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 $C_{10:0}$, $C_{12:0}$, $C_{14:0}$, $C_{14:1,n-7}$, $C_{16:0}$, $C_{16:1,n-7}$, $C_{18:0}$, $C_{18:1(t),n-9}$, $C_{18:1,n-9}$, $C_{18:2,n-6}$, $C_{18:3,n-3}$, $C_{18:3,n-6}$, $C_{18:3(5t,9,12)}$, $C_{22:0}$, $C_{22:1,n-9}$, $C_{22:2,n-6}$, $C_{22:3,n-3}$, $C_{22:4,n-6}$, $C_{22:5,n-6}$ or $C_{22:6,n-3}$ 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 carboxy 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 Kruffy, 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 columbinic acid ($C_{18:3(5t,9,12)}$) (Houtsmuller, 1981). Unlike $C_{18:3,n-3}$ and $C_{18:3,n-6}$, columbinic 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 columbinic acid may therefore help to distinguish the various roles of EFA as substrates for lipoxygenases and cyclo-oxygenase and as structural components of membranes.

The 22-carbon acids investigated contained up to six double bonds and included $C_{22:5,n-6}$ and $C_{22:6,n-3}$. 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 $C_{10:0}$, $C_{12:0}$, $C_{14:0}$, $C_{14:1,n-7}$, $C_{16:1,n-7}$, $C_{18:0}$, $C_{18:1(t),n-9}$, $C_{18:1,n-9}$, $C_{18:2,n-6}$, $C_{18:3,n-3}$, $C_{18:3,n-6}$, $C_{22:0}$, $C_{22:1,n-9}$, $C_{22:2,n-6}$, $C_{22:3,n-3}$, $C_{22:4,n-6}$ and $C_{22:6,n-3}$ were obtained from Nuchek Prep., Elysian, MN, U.S.A., and were more than 98% pure as shown by g.l.c. of their methyl esters, except for $C_{14:1,n-7}$, which contained 4% $C_{14:0}$, and $C_{16:1,n-7}$, which contained 2.5% $C_{18:1,n-9}$. Columbinic acid was generously given by Dr. U. M. T. Houtsmuller, Unilever, Vlaardingen, The Netherlands. It was 92% pure, containing 8% $C_{18:2,n-6}$.

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 H_2SO_4 /methanol (Tinoco *et al.*, 1967) and fractionated by argentation t.l.c. (Arvidson, 1968). G.l.c. analysis indicated that the fraction was 96.5% $C_{22:5,n-6}$ containing about 3.5% of what appeared to be a 24-carbon pentaene. It is probably $C_{24:5,n-6}$, 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 $[^2H]$ chloroform containing 1% tetramethylsilane gave the following signals: triplet at 0.89 (CH_3 , 3.0 H found), multiplet at 1.30 (CH_2 at carbon atoms 19, 20 and 21, 6.4 H found), quadruplet at 2.04 ($CH_2CH=$ at carbon atom 18, 2.1 H found), singlet at 2.42 (CH_2 at carbon atoms 2 and 3, 3.9 H found), multiplet at 2.85 ($=CHCH_2CH=$ 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 N_2 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% $C_{14:0}$ in $C_{16:0}/C_{14:1,n-7}$ PC, 1.1% $C_{18:1,n-9}$ in $C_{16:0}/C_{16:1,n-7}$ PC, 3.9% $C_{18:2,n-6}$ in $C_{16:0}/C_{18:3(5t,9,12)}$ PC and 1.6% $C_{24:5,n-6}$ in $C_{16:0}/C_{22:5,n-6}$ 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 ($C_{16:0}/C_{10:0}$, $C_{16:0}/C_{12:0}$, $C_{16:0}/C_{14:1,n-7}$ and $C_{16:0}/C_{16:1,n-7}$ PC) form expanded monolayers at all surface pressures. The results for $C_{16:0}/C_{16:0}$ 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 $10 \text{ mN} \cdot \text{m}^{-1}$. $C_{16:0}/C_{14:0}$ PC is an expanded molecule until at $34 \text{ mN} \cdot \text{m}^{-1}$ it undergoes a sharp transition to a condensed state that has, at higher pressures, a molecular area similar to that of $C_{16:0}/C_{16:0}$ PC. Nevertheless, $C_{16:0}/C_{14:0}$ PC resembles unsaturated and short-chain saturated PC in collapsing at surface pressures below $50 \text{ mN} \cdot \text{m}^{-1}$.

(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 $C_{16:0}/C_{18:0}$ PC. Introduction of one *cis* double bond ($C_{16:0}/C_{18:1,n-9}$ PC) causes a large expansion in area, but a second double bond ($C_{16:0}/C_{18:2,n-6}$ PC) has a much smaller effect. A third *cis* double bond ($C_{16:0}/C_{18:3,n-3}$ PC or $C_{16:0}/C_{18:3,n-6}$ PC) produces an additional large expansion in area.

The molecular area of $C_{16:0}/C_{18:1(t),n-9}$ PC was intermediate between that of $C_{16:0}/C_{18:0}$ PC and $C_{16:0}/C_{18:1,n-9}$ PC, except at extremes of surface pressure ($< 2 \text{ mN} \cdot \text{m}^{-1}$, $> 40 \text{ mN} \cdot \text{m}^{-1}$), when it closely resembled that of $C_{16:0}/C_{18:0}$ PC. Similarly, the molecular area of $C_{16:0}/C_{18:3(5t,9,12)}$ PC was intermediate between that of $C_{16:0}/C_{18:2,n-6}$ PC and the trienoic PCs ($C_{16:0}/C_{18:3,n-3}$ and $C_{16:0}/C_{18:3,n-6}$ PC), except at pressures below $10 \text{ mN} \cdot \text{m}^{-1}$, when it closely resembled that of $C_{16:0}/C_{18:2,n-6}$ PC.

Fig. 1. Pressure–area curves of short-chain 1-palmitoyl PCs at the air/water interface at $22 \pm 2^\circ\text{C}$

The subphase was glass-distilled water at a pH of about 5.1. Note: $1 \text{ \AA} = 0.1 \text{ nm}$.

Fig. 2. Pressure–area curves of sterols and $\text{C}_{16:0}/\text{C}_{18:x}$ PC at the air/water interface at $22 \pm 2^\circ\text{C}$

The subphase was glass-distilled water at a pH of about 5.1. Note: $1 \text{ \AA} = 0.1 \text{ nm}$.

(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 ($\text{C}_{16:0}/\text{C}_{22:2,n-6}$ PC), but not a third ($\text{C}_{16:0}/\text{C}_{22:3,n-3}$ PC), caused a large expansion in

molecular area, and that of the $\text{C}_{16:0}/\text{C}_{22:2,n-6}$ 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.

Fig. 3. Pressure–area curves of sterols and $C_{16:0}/C_{22:x}$ PC at the air/water interface at $22 \pm 2^\circ\text{C}$

The subphase was glass-distilled water, pH about 5.1. Note: $1 \text{ \AA} = 0.1 \text{ nm}$.

Comparison of molecular areas at $30 \text{ mN} \cdot \text{m}^{-1}$ 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 $30 \text{ mN} \cdot \text{m}^{-1}$ are shown in Fig. 4. The smallest area is occupied by $C_{16:0}/C_{20:0}$ PC [0.528 nm^2 ($52.8 \text{ \AA}^2$)] and only two other molecules, $C_{16:0}/C_{18:0}$ PC [0.560 nm^2 ($56.0 \text{ \AA}^2$)] and $C_{16:0}/C_{16:0}$ PC [0.571 nm^2 ($57.1 \text{ \AA}^2$)], have areas below $60 \text{ \AA}^2$. Fully saturated PCs containing short chains ($C_{16:0}/C_{10:0}$ PC and $C_{16:0}/C_{12:0}$ PC) are large molecules with areas similar to, or larger than, that of unsaturated PC containing one or two *cis* double bonds ($C_{16:0}/C_{14:1,n-7}$, $C_{16:0}/C_{16:1,n-7}$, $C_{16:0}/C_{18:1,n-9}$, $C_{16:0}/C_{18:2,n-6}$, $C_{16:0}/C_{20:1,n-9}$, $C_{16:0}/C_{20:2,n-6}$ or $C_{16:0}/C_{22:1,n-9}$ 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 $30 \text{ mN} \cdot \text{m}^{-1}$ 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

($C_{16:0}/C_{18:0}$ and $C_{16:0}/C_{22:0}$ PC) condensed 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 ($C_{16:0}/C_{18:0}$, $C_{16:0}/C_{18:1(t),n-9}$, $C_{16:0}/C_{22:0}$, $C_{16:0}/C_{22:1,n-9}$, $C_{16:0}/C_{22:2,n-6}$ and $C_{16:0}/C_{22:3,n-3}$ PC) and those for which condensation initially increased with increasing surface pressure until a maximum is reached at about $10\text{--}20 \text{ mN} \cdot \text{m}^{-1}$ ($C_{16:0}/C_{18:1,n-9}$, $C_{16:0}/C_{18:2,n-6}$, $C_{16:0}/C_{18:3(5t,9,12)}$, $C_{16:0}/C_{18:3,n-3}$, $C_{16:0}/C_{18:3,n-6}$, $C_{16:0}/C_{22:4,n-6}$, $C_{16:0}/C_{22:5,n-6}$ and $C_{16:0}/C_{22:6,n-3}$ 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 $60 \text{ mN} \cdot \text{m}^{-1}$ 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 $30 \text{ mN} \cdot \text{m}^{-1}$ for saturated, monoenoic, dienoic and *n*–3

Fig. 4. Molecular areas at $30 \text{ mN} \cdot \text{m}^{-1}$ of 1-palmitoyl PCs

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

Fig. 5. Variation of condensation as a function of composition in mixed monolayers of sterols and $\text{C}_{16:0}/\text{C}_{18:x}$ PC or $\text{C}_{16:0}/\text{C}_{22:x}$ PC

The temperature was $22 \pm 2^\circ \text{C}$, the surface pressure $30 \text{ mN} \cdot \text{m}^{-1}$ 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) $\circ$, $\text{C}_{16:0}/\text{C}_{18:0}$ PC/cholesterol; $\bullet$, $\text{C}_{16:0}/\text{C}_{18:0}$ PC/desmosterol; (b) $\circ$, $\text{C}_{16:0}/\text{C}_{18:1, n-9}$ PC; $\square$, $\text{C}_{16:0}/\text{C}_{18:1(t), n-9}$ PC; $\triangle$, $\text{C}_{16:0}/\text{C}_{18:2, n-6}$ PC; (c) $\circ$, $\text{C}_{16:0}/\text{C}_{18:3, n-3}$ PC; $\square$, $\text{C}_{16:0}/\text{C}_{18:3, n-6}$ PC; $\triangle$, $\text{C}_{16:0}/\text{C}_{18:3(5t,9,12)}$ PC; (d) $\circ$, $\text{C}_{16:0}/\text{C}_{22:0}$ PC/cholesterol; $\bullet$, $\text{C}_{16:0}/\text{C}_{22:0}$ PC/desmosterol; (e) $\circ$, $\text{C}_{16:0}/\text{C}_{22:1, n-9}$ PC; $\square$, $\text{C}_{16:0}/\text{C}_{22:2, n-6}$ PC; $\triangle$, $\text{C}_{16:0}/\text{C}_{22:3, n-3}$ PC; (f) $\circ$, $\text{C}_{16:0}/\text{C}_{22:4, n-6}$ PC; $\square$, $\text{C}_{16:0}/\text{C}_{22:5, n-6}$ PC; $\triangle$, $\text{C}_{16:0}/\text{C}_{22:6, n-3}$ PC. Note: $1 \text{ \AA} = 0.1 \text{ nm}$.

Fig. 6. Variation of condensation as a function of surface pressure at 22 ± 2 °C for approximately equimolar mixtures of cholesterol and $C_{16:0}/C_{18:x}$ or $C_{16:0}/C_{22:x}$ 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) $\circ$, $C_{16:0}/C_{18:0}$ PC; $\square$, $C_{16:0}/C_{18:1,n-9}$ PC; $\triangle$, $C_{16:0}/C_{18:1(t),n-9}$ PC; $\bullet$, $C_{16:0}/C_{18:3(5t,9,12)}$ PC; (b) $\circ$, $C_{16:0}/C_{18:2,n-6}$ PC; $\square$, $C_{16:0}/C_{18:3,n-6}$ PC; $\triangle$, $C_{16:0}/C_{18:3(5t,9,12)}$ PC; (c) $\circ$, $C_{16:0}/C_{22:0}$ PC; $\triangle$, $C_{16:0}/C_{22:1,n-9}$ PC; $\square$, $C_{16:0}/C_{22:2,n-6}$ PC; $\bullet$, $C_{16:0}/C_{22:3,n-3}$ PC; (d) $\circ$, $C_{16:0}/C_{22:4,n-6}$ PC; $\triangle$, $C_{16:0}/C_{22:5,n-6}$ PC; $\square$, $C_{16:0}/C_{22:6,n-3}$ PC. Note: $1 \text{ \AA} = 0.1 \text{ nm}$.

trienoic 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 $C_{16:0}/C_{14:0}$ PC at surface pressures below $34 \text{ mN} \cdot \text{m}^{-1}$, but at this pressure the pressure-area curve for $C_{16:0}/C_{14:0}$ PC (Fig. 3) undergoes a sharp inflection to a more compact molecule. Lundquist (1978) has reported that sharp inflections in the pressure-area curves of lipids indicate a change in orientation, whereas broad transitions, as evidenced by $C_{16:0}/C_{16:0}$ PC (Fig. 3), indicate the presence of two immiscible phases.

Monoenoic PCs. At $30 \text{ mN} \cdot \text{m}^{-1}$, 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 $10 \text{ mN} \cdot \text{m}^{-1}$, except that $C_{16:0}/C_{14:0}$ PC and $C_{16:0}/C_{14:1,n-7}$ PC occupied almost identical areas. The molecular areas of $C_{16:0}/C_{14:1,n-7}$ PC and $C_{16:0}/C_{16:1,n-7}$ PC are similar to those of $C_{16:0}/C_{18:1,n-9}$ PC and $C_{16:0}/C_{18:2,n-6}$ 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: $C_{16:0}/C_{18:0} < C_{16:0}/C_{18:1(t),n-9} < C_{16:0}/C_{18:1,n-9}$; $C_{16:0}/C_{18:2,n-6} < C_{16:0}/C_{18:3(5t,9,12)} < C_{16:0}/C_{18:3,n-6}$ PC (Fig. 4). The molecular area of $C_{16:0}/C_{18:3(5t,9,12)}$ PC [0.77 nm^2 ($77 \text{ \AA}^2$)] is similar to that of $C_{16:0}/C_{20:4,n-6}$ PC [0.80 nm^2 ($80 \text{ \AA}^2$)]. Thus columbinic 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*-Polyunsaturated 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 $30 \text{ mN} \cdot \text{m}^{-1}$ and 22 °C, all the areas are between 0.79 nm^2 ($79 \text{ \AA}^2$) and 0.87 nm^2 ($87 \text{ \AA}^2$) (Fig. 4). The failure of a fourth or additional double bonds to

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 Kruffy *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 Δ^9 C. 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 Δ^9 *cis* isomer.

It is well established that one double bond affects intramolecular motion (Stubbs *et al.*, 1981; Lancee-Hermkens & de Kruffy, 1977), and it is also clear from studies of surface viscosity that a single double bond greatly diminishes the intermolecular interaction of PC (Evans *et al.*, 1980, 1981). Saturated PCs, including $C_{16:0}/C_{18:0}$ 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, ($C_{16:0}/C_{18:1,n-9}$ 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 (C_{22} series) or third (C_{18} and C_{20} series) double bond, but it is noticeable that the effect of the third double bond is similar whether introduced towards either the methyl or carboxy end of the chain (compare $C_{16:0}/C_{18:2,n-6}$ PC with $C_{16:0}/C_{18:3,n-3}$ PC or $C_{16:0}/C_{18:3,n-6}$ PC; $C_{16:0}/C_{20:2,n-6}$ PC with $C_{16:0}/C_{20:3,n-6}$ PC or $C_{16:0}/C_{20:3,n-3}$ 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 $C_{18:1,n-9}/C_{18:1,n-9}$ PC, but not $C_{18:2,n-6}/C_{18:2,n-6}$ PC nor $C_{18:3,n-3}/C_{18:3,n-3}$ 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-saturated-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 continually decreases as surface pressure increases ($C_{16:0}/C_{18:0}$, $C_{16:0}/C_{18:1(t),n-9}$, $C_{16:0}/C_{22:0}$, $C_{16:0}/C_{22:1,n-9}$, $C_{16:0}/C_{22:2,n-6}$ and $C_{16:0}/C_{22:3,n-3}$ PC) and those for which condensation initially increases with increasing surface pressure until a maximum is reached at about $10\text{--}20\text{ mN}\cdot\text{m}^{-1}$ ($C_{16:0}/C_{18:1,n-9}$, $C_{16:0}/C_{18:2,n-6}$, $C_{16:0}/C_{18:3(5t,9,12)}$, $C_{16:0}/C_{18:3,n-3}$, $C_{16:0}/C_{18:3,n-6}$, $C_{16:0}/C_{22:4,n-6}$, $C_{16:0}/C_{22:5,n-6}$ and $C_{16:0}/C_{22:6,n-3}$ 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 $C_{16:0}/C_{18:1(t),n-9}$ PC (Fig. 1) is intermediate between that of $C_{16:0}/C_{18:0}$ PC and $C_{16:0}/C_{18:1,n-9}$ PC; the results for condensation as a function of surface pressure (Fig. 6) show that $C_{16:0}/C_{18:1(t),n-9}$ PC closely resembles $C_{16:0}/C_{18:0}$ PC, whereas the data for condensation as a function of monolayer composition (Fig. 5) and for collapse pressure (Fig. 1) suggest that $C_{16:0}/C_{18:1(t),n-9}$ PC is similar to the *cis*-unsaturated $C_{16:0}/C_{18:1,n-9}$ PC. In addition, we have observed (R. W. Evans, M. A. Williams & J. Tinoco, unpublished work) that the surface viscosity of $C_{16:0}/C_{18:1(t),n-9}$ PC is undetectable by an oscillating pendulum and thus mimics $C_{16:0}/C_{18:1,n-9}$ PC and not $C_{16:0}/C_{18:0}$ PC, which forms very viscous monolayers (Evans *et al.*, 1980). The ambivalent nature of elaidic acid is also observed metabolically in rat foetuses (Moore & Dhopeswarkar, 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 $C_{16:0}/C_{18:3(5t,9,12)}$ PC was intermediate between that of $C_{16:0}/C_{18:2,n-6}$ PC and $C_{16:0}/C_{18:3,n-6}$ PC, but in the nature of its interaction with sterols it resembled $C_{16:0}/C_{18:2,n-6}$ PC, indicating that in general its physical properties are dominated by its *cis* double bonds.

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