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A Comparison of the Packing Behavior of Egg Phosphatidylcholine with Cholesterol and Biogenically Related Sterols in Langmuir Monolayer Films

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Abstract

Cholesterol and selected derivatives were studied as mixed Langmuir monolayers with egg phosphatidylcholine (PC). As an extension of our earlier work, which employed binary sterol/PC mixtures, here we examined ternary mixed monolayers containing cholesterol along with an alternate sterol and PC in different molar ratios, using pressure-area isotherms. The ternary systems behaved similarly to the binary sterol/PC systems reported previously, with similar condensation noted for the sterol/PC films. To better understand how variations in sterol structure affect sterol packing in such membrane monolayers, binary mixtures containing cholestenone, cholestanol, and lanosterol with PC were also studied. Cholestanol behaved similarly to cholesterol when incorporated with PC, while cholestenone and lanosterol did not cause as much film condensation. The observed differences in molecular packing, and attributed sterol structural differences, are considered within the context of sterol/phospholipid mixtures in biological membranes.

Keywords

Cholesterol; cholestenone; 7-dehydrocholesterol; desmosterol; Langmuir monolayer; phospholipid

Introduction

Cholesterol (CHOL) is a ubiquitous and important cellular membrane component which assists in maintaining membrane integrity and fluidity, and participates in raft formation. CHOL clusters with sphingolipids in discrete, dynamic, liquid-ordered microdomains called “rafts” (reviewed in Edidin, 2003 and Boesze-Battaglia, 2005). Rafts are thought to serve as platforms for the organization of signaling complexes that control cellular physiology and homeostasis (reviewed in Brown and London, 1998; Simons and Toomre, 2000; Pike, 2003).

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Tissue substitution of alternate sterols, such as desmosterol (DES) and 7-dehydrocholesterol (7DHC), for CHOL is associated with phenotypically distinct human diseases (reviewed in Kelly, 2000 and Porter, 2003). 7DHC is a biogenic intermediate in the biosynthesis of CHOL and is the immediate precursor to CHOL via the Kandutsch-Russell pathway (reviewed in Schroepfer, Jr., 1981). Due to a defect in the enzyme that converts 7DHC to CHOL through reduction of the double bond between C7 and C8 of ring B in the sterol nucleus, 7DHC is the predominant sterol species and CHOL levels are abnormally low in all tissues of individuals affected with the Smith-Lemli-Optitz Syndrome (reviewed in Correa-Cerro and Porter, 2005). Likewise, when CHOL synthesis proceeds via the Bloch pathway (Schroepfer, Jr., 1981), DES is the immediate precursor of CHOL and failure to reduce the side-chain double bond in DES leads to abnormal accumulation of DES and abnormally low levels of CHOL, resulting in desmosterolosis (Clayton, et al., 1996).

It is possible that participation of DES and 7DHC in raft formation may contribute to failure of membrane function, and ultimately disease conditions (Tulenکو, et al., 2006). Two recent studies (Bittman, et al., 2001; Keller, et al., 2004) have shown that 7DHC promotes membrane raft formation at least as well as, if not better than, CHOL. However, replacement of CHOL with 7DHC in rat brain membranes in vivo was shown to cause marked differences in the protein composition of membrane rafts derived therefrom (Keller, et al., 2004), which would be expected to have profound consequences on membrane properties and cellular physiology. The exact pathobiological mechanism by which replacement of CHOL with DES and 7DHC leads to disease is not fully understood, although it appears to depend on both the chemistry of the CHOL precursor that accumulates as well as the magnitude of the CHOL deficiency (reviewed in Porter, 2003 and Porter, 2006).

In a previous study (Serfis, et al., 2001), we showed that DES and 7DHC behave similarly to CHOL in phospholipid Langmuir monolayers, which were used as model membranes. DES and 7DHC were found to occupy similar molecular areas on the water surface, although 7DHC takes up slightly more space due to its loss of planarity with the additional double bond in the sterol ring system. Both DES and 7DHC caused condensation of egg phosphatidylcholine (PC) monolayers, with similar condensing abilities to CHOL. However, in the disease states mentioned above, CHOL and its biogenic precursors coexist in various ratios in cells and tissues, solvated by phospholipids. Hence, in the present study, we have extended our prior work to include ternary mixtures of CHOL and PC mixed with either DES or 7DHC in varying molar ratios. Here we report pressure-area isotherms for these ternary systems and compare them to those reported in our previous study (Serfis, et al., 2001). In addition, to assist in the interpretation of the sterol structural affect on packing ability, we also present isotherms for mixed monolayers of PC with other sterols, including cholest-4-en-3-one (cholestenone; CHONE), 3 β -hydroxycholestanol (cholestanol; CHANOL), and lanosterol (LAN). These three sterols have discrete structural features that distinguish them from CHOL (see Fig. 1): CHONE is the same size (C27) as CHOL, but has a keto group at C3 in place of the 3 β -hydroxy group present in CHOL; cholestanol lacks the Δ^5 double bond found in CHOL; and LAN is a larger (C30) 3 β -hydroxy sterol that contains additional methyl groups (4 α -, 4 β -, and 14 α -methyl) and has an isomeric $\Delta^{8(9)}$ nuclear double bond instead of the Δ^5 double bond found in CHOL plus a side-chain double bond (Δ^{24}) as is also present in DES. The goal of this work is to compare CHOL and the alternate sterols in their ability to pack with, and cause condensation of, PC Langmuir monolayers.

Materials and Methods

L- α -PC, from egg yolk (> 99% pure), CHOL, DES, LAN, and CHONE were obtained from Sigma-Aldrich (St. Louis, MO, USA) and were used as received. The fatty acid composition of the egg PC employed throughout this study was reported by the manufacturer to be as

follows: 33% C16:0, 13% C18:0, 31% C18:1, and 15% C18:2 (with 8% from other, non-specified fatty acid species). 7DHC (a generous gift from Dr. Seiichi P.T. Matsuda, Rice University, Houston, TX, USA) was recrystallized repeatedly from acetone-water prior to use. All sterols were analyzed by reverse-phase HPLC and found to be >99% pure. Sterols were stored in dry form under argon atmosphere in amber glass vials at -20°C until ready for use. Spreading solutions containing the desired pure lipid (sterol or PC) or PC-sterol mixture were prepared in chloroform (HPLC grade; Fisher Scientific, Pittsburgh, PA, USA). Stock solutions of PC, CHOL, DES, CHONE, 7DHC, CHANOL, and LAN (*ca.* 1 mg PC or sterol per mL chloroform) were prepared. From these stock solutions, the PC-sterol mixtures were made to achieve final concentrations of 10, 20, or 30 mol% sterol. For binary mixtures the notation 10:90 indicates the sterol-phospholipid mixture contains 10 mol % sterol and 90 mol % PC; likewise, mixtures with 20:80 and 30:70 ratios indicate 20 and 30 mol % sterol with 80 and 70 mol % PC, respectively. Ternary mixtures contained CHOL plus an additional sterol (either DES or 7DHC) mixed with PC. The combined sterol content was either 10, 20, or 30 mol %, with a PC content corresponding to 90, 80, or 70 mol %, respectively. The ratio of CHOL to DES (or 7DHC) in any given ternary system was also varied. The ratios of CHOL to DES (or 7DHC) are noted here as 20:80, 50:50, and 80:20 as the ratio of CHOL:DES (or CHOL:7DHC) in the ternary mixtures with PC. The notation CHOL/7DES:PC indicates a ternary mixture containing CHOL, DES, and PC; in the presentation of the results it will be indicated what the total ratio of sterol to PC is, as well as the ratio of CHOL to DES (or 7DHC). Water used for the subphase was purified with a Barnstead Nanopure system (Barnstead/Thermolyne, Dubuque, IA, USA), and had a specific resistivity of 18 M Ω -cm. Tris base (Fisher Scientific) was added to the subphase to achieve a final concentration of 0.01 M, and the pH was adjusted to 7.4 with hydrochloric acid (Fisher Scientific).

Langmuir films were prepared using a NIMA Model 611D Langmuir-Blodgett trough (Coventry, UK), essentially as described previously (Serfis, et al., 2001). In brief, a lipid monolayer was formed by dropping approximately 0.1 mL of a chloroform-solvated lipid spreading solution (containing either pure sterol, pure PC, or a sterol-PC mixture) onto the aqueous subphase with a glass syringe. Ten minutes were allowed for solvent evaporation before film compression was initiated. Using the software provided by NIMA, pressure-area isotherms were collected continuously for the compression and expansion cycles at a rate of 12 $\text{\AA}^2/\text{molecule}/\text{min}$. Trough areas were recorded as a function of surface pressure, and Excel[®] spreadsheets were utilized to calculate the pressure-area isotherms in mean molecular area per molecule.

For each solution examined, measurements were made at least ten times on two days ($N=10$ for each lipid film) with at least two solutions, and representative isotherms are displayed. The subphase was maintained at 37°C with a heated circulating water bath, which pumped water through coils in the base of the trough. A NIMA PS4 surface pressure sensor was employed in all studies.

Ideal isotherms for mixed monolayers were calculated using the equations for the ‘additivity rule’

$$\underline{A} = x_1 A_1 + x_2 A_2 + x_3 A_3 \quad (1)$$

where $\underline{A}$ is the average area occupied per molecule in a mixed monolayer at a given pressure, x_1 , x_2 and x_3 are the mole fractions of the three components in the mixed film, and A_1 , A_2 and A_3 were the areas determined at a given pressure from the isotherms obtained for each pure

component monolayer. A complete ideal isotherm was constructed by calculating $\bar{A}$ through a range of pressures.

Results

Pressure-area isotherms for 10:90 sterol:PC mixtures for 7DHC mixed with CHOL and PC are shown in Fig. 2. The experimental isotherms (solid lines) for all mixtures (20:80, 50:50, and 80:20 CHOL:7DHC) indicate liquid phase films with areas that lie to the left of the calculated ideal isotherms (dashed lines). The ideal isotherms were calculated from the pure isotherms of CHOL, 7DHC, and PC according to Eq. 1. The pure isotherms have been reported previously, and the reader is referred to those studies (Serfis, et al., 2001). The experimental isotherms for each of the CHOL:7DHC ratios overlap well throughout the compression range, while some deviation for the 50:50 isotherm (b) is noted above 15 mN/m. Since these isotherms have experimental error of about $2 \text{ \AA}^2/\text{molecule}$, this deviation was deemed to be within experimental error. Errors in making isotherm measurements arise from various sources, including solution preparation, solution delivery to the surface, and temperature variations during solution preparation and the experiment. The shift in the experimental isotherms to the left of the ideal isotherms demonstrates the well-known “condensing effect” of sterols in such lipid films (reviewed in Yeagle, 2005): the addition of sterols to the PC monolayer causes tighter packing than would be expected if the molecules pack the same way as in their pure component films. This effect was reported for CHOL and 7DHC in our previous work with PC monolayers (Serfis, et al., 2001); in the current studies, it seems that mixing CHOL and 7DHC together with PC causes similar condensation with similar deviations throughout the compression region. Table 1 displays the deviation of CHOL/7DHC:PC isotherms from ideal behavior, and the deviations found for CHOL:PC films in previous work (Serfis, et al., 2001) are shown for comparison. For all three CHOL/7DHC:PC films with a total of 10 mol % sterol, the experimental isotherms deviate from ideal by about $3\text{--}7 \text{ \AA}^2/\text{molecule}$ through the compression range. For CHOL:PC binary films, the deviation from ideal for a 10:90 system was found to be $2\text{--}4 \text{ \AA}^2/\text{molecule}$ through the compression range. Table 2 displays the mean molecular areas for these same films. The ternary CHOL/7DHC:PC films occupy slightly smaller mean molecular areas compared to the CHOL:PC binary system with this small amount (10 mol %) of sterol in the mixed films. The ternary films occupy roughly $5\text{--}7 \text{ \AA}^2/\text{molecule}$ less at any given pressure compared to the areas occupied by CHOL:PC films, as reported previously. The difference in deviation from ideal and mean molecular areas for the ternary versus binary films is not believed to be significant; given that different researchers performed these experiments compared to the previous, the general experimental error associated with these experiments, and the fact that the 20 and 30 mol % ternary films agree quite well with the CHOL:PC binary systems (shown below).

The isotherms for 20:80 sterol:PC ternary mixtures (CHOL, 7DHC, PC) are shown in Fig. 3. There is good overlap of the three experimental isotherms, but some separation is noted at high pressure. At 30 mN/m the 80:20 and 20:80 CHOL:7DHC films are within one $\text{\AA}^2/\text{molecule}$, while the 50:50 film occupies a slightly smaller area ($3 \text{ \AA}^2/\text{molecule}$ less). This is not a significant difference, and overall these isotherms are reproducible within $2 \text{ \AA}^2/\text{molecule}$. The experimental isotherms are shifted from the ideal isotherms by about $7\text{--}15 \text{ \AA}^2/\text{molecule}$ throughout the compression range (Table 1). As the total sterol content has increased to 20 mol %, a greater condensing effect is observed, and greater shifts from ideal are thus noted. The greater number of sterol molecules causes tighter packing of PC molecules compared to having only 10 mol % of sterols as discussed above. It was found previously (Table 1) that CHOL:PC isotherms were shifted to smaller areas from the ideal isotherms by about $8 \text{ \AA}^2/\text{molecule}$ at this composition. The mean molecular areas for the CHOL/7DHC:PC films agree reasonably well with the areas for the CHOL:PC areas at the 20 mol % sterol composition (Table 2). For example, the 80:20 CHOL/7DHC:PC film occupies an area of $76 \text{ \AA}^2/\text{molecule}$ at a pressure

of 10 mN/m. The CHOL:PC binary film was found to occupy $79.6 \text{ \AA}^2/\text{molecule}$ at this pressure. For each of the ternary systems, the mean molecular areas are within about $6 \text{ \AA}^2/\text{molecule}$ of those areas found for a CHOL:PC binary monolayer.

While there are some small differences in film behavior for the ternary systems with 10 and 20 mol % sterols reported above as compared to binary CHOL:PC films studied earlier, incorporation of 30 mol percent sterol with different ratios of CHOL and 7DHC agrees extremely well with previous data on a 30 % CHOL: 70% PC monolayer. The isotherms for the ternary 30 % CHOL/7DHC:PC films are shown in Fig. 4. The three experimental isotherms overlap very well throughout the compression ranges, and are consistently shifted from the calculated ideal isotherms. The deviations from ideal are $7\text{--}12 \text{ \AA}^2/\text{molecule}$ throughout the compression range, while the deviation from ideal for 30 % CHOL with PC was about $13 \text{ \AA}^2/\text{molecule}$ throughout the compression range. It should also be noted that at low pressure these isotherms show deviations from ideal that are greater than the 20 mol% ternary films; however, at high pressure the deviations from ideal for the 30 mol % films are similar to the deviations for the 20 mol % films. At high pressures near 20 mN/m and above, the experimental isotherms overlap completely, and the best agreement with previous CHOL data is noted in terms of deviation from ideal (Table 1) and mean molecular areas (Table 2). For these 30 mol % ternary films, the molecules occupy the same area on the surface as the binary CHOL:PC films previously studied. Isotherms for ternary systems of 10:90 sterol:PC with DES and CHOL are shown in Fig. 5. The three experimental isotherms overlap well throughout the compression range; in addition, these isotherms overlap very well with the ternary CHOL/7DHC:PC isotherms having a sterol content of 10 mol % as shown in Fig. 2. The CHOL/DES:PC isotherms in Fig. 5 also deviate from ideal behavior, with a shift to lower molecular areas, and a significant condensation effect. This isotherm deviation from ideal ranges from $2\text{--}5 \text{ \AA}^2/\text{molecule}$ for all three CHOL:DES ratios, and compares very well to the $2\text{--}4 \text{ \AA}^2/\text{molecule}$ deviation noted for binary 10 % CHOL:PC films in the previous study (Table 1). These deviations are also similar to those noted above for the 10 mol % ternary mixtures with 7DHC. In addition, the mean molecular areas for the CHOL/DES:PC films (Table 2) show that monolayers having all three compositions of CHOL:DES occupy similar areas not only with each other, but also with 10 % CHOL:PC films from the previous study, and compare well with the areas found for the CHOL/7DHC:PC films.

Isotherms for 20 mol % sterol composed of CHOL and DES with 80 % PC are shown in Fig. 6. The isotherms for the varying compositions of CHOL and DES overlap well throughout the compression range, with some slight deviation at high pressure for the 20:80 CHOL:DES mixture. However, a difference of only about $4 \text{ \AA}^2/\text{molecule}$ is likely not significant given the sources of error. An increased condensation effect is observed with higher sterol concentrations in the film (compared with the 10 mol % system described above), and the experimental isotherms are consistently shifted from the ideal isotherms throughout the compression range. The deviation from ideal is similar for the three mixtures (Table 1), and ranges from about $5\text{--}12 \text{ \AA}^2/\text{molecule}$. For the 20 % CHOL:80 % PC system in the previous study, the deviation from ideal was consistently $8 \text{ \AA}^2/\text{molecule}$. The deviations for this set of CHOL:DES films is also similar to those found for the same compositions of CHOL:7DHC in ternary films (Table 1). The mean molecular areas for the CHOL:DES/PC ternary films at 20 mol % sterol are also very similar to those tabulated for the CHOL:7DHC/PC films, and the isotherms for these two sets do indeed overlap well. The mean molecular areas for CHOL:DES:PC films also correspond well to those reported previously for 20% CHOL in PC films (Table 2).

Isotherms for CHOL:DES ternary films with PC at a sterol concentration of 30 mol % are shown in Fig. 7. The experimental isotherms for the three varying ratios of CHOL with DES are similar within experimental error. This set of experimental isotherms is shifted to smaller areas than the ideal isotherms, indicating a strong condensing effect. The condensing effect is

similar to that observed with CHOL:7DHC/PC isotherms as shown in Fig. 4. In fact, a comparison of experimental isotherms from fig. 4 with the corresponding CHOL:DES/PC isotherms shown in Fig. 7 indicates good overlap of these data sets. The deviations from ideal (Table 1) for the 30 % sterol systems with CHOL:DES are similar to CHOL:7DHC at 30 mol % sterol, and to the 30 % CHOL:70 % PC deviations reported previously (within experimental error). All three data sets for a sterol concentration of 30 mol % show deviation from ideal of 7–13 Å²/molecule. The mean molecular areas for the CHOL:DES films are similar to the areas noted for the 30 mol % CHOL:7DHC ternary films with PC, and the 30 mol% CHOL:70% PC data reported previously throughout the compression range (all data in Table 2).

To assist in the interpretation of this data and learn more about how sterol structure affects sterol packing with phospholipids, three additional sterols were studied with PC in binary monolayers. CHANOL, which has the same structure as CHOL but lacks a double bond in the ring system, was studied in monolayers with PC at concentrations of 10, 20, and 30 mole percent sterol. Isotherms are presented in Fig. 8. The isotherm for pure CHANOL (isotherm a) shows solid phase film behavior, with a lift off area at 42 Å²/molecule followed by a steep rise in the isotherm through compression. This isotherm lies to the left (smaller areas) than a pure CHOL isotherm (Serfis, et al., 200; Demel and Dekruyff, 1976), indicating CHANOL molecules can pack even tighter in their pure film compared to CHOL. CHOL isotherms also show condensed phase behavior, but have a lift off area of nearly 50 Å²/molecule, a little higher than CHANOL. When incorporated into a mixed film with PC at 10 mol %, CHANOL packs tightly with phospholipids, and a condensing effect is observed. The experimental 10:90 isotherm (d) falls to smaller areas compared to the ideal (g) isotherm. Incorporation of 20 (c) and 30 (b) mol % CHANOL with PC leads to a greater condensing effect with higher sterol incorporation. The deviations from ideal for each CHANOL:PC system throughout the compression range are tabulated in Table 3. CHANOL:PC monolayers show deviations from ideal that are much larger than CHOL:PC in the 10 mol % sterol system, but agrees better with CHOL:PC systems at higher sterol concentrations of 20 and 30 mol %. In fact, the CHANOL and CHOL mixed PC films show the same (within experimental error) deviations at high pressure for the 30 mol % sterol systems (12–14 Å²/molecule). CHANOL seems to pack with phospholipids a little better at smaller sterol incorporation, but similar to CHOL at higher sterol concentrations. The lack of a double bond in the steroid ring does not inhibit CHANOL's ability to pack with phospholipids, just as the additional double bond in the ring of 7DHC does not seem to compromise its ability to pack tightly with phospholipids.

When the 3 β-OH group is converted to a carbonyl on ring A of the sterol nucleus, as in CHONE, the molecule is still able to accommodate close packing with phospholipids, although its ability is lessened compared to CHOL. Monolayer isotherms for CHONE mixtures with PC are shown in Fig. 9. The isotherm for pure CHONE exhibits condensed phase behavior, and has a lift off area of 61 Å²/molecule. This isotherm falls to larger molecular areas compared to CHOL and CHANOL, as presented above. CHONE takes up slightly larger surface areas compared to CHOL and CHANOL, as the carbonyl group on the steroid ring does not allow for hydrogen bonding with the water and other CHANOL molecules to the same extent. When incorporated with PC in binary films, fluid phase films result, and the experimental isotherms deviate from ideal behavior. For the 10 mol % CHONE film, the deviations from ideal are small, and the experimental isotherm approaches that for the ideal at pressures above 20 mN/m. Table 3 indicates deviations from ideal are larger at greater areas, but are minimal at high pressures. The deviations from ideal for CHONE:PC mixtures are less than those for CHOL:PC mixtures at high pressures, which are more biologically relevant pressures. As the amount of CHONE is increased to 20 and 30 mol %, a greater condensing effect is observed. The isotherms are shifted to much smaller areas as the amount of CHONE in the monolayer increases. The tabulated deviations from ideal also indicate a strong condensing effect which is similar to

CHOL:PC at 20 mol % sterol, but perhaps slightly less than CHOL:PC at 30 mol % sterol incorporation.

As mentioned in the Introduction, LAN has three structural features that distinguish it from CHOL: a Δ^{24} double bond in the hydrocarbon chain, altered location of the double bond in the steroidal nucleus ($\Delta^{8(9)}$, instead of Δ^5), and three additional methyl groups (two at the C4 position, and one at the C14 position of the steroid nucleus). It is anticipated that the C4 methyl groups would have the largest effect on LAN packing with phospholipids, as the side-chain double bond did not affect DES packing, and changes in steroid double bond presence did not greatly affect 7DHC packing (Serfis, et al., 2001). The isotherms for LAN are displayed in Fig. 10. The isotherm for pure LAN exhibits a more fluid like behavior compared to the stiff and condensed films of CHOL, 7DHC, DES, CHANOL, and CHONE. The lift off area is observed at $64 \text{ \AA}^2/\text{molecule}$, with a steady rise in pressure through compression. The isotherm falls at larger areas compared to the other sterol isotherms studied, and this is primarily due to the inability for LAN molecules to pack tightly with the bulky head groups in the interface. The additional methyl groups make the head group bulky, and close approach of head groups is prohibited compared to CHOL and the other systems studied. When mixed with PC, LAN forms fluid phase films, but a strong condensing effect is not observed. For the 10 mol % LAN:PC film, the experimental isotherm falls to the left of the ideal isotherm, with deviations from ideal (Table 3) that are similar to CHOL:PC films at 10 mol % CHOL incorporation. However, as the amount of LAN is increased to 20 and 30 mol % in the PC films, the condensing effect is greatly reduced compared to CHOL:PC systems. The experimental isotherms fall to the left of the ideals, however, the deviations from ideal are significantly less compared to the deviations seen for CHOL. At 20 mol % LAN incorporation, the deviations are one half that observed for CHOL:PC films; at 30 mol % they are about one fourth the magnitude of the corresponding CHOL:PC system. The mean molecular areas for the 10 mol % LAN film are similar to the 10 mol % CHOL film (Table 4). This result correlates with the similarity in deviation from ideal behavior for the two systems, and also indicates the isotherms overlap reasonably well. This also indicates LAN behaves similarly to CHOL at this concentration. However, the mean molecular areas for LAN:PC films containing 20 and 30 mol % LAN occupy larger mean molecular areas, indicating the isotherms fall to larger molecular areas compared to CHOL:PC films at the same concentrations. As the amount of LAN in the mixed films is increased, tight packing of LAN with PC is inhibited due to the bulky LAN head group. At high concentrations of LAN (30 mol %), there is a minimal condensing effect as tight packing is prohibited. LAN behavior in PC films deviates strongly from CHOL in PC films.

Discussion

For all systems containing CHOL/7DHC:PC and CHOL/DES:PC, it appears that regardless of the identity of the sterol molecules incorporated in the film, all systems at a given total sterol concentration behave similarly in terms of their molecular areas and ability to condense PC films. All 30 mol % films show similar isotherms, condensing effect (evidenced by deviations from ideal), and mean molecular areas, whether the film contains 30 % CHOL:70% PC, 30 % CHOL/7DHC sterol mixtures (20:80, 50:50, or 80:20 CHOL:7DHC molar ratios) or 30 % CHOL/DES sterol mixtures (20:80, 50:50, or 80:20 CHOL:DES molar ratios). The same trends were also noted for the 10 and 20 sterol mol % systems, although the greatest agreement amongst the sets occurs with the 30 % sterol systems. It appears that 7DHC and DES can not only substitute for CHOL (Serfis, et al., 2001) in terms of its ability to pack with, and condense, phospholipid monolayers, but also mix with CHOL in model membrane systems with minimal perturbation. There are some small differences noted for some different ratios, but within experimental error seem to behave similarly enough to draw this conclusion.

It is interesting to note that DES, with its additional double bond in the side chain, and 7DHC, with its additional double bond in the ring system, introduces minimal perturbation in their packing with phospholipids compared to CHOL in Langmuir films. 7DHC may pucker and lose some planarity due to the additional double bond, but this introduces minimal perturbation to its packing with PC, as noted in the isotherms presented in this study. Likewise, the stiffness in the hydrophobic chain that is introduced with the additional double bond in DES, does not overly restrict tight packing with phospholipids, and behaves similarly to CHOL in PC films, as found in this current study, and in our previous study (Serfis, et al., 2001).

Other studies utilizing the same alternate sterols used in this work have been reported. There are some works that have incorporated these alternate sterols into mono- or bi-layer systems to investigate their ability to form domains (raft) structures. The ability of CHOL (or alternate sterols) to participate in close packing with saturated phospholipids is the critical feature required for lipid domain formation (Xu and London, 2000). Thus, sterols that lack the ability to pack tightly with phospholipids will likely not promote domain formation. CHOL has been identified as a promoter sterol, or one that causes formation of a second liquid phase when incorporated into phospholipid bilayers (Beattie, et al., 2005). CHANOL behaves similarly to CHOL in its ability to promote this phase separation as a promoter sterol and form domains in phospholipid bilayers (Xu and London, 2000). CHANOL is a relatively flat molecule, allowing tight packing with phospholipids, and is structurally similar to CHOL in terms of planarity. CHOL and CHANOL have also been identified as membrane active sterols (Barenholz, 2002), due to their ability to decrease membrane permeability, increase lipid acyl chain order, and enable growth of sterol auxotroph microorganisms. Membrane active sterols have a flat structure of fused rings, a hydroxyl headgroup, and small molecular areas below 40 Å²/molecule at 12mN/m. CHANOL exhibits a molecular area of 36 Å²/molecule at a pressure of 12 mN/m, and fits the definition of a promoter molecule. Our isotherm results correlate well with these other studies, in that CHANOL behaves similarly to CHOL in its ability to condense phospholipid monolayers and occupies similar molecular areas at the air-water interface.

CHONE was found to inhibit formation of a second liquid phase in phospholipid bilayers (Xu and London, 2000; Beattie, et al., 2005), and is very different from CHOL in its ability to form rafts with co-existing phases. Instead, it causes separation with formation of a solid phase in co-existence with a liquid phase. It has been classified as non-membrane active (Stottrup and Keller, 2006) due to its lessened ability to order phospholipids acyl chains and decrease membrane permeability. CHONE also failed to form liquid condensed domains with DPPC in Langmuir monolayers when mixed with DPPC at 20 mol% (Slotte, 1995a), while CHOL forms extensive liquid condensed domains with DPPC in Langmuir monolayers (Slotte, 1995a; Berring, et al., 2005; Mattjus and Slotte, 1996; Mattjus, et al., 1995; Mattjus, et al., 1996; McConnell and Radhakrishnan, 2003; Slotte, 1995b; Slotte, 1995c; Slotte, 1995d; Subramaniam and McConnell, 1987). CHONE's inability to pack tightly with phospholipids and deviate from CHOL behavior is attributed to the ketone head group, which may lack the ability of a hydroxyl group to directly hydrogen bond with the ester linkage of phospholipid acyl chains (Ben-Yashar and Barenholz, 1989). This may also affect the immersion depth for this sterol in the bilayer (Xu and London, 2000). In our work, CHONE did not condense monolayers to the same extent as CHOL, likely due to the ketone head group and its effect on phospholipid packing.

LAN was found to not produce any co-existing solid or liquid phases over a wide range of temperatures in phospholipid bilayers (Beattie, et al., 2005), or in phospholipid vesicles (Xu and London, 2000), and has also been classified as non-membrane active (Stottrup and Keller, 2006). LAN does not pack tightly and condense phospholipids to the extent of CHOL due to the additional methyl groups on the sterol head. In addition, LAN failed to reduce surface viscosities of saturated phospholipid Langmuir monolayers; CHOL, CHANOL, and DES were

successful in decreasing surface viscosities of these films (Evans, 1995). LAN was found to be less effective than CHOL in increasing membrane rigidity in POPC bilayers, although it did promote some rigidity (Henriksen, et al., 2006). A recent study focused on the incorporation of LAN and ergosterol into DPPC and DMPC Langmuir films (Sabatini, et al., 2008). It was reported that LAN did not condense these phospholipid monolayers to the same extent as CHOL, while expansion of the LAN/phospholipid films was observed at high pressures. They concluded that the bulkier LAN head group prohibited effective packing with phospholipids compared to CHOL, and supports the findings reported in this higher temperature study.

DES was found to be weaker than CHOL in promoting the formation or stability of ordered domains in phospholipid bilayers and in mammalian cell membranes (Vainio, et al., 2006). The biophysical and functional characteristics of CHOL and DES differ due to the double bond at C24, which significantly weakens the sterol ordering potential. The DES molecules were found to tilt differently from CHOL due to conformational changes that occur when the chain fails to accommodate close packing with phospholipids. While the hydroxyl group anchors the sterol in the monolayer in the polar phosphate region, the hydrophobic tail interacts with the hydrophobic phospholipid acyl chains. The kink in the DES chain that is introduced with a double bond prevents close contact with phospholipids. However, in our monolayer studies, those hydrophobic interactions do not seem to affect the area occupied per molecule on the water surface. The chains seem to be able to accommodate DES in the hydrophobic region above the water surface.

7DHC was found to form domains that were significantly more stable than those formed with CHOL in phospholipid vesicles (Porter, 2006). It was also determined in that work that sterols with a double bond between C7 and C8 (7DHC and ergosterol) promote domain/raft formation more strongly than CHOL. In our work, 7DHC seems to occupy similar molecular areas as CHOL, and induce phospholipid condensation to a similar extent. This correlates with the bilayer studies where 7DHC and CHOL form stable domains to a similar extent. This is quite interesting when considering the impact on disease conditions. In the Smith Lemli Opitz Syndrome, 7DHC levels are markedly elevated at the expense of CHOL; it is not known whether the 7DHC incorporation in rafts affects signaling processes. Developmental effects in this syndrome have been explained by a proposed perturbation of signal transduction mediated by the Hedgehog protein (Porter, et al., 1996; Osterlund and Kogerman, 2006), which is covalently modified with CHOL, and it is possible 7DHC incorporation in rafts affects Hedgehog binding to its cognate membrane-bound receptor, Patched, or subsequent interactions between Patched and Smoothened in the signaling cascade.

The referenced studies have investigated CHOL and its derivatives in model membranes, namely vesicles, and reported how structural deviations from CHOL affect the ability to form rafts and domains. DES and 7DHC behave similarly to CHOL in their ability to pack in Langmuir monolayers, as described in this work. However, their ability to participate in raft formation and maintain cellular function in a bilayer system could be somewhat different. The presence of sterols other than CHOL in the context of a more complex membrane microenvironment containing sphingolipids and raft proteins may also be important for disease promotion. From the fundamental standpoint of structure vs. packing ability with lipids, it is clear that DES and 7DHC are comparable to CHOL and, thus, introduce minimal perturbation in the ability of sterol to pack tightly with glycerophospholipids. Minor structural deviations as presented in CHANOL lead to similar abilities to pack while the ketone group (as in CHONE) and bulky head groups (as in LAN) have the greatest deviation in packing ability compared to CHOL. Our work is in reasonable agreement with referenced studies and presents a foundation on which further investigation should build.

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Figure 1.

Structures of sterols used in this study: cholesterol (CHOL), desmosterol (DES), lanosterol (LAN), 7-dehydrocholesterol (7DHC), cholestanol (CHANOL), and cholestenone (CHONE).

Figure 2. Pressure-area isotherms for ternary monolayers composed of 10 mol% sterols and 90 mol% PC. The 10 mol % sterol mixture contains CHOL and 7DHC in ratios of CHOL:7DHC of (a) 80:20, (b) 50:50, and (c) 20:80. Isotherms in (d) represent the ideal calculated isotherms for these ternary mixtures, and overlap to the extent they cannot be easily identified separately.

Figure 3.

Pressure-area isotherms for ternary monolayers composed of 20 mol% sterols and 80 mol% PC. The 20 mol % sterol mixture contains CHOL and 7DHC in ratios of CHOL:7DHC of (a) 50:50, (b) 20:80, and (c) 80:20. Isotherms in (d) represent the ideal calculated isotherms for these ternary mixtures, and overlap to the extent they cannot be easily identified separately.

Figure 4.

Pressure-area isotherms for ternary monolayers composed of 30 mol% sterols and 70 mol% PC. The 30 mol % sterol mixture contains CHOL and 7DHC in ratios of CHOL:7DHC of (a) 50:50, (b) 20:80, and (c) 80:20. Isotherms in (d) represent the ideal calculated isotherms for these ternary mixtures, and overlap to the extent they cannot be easily identified separately.

Figure 5.

Pressure-area isotherms for ternary monolayers composed of 10 mol% sterols and 90 mol% PC. The 10 mol % sterol mixture contains CHOL and DES in ratios of CHOL:DES of (a) 50:50, (b) 20:80, and (c) 80:20. Isotherms in (d) represent the ideal calculated isotherms for these ternary mixtures, and overlap to the extent they cannot be easily identified separately.

Figure 6.

Pressure-area isotherms for ternary monolayers composed of 20 mol% sterols and 80 mol% PC. The 20 mol % sterol mixture contains CHOL and DES in ratios of CHOL:DES of (a) 20:80, (b) 50:50, and (c) 80:20. Isotherms in (d) represent the ideal calculated isotherms for these ternary mixtures, and overlap to the extent they cannot be easily identified separately.

Figure 7. Pressure-area isotherms for ternary monolayers composed of 30 mol% sterols and 70 mol% PC. The 30 mol % sterol mixture contains CHOL and DES in ratios of CHOL:DES of (a) 80:20, (b) 20:80, and (c) 50:50. Isotherms in (d) represent the ideal calculated isotherms for these ternary mixtures, and overlap to the extent they cannot be easily identified separately.

Figure 8.

Pressure-area isotherms for CHANOL and CHANOL/PC mixed monolayers. The pure CHANOL film is shown in (a). The experimental isotherms for mixtures of CHANOL/PC are shown for (b) 30 mol %, (c) 20 mol%, and (d) 10 mol% of CHANOL. Ideal isotherms for mixtures of CHANOL/PC are shown for (e) 30 mol%, (f) 20 mol%, and (g) 10 mol% CHANOL. The pure PC isotherm is shown in (h).

Figure 9.

Pressure-area isotherms for CHONE and CHONE/PC mixed monolayers. The pure CHONE film is shown in (a). The experimental isotherms for mixtures of CHONE/PC are shown for (b) 30 mol %, (c) 20 mol%, and (d) 10 mol% of CHONE. Ideal isotherms for mixtures of CHONE/PC are shown for (e) 30 mol%, (f) 20 mol%, and (g) 10 mol% CHANOL. The pure PC isotherm is shown in (h).

Figure 10.

Pressure-area isotherms for LAN and LAN/PC mixed monolayers. The pure LAN film is shown in (a). The experimental isotherms for mixtures of CHANOL/PC are shown for (b) 30 mol %, (c) 20 mol%, and (d) 10 mol% of LAN. Ideal isotherms for mixtures of CHANOL/PC are shown for (f) 30 mol%, (g) 20 mol%, and (h) 10 mol% CHANOL. The pure PC isotherm is shown in (e).

Deviations from ideal behavior in $\text{\AA}^2/\text{molecule}$ for ternary sterol:PC monolayers. CHOL:PC data are from previous study (Serfis, et al., 2001).

Table 1

10:90 Sterols:PC															
CHOL:x-Sterol		20/80				50/50				80/20					
Surface Pressure (mN/m)	5	10	15	20	25	5	10	15	20	25	5	10	15	20	25
CHOL:7DHC	6.0	2.7	4.3	5.8	4.6	5.5	2.8	6.0	7.4	7.3	7.6	3.3	4.3	5.8	4.6
CHOL:DES	5.2	3.6	3.5	3.2	3.3	7.1	5.7	5.0	4.7	4.9	5.6	2.7	2.8	2.7	3.0
CHOL:PC		2.3	2.9	3.9			2.3	2.9	3.9			2.3	2.9	3.9	
20:80 Sterols:PC															
CHOL:x-Sterol		20/80				50/50				80/20					
Surface Pressure (mN/m)	5	10	15	20	25	5	10	15	20	25	5	10	15	20	25
CHOL:7DHC	13.3	9.9	11.8	12.9	12.3	13.9	11.2	13.6	15.0	14.7	11.3	6.9	9.0	10.3	10.6
CHOL:DES	10.5	10.6	10.6	10.8	12.3	10.8	9.3	8.7	8.3	8.5	11.6	10.0	9.1	8.2	7.0
CHOL:PC		7.8	8.1	8.2			7.8	8.1	8.2			7.8	8.1	8.2	
30:70 Sterols:PC															
CHOL:x-Sterol		20/80				50/50				80/20					
Surface Pressure (mN/m)	5	10	15	20	25	5	10	15	20	25	5	10	15	20	25
CHOL:7DHC	14.5	10.4	10.9	12.9	10.8	16.7	11.8	12	11.7	11	11.0	7.4	8.4	9.5	9.3
CHOL:DES	10.9	9.3	8.6	7.7	7.6	8.7	7.4	7.1	7.7	7.6	12.6	11.1	10.2	9.3	9.5
CHOL:PC		13.1	13.3	13.0			13.1	13.3	13.0			13.1	13.3	13	

Table 2

Mean molecular area in Å²/molecule for ternary sterol:PC monolayers. CHOL:PC data are from previous study (Serfis, et. Al, 2001).

10:90 Sterols:PC															
CHOL:x-Sterol		20/80			50/50			80/20							
Surface Pressure (mN/m)	5	10	15	20	25	5	10	15	20	25	5	10	15	20	25
CHOL:7DHC	97.6	85.9	77.7	70.7	65.3	97.9	85.6	75.8	68.9	62.9	96.1	83.8	77.2	68.8	65.4
CHOL: DES	98.5	87.7	79.2	72.4	67.9	96.8	85.7	77.7	71.3	66.1	98.6	88.0	80.1	73.5	66.5
CHOL:PC		90.6	82.9	76.2			90.6	82.9	76.2			90.6	82.9	76.2	
20:80 Sterols:PC															
CHOL:x-Sterol		20/80			50/50			80/20							
Surface Pressure (mN/m)	5	10	15	20	25	5	10	15	20	25	5	10	15	20	25
CHOL:7DHC	84.0	73.7	65.7	59.7	54.2	85.9	71.2	62.6	57.3	51.5	85.6	76.0	67.8	61.6	55.3
CHOL:DES	87.1	75.5	67.7	61.4	54.3	86.3	76.6	69.1	63.8	58.9	85.5	75.7	68.8	63.6	59.3
CHOL:PC		79.6	72.2	66.5			79.6	72.2	66.5			79.6	72.2	66.5	
30:70 Sterols:PC															
CHOL:x-Sterol		20/80			50/50			80/20							
Surface Pressure (mN/m)	5	10	15	20	25	5	10	15	20	25	5	10	15	20	25
CHOL:7DHC	76.5	68.2	62.3	57.0	52.4	73.9	66.3	60.7	56.5	51.7	79.9	70.6	63.8	58.2	52.9
CHOL:DES	79.4	70.8	64.6	60.0	55.9	81.9	73	66.3	61.2	57.2	78.4	69.6	63.6	58.9	55.2
CHOL:PC		68.9	63.5	58.5			68.9	63.5	58.5			68.9	63.5	58.5	

Table 3

Mole percent sterol in sterol/ PC mixture		10				20				30						
		5	10	15	20	25	5	10	15	20	25	5	10	15	20	25
Surface Pressure (mN/m)																
Sterol:																
CHOL	2.4	2.3	2.9	3.9			8.0	7.8	8.1	8.2		13.2	13.1	13.3	13.0	
CHANOL	15.6	11.2	8.8	7.2	4.9		18.7	14.8	12.4	10.3	7.8	18.9	16.2	14.2	12.3	10.1
CHONE	4.0	1.4	0.9	1.1	0.4		12.9	11.2	10.6	10.6	10.1	11.3	9.9	9.5	9.1	8.2
LAN	4.8	3.1	3.1	3.1			5.3	4.1	4.6	5.1	5.3	3.0	2.7	4.0	5.2	5.2

Table 4

Mean molecular area in Å²/molecule for binary sterol:PC monolayers.

Mole percent sterol in sterol/ PC mixture		10			20			30								
Surface Pressure (mN/m)		5	10	15	20	25	5	10	15	20	25	5	10	15	20	25
Sterol:																
CHOL	96.2	90.6	82.9	76.2	88.6	79.6	72.2	66.5	76.8	68.9	63.5	58.5				
CHANOL	88.1	79.8	73.6	68.6	64.7	77.8	70.4	65.1	58.1	70.5	63.1	58.2	54.8	52.1		
CHONE	101.2	90.9	82.5	75.5	69.8	86.7	76.5	68.9	56.9	82.8	73.3	66.1	60.5	55.8		
LAN	99.8	88.6	79.8	72.9	63.3	93.1	82.4	73.6	60.7	89.2	78.6	69.8	62.6	56.8		