p. 40–60 °C), and desmosterol (Steraloids, Pawling, NY, U.S.A.) each gave only one component during g.l.c. The sterols were dried over phosphorus pentoxide under vacuum at room temperature overnight, and stock solutions in reagent-grade chloroform were prepared by weight. These solutions were stored at −16 °C under nitrogen and used within 3 days of preparation.

Fatty acids C10:0, C12:0, C14:0, C14:1, n-7, C15:0, C15:1, n-7, C16:0, C16:1(t), n-9, C16:1, n-9, C16:2, n-6, C16:3, n-3, C16:3, n-6, C18:0, C18:1, n-9, C18:2, n-6, C18:3, n-3, C18:3, n-6, C22:0, C22:1, n-9, C22:2, n-6, C22:3, n-3, C22:4, n-6, and C22:5, n-6 were obtained from Nuchek Prep., Elyssian, MN, U.S.A., and were more than 98% pure as shown by g.l.c. of their methyl esters, except for C14:1, n-7, which contained 4% C14:0, and C16:1, n-7, which contained 2.5% C16:1, n-9. Columbic acid was generously given by Dr. U. M. T. Houtschmuller, Unilever, Vlaardingen, The Netherlands. It was 92% pure, containing 8% C18:2, n-6.

Docosapentaenoic acid was isolated from rat testes. Lipids were extracted using the technique of Bligh & Dyer (1959) and then saponified (Kates, 1972). Non-esterified fatty acids were converted into methyl esters in H2SO4/MeOH (Tinoco et al., 1967) and fractionated by argentation t.l.c. (Arvidson, 1968). G.l.c. analysis indicated that the fraction was 96.5% C22:5, n-6 containing about 3.5% of what appeared to be a 24-carbon pentaene. It is probably C24:5, n-6, as this acid has been reported in rat testes (Bridges & Coniglio, 1970; Coniglio et al., 1976). The methyl ester was saponified, and proton n.m.r. analysis of the non-esterified fatty acid in [3H]chloroform containing 1% tetramethylsilane gave the following signals: triplet at 0.89 (CH3, 3.0 H found), multiplet at 1.30 (CH3 at carbon atoms 19, 20 and 21, 6.4 H found), quadruplet at 2.04 (CH2CH3 = at carbon atom 18, 2.1 H found), singlet at 2.42 (CH2 at carbon atoms 2 and 3, 3.9 H found), multiplet at 2.85 (=CH2CH2 = at carbon atoms 6, 9, 12 and 15, 8.0 H taken as reference for integration), multiplet at 5.38 (CH=CH at carbon atoms 4, 5, 7, 8, 10, 11, 13, 14, 16 and 17, 10.3 H found). A noteworthy aspect of the spectrum is the signal at 2.42 p.p.m. A peak here equivalent to four protons is characteristic of fatty acids with a double bond at position 4. In conjunction with the absorbances at 5.38 and 2.85 p.p.m., it allows the number and position of the double bonds in the fatty acid to be determined.

1,2-Dipalmitoyl phosphatidylcholine was obtained from Calbiochem, Los Angeles, CA, U.S.A. Preparations of 1-palmitoyl lyso-PC and acylation with fatty acid anhydrides have been described (Evans & Tinoco, 1978). All PCs were stored at −16 °C in chloroform/methanol under N2 and used within 2 days of preparation. In the PCs synthesized, the percentages of palmitate ranged from 46.5 to 54.3%, and the percentages of the variable fatty acid ranged from 45.7 to 53.5%. Contaminants were 1.6% C14:0 in C16:0/C14:1, n-7 PC, 1.1% C18:1, n-9 in C16:0/C16:1, n-7 PC, 3.5% C18:2, n-6 in C16:0/C18:3(5, 6, 12) PC and 1.6% C22:5, n-6 in C16:0/C22:5, n-6 PC.

Pressure–area measurements

A surface balance (Cenco Hydrophil Balance; Central Scientific Co., Chicago, IL, U.S.A.), with glass-distilled water, pH about 5.1, as subphase, was used, as described previously (Evans & Tinoco, 1978). Pressure–area measurements were made at 22 ± 2 °C (the temperature for each group of phospholipid/sterol studies did not vary more than ±0.5 °C). Published results (Phillips & Chapman, 1968) indicate that this temperature variation would have insignificant effects on the data.

RESULTS

Pressure–area curves of 1-palmitoyl PCs

(a) PC containing 10–16-carbon fatty acids at the 2-position. The molecular areas of these PCs are shown in Fig. 1. Four of the molecules (C14:0/C10:0, C16:0/C12:0, C16:0/C14:1, n-7 and C16:0/C16:1, n-7 PC) form expanded monolayers at all surface pressures. The results for C16:0/C16:0 PC are consistent with those in the literature (Phillips & Chapman, 1968), and this lipid gradually undergoes a transition from an expanded to a condensed phase as the pressure is raised to about 10 mn–1. C16:0/C14:0 PC is an expanded molecule until at 34 mn–1 it undergoes a sharp transition to a condensed state that has, at higher pressures, a molecular area similar to that of C16:0/C16:0 PC. Nevertheless, C16:0/C16:0 PC resembles unsaturated and short-chain saturated PC in collapsing at surface pressures below 50 mn–1.

(b) PC containing 18-carbon fatty acids at the 2-position. Pressure–area curves for these phosphatidylcholines are shown in Fig 2. Values for the saturated and cis-unsaturated molecules fell into three groups: saturated, mono- and di-enoic, and trienoic. A similar grouping was observed for a 20-carbon series of 1-palmitoyl PCs (Evans & Tinoco, 1978). The smallest molecular area is occupied by the fully saturated C16:0/C18:0 PC. Introduction of one cis double bond (C16:0/C18:1, n-9 PC) causes a large expansion in area, but a second double bond (C16:0/C18:2, n-6 PC) has a much smaller effect. A third cis double bond (C16:0/C18:3, n-13 PC or C16:0/C18:1, n-9 PC in C18:1, n-9 PC) causes a substantial additional expansion in area.

The molecular area of C16:0/C18:1, n-9 PC was intermediate between that of C16:0/C18:0 PC and C16:0/C18:1, n-9 PC, except at extremes of surface pressure (< 2 mn–1, > 40 mn–1), when it closely resembled that of C16:0/C18:0 PC. Similarly, the molecular area of C16:0/C18:3, n-13 PC was intermediate between that of C16:0/C18:0 PC and the trienoic PCs (C16:0/C18:3, n-13 PC, C16:0/C18:3, n-6 PC, except at pressures below 10 mn–1, when it closely resembled that of C16:0/C18:2, n-6 PC.)

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Fig. 1. Pressure–area curves of short-chain 1-palmitoyl PCs at the air/water interface at 22 ± 2 °C
The subphase was glass-distilled water at a pH of about 5.1. Note: 1 Å = 0.1 nm.

Fig. 2. Pressure–area curves of sterols and C_{16:0}/C_{18:2} PC at the air/water interface at 22 ± 2 °C
The subphase was glass-distilled water at a pH of about 5.1. Note: 1 Å = 0.1 nm.

(c) PC containing 22-carbon fatty acids at the 2-position. The pressure–area curves of these PCs (Fig. 3) can also be placed into three groups, but these are saturated, monoenoic, and dienoic to hexaenoic. A second cis double bond (C_{16:0}/C_{22:2} PC), but not a third (C_{16:0}/C_{22:3} PC), caused a large expansion in molecular area, and that of the C_{16:0}/C_{22:2} PC is as large as that of the more unsaturated molecules. This result is in contrast with the results obtained with the 18- and 20-carbon (Evans & Tinoco, 1978) series of PCs, in which a third double bond, but not the second, caused a large increase in molecular area.
Comparison of molecular areas at 30 mN·m⁻¹ of 1-palmitoyl PCs containing saturated or unsaturated fatty acids at the 2-position

The molecular areas of the PCs studied at a surface pressure of 30 mN·m⁻¹ are shown in Fig. 4. The smallest area is occupied by C₁₆:₀/C₂₀:₀ PC [0.528 nm² (52.8 Å²)] and only two other molecules, C₁₈:₀/C₁₈:₀ PC [0.560 nm² (56.0 Å²)] and C₁₆:₀/C₁₆:₀ PC [0.571 nm² (57.1 Å²)], have areas below 60 Å². Fully saturated PCs containing short chains (C₁₆:₀/C₁₀:₀ PC and C₁₆:₀/C₁₂:₀ PC) are large molecules with areas similar to, or larger than, that of unsaturated PC containing one or two cis double bonds (C₁₆:₀/C₁₄:₁,n-7,PC C₁₆:₀/C₁₈:₁,n-7,PC C₁₆:₀/C₁₈:₁,n-9,PC C₁₆:₀/C₁₈:₂,n-6,PC C₁₆:₀/C₂₀:₁,n-9,PC C₁₆:₀/C₂₀:₂,n-6,PC or C₁₆:₀/C₂₂:₁,n-9,PC). The data in Fig. 4 also emphasize the similarity in molecular area of molecules containing three or more double bonds, independent of chain length (18–22 carbon atoms).

Condensation in mixed monolayers

Interaction of sterol and phospholipid in mixed monolayers is indicated when the area/molecule for the mixed monolayer is different from the sum of the molecular areas of the pure components. The influence of monolayer composition on the extent of condensation at 30 mN·m⁻¹ is shown in Fig. 5 for the 18- and 22-carbon series of PCs. Similar results were obtained with either cholesterol or desmosterol and the response of the PC can be divided into two groups: (i) saturated or (ii) cis- or trans-unsaturated. The differences between the two groups are noteworthy for the extent of condensation above 60 mol % sterol, which was very small for the saturated group. The saturated molecules (C₁₆:₀/C₁₈:₀ and C₁₆:₀/C₂₂:₀ PC) condense maximally in mixtures containing about 70 mol % PC, whereas the unsaturated PC condensed maximally in approximately equimolar solutions. Similar results were obtained previously with the 20-carbon series of PCs (Evans & Tinoco, 1978).

Fig. 6 shows the variation in condensation with surface pressure for approximately equimolar mixtures of cholesterol and 18- and 22-carbon PCs. The PCs can again be divided into two groups: molecules for which condensation decreased continually as surface pressure increased (C₁₆:₀/C₁₈:₀ PC, C₁₆:₀/C₁₈:₁(n),n-9,PC C₁₆:₀/C₂₂:₀ PC C₁₆:₀/C₂₂:₁,n-9,PC C₁₆:₀/C₂₂:₂,n-6,PC and C₁₆:₀/C₂₂:₃,n-3,PC) and those for which condensation initially increased with increasing surface pressure until a maximum is reached at about 10–20 mN·m⁻¹ (C₁₆:₀/C₁₈:₁,n-9,PC C₁₆:₀/C₁₈:₂,n-6,PC C₁₆:₀/C₁₈:₃(n),n-3,PC C₁₆:₀/C₁₈:₃,n-6,PC C₁₆:₀/C₂₀:₁,n-6,PC C₁₆:₀/C₂₂:₁,n-6,PC and C₁₆:₀/C₂₂:₃,n-3,PC).

DISCUSSION

Pressure–area curves of 1-palmitoyl PCs

Saturated PCs. Saturated PCs with 16 or more carbon atoms in each acyl chain have the smallest molecular areas and can be compressed to surface pressures above 60 mN·m⁻¹ at 22 °C (Figs. 1, 2, and 3). The longer saturated chains can pack more efficiently, as the extent of van der Waals attraction between chains increases with increasing chain length. For 1-palmitoyl PCs the attractive force reaches a maximum when the sn-2 chain is 20 carbon atoms long (Fig. 4). The minimum area, at 30 mN·m⁻¹ for saturated, monoenoic, dienoic and n = 3

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The subphase was glass-distilled water, pH about 5.1. Note: 1 Å = 0.1 nm.
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**Fig. 4.** Molecular areas at 30 mN·m⁻¹ of 1-palmitoyl PCs

The subphase was glass-distilled water at a pH of about 5.1. The temperature was 22 ± 2 °C. The vertical bars represent the S.E.M. of two to five determinations. Values for C_{16:0}/C_{20:4} PC are taken from Evans & Tinoco (1978).

**Fig. 5.** Variation of condensation as a function of composition in mixed monolayers of sterols and C_{16:0}/C_{18:1} PC or C_{18:0}/C_{22:0} PC

The temperature was 22 ± 2 °C, the surface pressure 30 mN·m⁻¹ and the subphase was glass-distilled water at a pH of about 5.1. The vertical bars represent the S.E.M. of two or three determinations. (a) ○, C_{16:0}/C_{18:1} PC/cholesterol; ■, C_{16:0}/C_{18:1} PC/desmosterol; (b) ○, C_{16:0}/C_{18:1, n-9} PC; □, C_{16:0}/C_{18:1(10)}, n-9 PC; △, C_{16:0}/C_{18:2,n-6} PC; (c) ○, C_{16:0}/C_{18:3, n-3} PC; □, C_{16:0}/C_{18:3, n-6} PC; △, C_{16:0}/C_{18:3(5,9,12)} PC; (d) ○, C_{16:0}/C_{22:0} PC/cholesterol; ■, C_{16:0}/C_{22:0} PC/desmosterol; (e) ○, C_{16:0}/C_{22:1, n-9} PC; □, C_{16:0}/C_{22:2, n-9} PC; △, C_{16:0}/C_{22:3, n-6} PC; (f) ○, C_{16:0}/C_{22:4,n-6} PC; □, C_{16:0}/C_{22:5,n-6} PC; △, C_{16:0}/C_{22:6,n-3} PC. Note: 1 Å = 0.1 nm.
Fig. 6. Variation of condensation as a function of surface pressure at 22 ± 2 °C for approximately equimolar mixtures of cholesterol and C_{16:0}/C_{18:1} or C_{16:0}/C_{22:0} PC.

The subphase was glass-distilled water at a pH of about 5.1. The vertical bars represent the S.E.M. of two or three determinations.

(a) ○, C_{16:0}/C_{18:0} PC; □, C_{16:0}/C_{18:1},n=9 PC; △, C_{16:0}/C_{18:1},n=9 PC; ●, C_{16:0}/C_{18:3},n=9 PC; ○, C_{18:0}/C_{18:1},n=9 PC; □, C_{18:0}/C_{18:3},n=9 PC; ○, C_{16:0}/C_{22:0} PC; △, C_{16:0}/C_{22:1},n=9 PC; ●, C_{16:0}/C_{22:3},n=9 PC; ○, C_{16:0}/C_{22:4},n=9 PC: △, C_{18:0}/C_{22:5},n=9 PC; □, C_{16:0}/C_{22:6},n=9 PC. Note: 1 Å = 0.1 nm.

trioenoic 1-palmitoyl PCs involves, in each series, the 20-carbon homologue. The observation that the 22-carbon PC was larger than the 20-carbon homologue was unexpected, and the marked difference in chain length between the two acids in the 22-carbon homologue may account for this result. A segment of the sn-2 22-carbon chain extending beyond the sn-1 16-carbon chain may have greater freedom of motion, as it is not involved in van der Waal’s interaction with the adjoining chain. This interpretation is consistent with the results for a series of PCs containing two identical acyl chains which showed that the molecular area of these PCs continuously decreased as chain length increased, until a minimum was reached with C_{18:0}/C_{18:0} PC. Further increases in chain length caused no expansion in molecular area (van Deenen et al., 1962; Phillips & Chapman, 1968).

1-Palmitoyl PCs containing short saturated chains, C_{10:0} or C_{12:0}, have too little van der Waal’s interaction at 22 °C to produce small molecular areas (Fig. 4). Large areas are also observed for C_{18:0}/C_{14:0} PC at surface pressures below 34 mN·m⁻¹, but at this pressure the pressure–area curve for C_{18:0}/C_{14:0} PC (Fig. 3) undergoes a sharp inflexion to a more compact molecule. Lundquist (1978) has reported that sharp inflexions in the pressure–area curves of lipids indicate a change in orientation, whereas broad transitions, as evidenced by C_{18:0}/C_{16:0} PC (Fig. 3), indicate the presence of two immiscible phases.

**Monoenoic PCs.** At 30 mN·m⁻¹, all PCs with one cis double bond in the sn-2 chain have, as expected, larger molecular areas than the corresponding saturated PCs (Fig. 4). Similar results (not shown) were obtained at 10 mN·m⁻¹, except that C_{16:0}/C_{14:0} PC and C_{18:0}/C_{14:1},n=7 PC occupied almost identical areas. The molecular areas of C_{16:0}/C_{14:1},n=7 PC and C_{18:0}/C_{14:1},n=7 PC are similar to those of C_{18:0}/C_{18:1},n=9 PC and C_{18:0}/C_{18:3},n=9 PC, which are common membrane components, and indeed C_{14:1},n=7 and particularly C_{16:1},n=7 are constituents of yeast PCs (Sprecher, 1977).

**PCs with a trans double bond.** A trans double bond is not as effective as a cis double bond in increasing the molecular area of a PC: C_{18:0}/C_{18:0} < C_{16:0}/C_{18:0} (d) < C_{16:0}/C_{16:0} (c) < C_{16:0}/C_{18:0} (b). PC (Fig. 4). The molecular area of C_{16:0}/C_{18:3} (c), which is similar to that of C_{16:0}/C_{20:4},n=6 PC (0.80 nm² (80 Å²)]. Thus colubinic acid appears structurally to be a satisfactory replacement for arachidonic acid, and this may account for its ability to prevent the scaly skin which is characteristic of EFA deficiency (Houtsmuller & van der Beek, 1981). It is known to be a satisfactory substrate for lecithin:cholesterol acyltransferase and is well incorporated into plasma phospholipids and cholesteryl esters (Houtsmuller, 1981).

**cis-Polysaturated PCs.** 1-Palmitoyl PCs containing three to six double bonds have similar molecular areas irrespective of the unsaturated chain length (18 to 22 carbon atoms). At 30 mN·m⁻¹ and 22 °C, all the areas are between 0.79 nm² (79 Å²) and 0.87 nm² (87 Å²) (Fig. 4). The failure of a fourth or additional double bonds to
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affect molecular area is puzzling, but expected, since previous reports (Coolbear et al., 1983; Stubbs et al., 1981) have reported little influence of multiple double bonds on physical properties.

Factors affecting molecular area involve inter- and intra-molecular packing, including the interaction between the two acyl chains on the same PC molecule. This latter interaction may be particularly relevant, as many of the acyl chains present in the 1-palmitoyl PCs studied would cause phase separation if mixed with dipalmitoyl PC as separate molecular species of PC (de Kruyff et al., 1975).

A large effect of the first double bond on molecular area is expected, particularly as in all the molecular species we studied the first double bond was introduced near the middle of the molecule. Barton & Gunstone (1975) have reported that, in a series of monoenoic octadecenoyl PCs, the effect of the double bond on the temperature of the gel-to-liquid-crystal phase transition was greatest when it was situated in the middle of the chain at ΔTc. To interpret their results they assumed that the interaction potential energy (Shapiro & Ohki, 1974) for mono-unsaturated chains equals the sum of the interaction energies of the two constituent all-trans segments, and they showed that the calculated values were also minimal with the Δc cis isomer.

It is well established that one double bond affects intramolecular motion (Stubbs et al., 1981; Lancee-Hermkens & de Kruyff, 1977), and it is also clear from studies of surface viscosity that a single double bond greatly diminishes the intramolecular interaction of PC (Evans et al., 1980, 1981). Saturated PCs, including C14:0/C18:0 PC, have very high surface viscosities, and the data were interpreted as indicating that, in monolayers, saturated PCs exist as long linear polymers. The introduction of one double bond, however, (C14:1/C18:1,n-9 PC) rendered the surface viscosity undetectable by an oscillating pendulum and implied that the unsaturated PCs do not exist as polymers. We therefore conclude that the first double bond elicits a large expansion in molecular area via intra- and inter-molecular effects.

If monoenoic PCs exist as monomers, additional double bonds would be expected to exert their influence via intramolecular effects. It is not clear, however, why the response reaches a minimum with the second (C22 series) or third (C18 and C20 series) double bond, but it is noticeable that the effect of the third double bond is similar whether introduced towards either the methyl or carboxyl end of the chain (compare C16:0/C18:2,n-6 PC with C16:0/C18:2,n-6 PC or C16:0/C18:3,n-6 PC; C16:0/C20:2,n-6 PC with C16:0/C20:3,n-6 PC or C16:0/C20:3,n-3 PC).

Interactions with cholesterol

Saturated PCs condensed most with cholesterol at molar ratios of about 2:1, but PCs containing one unsaturated chain condensed maximally with cholesterol in approximately equimolar mixtures (Fig. 5). Similar results were previously obtained with a 20-carbon series of PCs (Evans & Tinoco, 1978). Reports in the literature also suggest that C16:0 PC, but not C18:1,1-n-9/C18:1,1-n-9 or C18:2,3-6/C18:2,3-6 PC nor C16:0/C18:3,n-3 PC condenses with cholesterol. In addition, it is known that cholesterol condenses with 1-unsaturated-2-saturated PC to about the same extent as it does with the normal 1-saturated-2-unsaturated structure (Demel et al., 1972; Ghosh et al., 1973). These observations suggest that only PCs containing a saturated or oleyl chain condense with cholesterol. Furthermore, the molar ratios suggest that one cholesterol molecule can interact with two saturated/oleyl chains only if they are present on two separate PC molecules at the sn-1 and the sn-2 positions. This latter condition is necessary to account for the observations that two saturated PC molecules, but only one 1-unsaturated-2-unsaturated PC molecule, can interact with cholesterol. We suggest that the interaction of two saturated PC molecules with one cholesterol molecule involves the saturated chain at sn-1 of one PC and the saturated chain at sn-2 of the other molecule.

Although we suggest that unsaturated chains (except oleic) are not directly involved in PC-cholesterol interaction, the data for condensation as a function of surface pressure (Fig. 6) demonstrate that unsaturated chains must have at least an indirect effect on condensation. The PCs could be divided into two groups: molecules for which condensation continuously decreases as surface pressure increases (C16:0/C18:0, C16:0/C18:1,n-9, C16:0/C22:0, C16:0/C22:1,n-9, C16:0/C22:2,n-6, and C16:0/C18:0, PC) and those for which condensation initially increases with increasing surface pressure until a maximum is reached at about 10-20 mN·m⁻¹ (C16:0/C18:1,n-9, C16:0/C18:2,n-6, C16:0/C18:2(4,9,12), C16:0/C18:2,3,6, C16:0/C18:2,3,6, and C16:0/C22:5,n-3, PC). All the lipids in the latter group possess at least one cis double bond within 10 methylene units of the carboxy group, indicating that these double bonds interfere with the orientation of the phospholipid/cholesterol molecules. A small effect may elicit a large response as van der Waals forces vary inversely with the sixth power of the distance (Eggers et al., 1964).

The results presented here indicate a varying influence of a trans double bond on the physical properties of a PC, even in simple one- or two-component systems. The pressure–area curve for C16:0/C18:1(n-9) PC (Fig. 1) is intermediate between that of C16:0/C18:0 PC and C16:0/C18:1,n-9 PC; the results for condensation as a function of surface pressure (Fig. 6) show that C16:0/C18:1(n-9) PC closely resembles C16:0/C18:0 PC, whereas the data for condensation as a function of monolayer composition (Fig. 5) and for collapse pressure (Fig. 1) suggest that C16:0/C18:1(n-9) PC is similar to the cis-unsaturated C16:0/C18:1,n-9 PC. In addition, we have observed (R. W. Evans, M. A. Williams & J. Tinoco, unpublished work) that the surface viscosity of C16:0/C18:1(n-9) PC is undetectable by an oscillating pendulum and thus mimics C16:0/C18:1,n-9 PC and not C16:0/C18:0 PC, which forms very viscous monolayers (Evans et al., 1980). The ambivalent nature of elaidic acid is also observed metabolically in rat foetuses (Moore & Dhopheswarkar, 1981). Whereas saturated and cis-unsaturated fatty acids favour the sn-1 and sn-2 positions of phospholipids respectively, elaidic acid was almost equally distributed between the two positions in rat foetal body PC.

The surface area of C16:0/C18:1(3,0,4,12) PC was intermediate between that of C16:0/C18:2,n-6 PC and C16:0/C18:3,n-6 PC, but in the nature of its interaction with sterols it resembled C16:0/C18:2,n-6 PC, indicating that in general its physical properties are dominated by its cis double bonds.  

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