ELSEVIER

Biochimica et Biophysica Acta 1237 (1995) 127–134

BBA Biochimica et Biophysica Acta

Effect of sterol structure on molecular interactions and lateral domain formation in monolayers containing dipalmitoyl phosphatidylcholine

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Received 15 November 1994; revised 16 February 1995; accepted 30 March 1995

Abstract

Molecular associations between different sterols and dipalmitoyl phosphatidylcholine (DPPC) were examined in monolayers at the air/water interface. The sterols examined included cholesterol, 5-cholesten-3-one, 4-cholesten-3β-ol, 4-cholesten-3-one, cholesteryl acetate, and cholesteryl methyl-and ethyl ether. Information about the long-range order in pure sterol monolayers, as well as lateral domain-formation in mixed sterol/DPPC monolayers was obtained from the lateral miscibility or distribution of NBD-cholesterol (present at 0.5 mol%), as determined by monolayer epifluorescence microscopy. It was observed that the miscibility of NBD-cholesterol with the host sterol was limited in all monolayers except those of 5-cholesten-3-one and 4-cholesten-3-one, suggesting that only these monolayers lacked a long-range order present in the other sterol monolayers. Note that the term long-range order does not necessarily imply that the monolayer is solid. In mixed monolayers containing 3β-OH sterols and DPPC, cholesterol formed laterally condensed domains whereas 4-cholesten-3β-ol did not. This finding suggest that the sterol/DPPC interaction is sensitive to the position of the double-bond of the sterol molecule (Δ^5 versus Δ^4). Neither of the 3-keto sterols formed laterally condensed domains with DPPC. Cholesteryl acetate, however, formed lateral domains with DPPC which were in part similar to those seen in the cholesterol/DPPC system. The domains formed were circular, indicating their fluid nature. Mixed monolayers containing either of the ether sterol derivatives failed to produce clearly defined condensed domains with DPPC, although both mixed monolayers had a surface texture which suggested some degree of nonuniform distribution of the fluorescent probe. In summary, these novel results directly demonstrate the selective importance of both the Δ^5 double bond, as well as of specific functional groups at the 3-position, for the molecular association with DPPC, and consequently for the formation of sterol/phospholipid-rich lateral domains.

Keywords: Sterol; Cholesteryl ether; Cholesteryl acetate; Mixed monolayer; Lateral domain; Miscibility; Condensation; Surface potential

1. Introduction

The preferred sterol species in the membranes of most mammalian cells is cholesterol [1]. It has a hydrophobic and planar fused tetracyclic ring structure with two β-oriented methyl groups at positions 10 and 13, a branched extended side chain (isooctyl) at position C17, and a hydrophilic β-oriented hydroxy group at position 3. Due to its amphiphilic nature, cholesterol orients itself parallel with the acyl chains of membrane phospholipids, with its 3β-OH function in close proximity to the phospholipid ester carbonyl oxygen at the aqueous/membrane interface [2,3]. Although the 3β-OH function of cholesterol is a potential hydrogen-donating group, and the ester oxygens of glycerolipids are potential hydrogen acceptors, thus making hydrogen-bond formation possible between cholesterol and adjacent glycerolipids, direct evidence for the formation of hydrogen bonding is lacking [4]. It appears that van der Waals interactions, acting at short distances between the acyl chains of phospholipids and the sterol ring structure as well as the branched side chain of cholesterol, are more important in stabilizing cholesterol/phospholipid interactions in membranes [5–7].

Although cholesterol is the sterol preferred by most organisms, it has been established that sterol analogues having blocked 3β-OH functions (e.g., cholesteryl methyl ether and cholesteryl acetate) can support the growth of *Mycoplasma capricolum* nearly as well as cholesterol can [8,9]. Similarly, ergosterol methyl ether can support the

Abbreviations: DPPC, L-α-1,2-dipalmitoyl-*sn*-glycero-3-phosphocholine; NBD, cholesterol-22-(*N*-(7-nitrobenz-2-oxa-1,3-diazol-4-yl)amino)-23,24-bisnor-5-cholen-3-ol.

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SSDI 0005-2736(95)00096-8

growth of *Saccharomyces cerevisiae* (strain GL 7) nearly as efficiently as the native yeast sterol ergosterol [9]. Sterols with a blocked 3β-OH function can to some extent mimic the properties of cholesterol in model membranes. It has been observed that cholesteryl methyl and ethyl ether can condense the lateral packing of dioleoyl phosphatidylcholine, whereas the condensing effect of cholesteryl acetate was very marginal [10]. We have previously shown that both 5-cholesten-3-one and 4-cholesten-3-one can condense the lateral packing of 1-palmitoyl-2-oleoyl-*sn*-glycero-3-phosphocholine mixed monolayers [11], suggesting some interaction in the monolayers. Glucose permeability experiments in vesicles have demonstrated that whereas cholesteryl methyl and ethyl ethers can reduce membrane permeability to glucose in a manner similar to cholesterol, cholesteryl acetate cannot [10]. Despite many different model membrane studies, it has not been possible to definitely correlate the properties of sterols in model membranes with the growth-promoting effects seen with living organisms.

The aim of this study was to examine the lateral domain-forming properties of different sterols in a mixed monolayer with dipalmitoyl phosphatidylcholine. Lateral domains were visualized using monolayer epifluorescence microscopy. Using this technique it is possible observe the selective partitioning of a fluorescent probe among lateral domains [12–15]. The assumption taken in this study is that sterols, which form specific lateral domains with a colipid, also must engage in a specific intermolecular association, which differ from unspecific lateral miscibility.

2. Materials and methods

2.1. Materials

Cholesterol (5-cholesten-3β-ol), 5-cholesten-3-one, 4-cholesten-3-one, cholesteryl acetate, cholesteryl methyl ether, cholesteryl ethyl ether, and DPPC were obtained from Sigma (St. Louis, MO, USA). 4-Cholesten-3β-ol was purchased from Steraloids (Wilton, NH, USA). The sterols were 99% pure when used. 5-Cholesten-3-one, cholesteryl acetate, and cholesteryl methyl ether had to be purified prior to use. DPPC gave a single spot when analyzed by thin-layer chromatography. NBD-Cholesterol (22-(*N*-(7-nitrobenz-2-oxa-1,3-diazol-4-yl)amino)-23,24-bisnor-5-cholen-3-ol) was purchased from Molecular Probes (Oregon, USA). It was found to give single spot in thin-layer chromatography, and was used as delivered. The water used as subphase was purified by reverse osmosis followed by passage through a Millipore UF Plus water purification system, to yield a product with a resistivity better than 18 MΩ/cm.

2.2. Force-area isotherms

Force-area isotherms of pure or mixed lipid monolayers were collected at the air/water interface at 22° C with a KSV 3000 surface barostat (KSV Instruments, Helsinki). Briefly, stock solutions of individual lipids were prepared to a concentration of about 2 mM in hexane/2-propanol. 30 nmol of pure or mixed lipids were spread on the pure water interface, whereafter the monolayer was subjected to asymmetric compression (i.e., only from one side) with a speed not exceeding 6 Å^2/molecule, min. Triplicate determinations were obtained for each lipid composition.

2.3. Surface dipole measurements

Surface dipole versus mean molecular area isotherms of pure sterol monolayers on water were obtained using a vibrating plate potentiometer (KSV Instruments).

2.4. Epifluorescence microscopy

Pure sterol or mixed sterol/DPPC monolayers (at 20 mol% sterol) were doped with 0.5 mol% NBD-cholesterol,

Table 1
Interfacial properties of pure sterol monolayers

Sterol	Π_c [a] (mN/m)	A_c [b] (Å^2)	$A_{(1.5 \text{ or } 3.0 \text{mN/m})}$ [c] (Å^2)	k [d] (m/mN)	ΔV [e] (mV)	$\Delta A_{X\text{chol}=0.2}$ [f] (Å^2)
5-Cholesten-3ß-ol	47 (43)	37.5	40	$1.3 \cdot 10^{-3}$	+400	13.2
5-Cholesten-3-one	33	38	42.5	$3.1 \cdot 10^{-3}$	+700	12.4
4-Cholesten-3ß-ol	57 (53)	37.5	41.5	$1.3 \cdot 10^{-3}$	+500	12.5
4-Cholesten-3-one	27	40	54	$10 \cdot 10^{-3}$	+790	3.2
Cholesteryl acetate	16	41	43.5	$4 \cdot 10^{-3}$	+575	16.4
Cholesteryl methyl ether	30	39.5	41	$2.5 \cdot 10^{-3}$	+605	8.2
Cholesteryl ethyl ether	25	40	41.5	$2.5 \cdot 10^{-3}$	+570	10.2

The monolayers were spread on pure water at 22° C and were compressed with a barrier speed of about 10 Å^2/molecule, min.
[a] Collapse initiation pressure (equilibrium collapse pressure), [b] area at collapse, [c] mean molecular area at the surface pressure of 1.5 mN/m (the 3ß-OH or 3-keto sterols) or 3.0 mN/m (cholesteryl acetate, methyl-and ethyl-ether) in pure sterol monolayers, [d] compressibility (between 5–20 mN/m, [e] surface dipole potential at monolayer collapse, [f] deviation from ideal additivity (i.e., condensation) of the mean molecular area in a mixed monolayer of DPPC containing 20 mol% of the sterol, the mean molecular area was determined at a surface pressure of 1.5 mN/m.

and observed with an inverted epifluorescence microscope, as described previously [15]. Documentation of the monolayer surface texture was accomplished by video camera, which was connected to a DT3851 (Data Translation, Marlboro, MA, USA) digitizing board in a personal computer.

3. Results

3.1. Monolayer characteristics of pure sterols

In order to compare how the functional group at position 3 (a hydroxy, a keto, an acetate, or an ether function), and the double bond position (Δ^5 or Δ^4) affected the molecular packing properties of the sterols in pure sterol monolayers, force-area isotherms were obtained at the air/water interface at 22° C. The monolayer properties of the sterols used in this study have been reported to some extent previously by us and others [6,10,11], and are included here for the convenience of the reader. The packing density, as evidenced both from the molecular area at collapse, and from the compressibility of the force-area isotherm function, was highest with the 3β-OH sterols (Table 1), irrespective of the position of the double bond. The collapse areas and compressibilities of the two 3-keto sterols were slightly or markedly higher compared to the 3β-OH analogues (Table 1). Cholesteryl acetate, and cholesteryl methyl-and ethyl-ether had collapse areas which were slightly higher than that for cholesterol, and the calculated compressibilities for these sterols were also slightly higher than that for cholesterol (Table 1). Monolayers with the highest stability (i.e., with the highest collapse pressure) were seen with 4-cholesten-3β-ol followed by cholesterol, whereas the two 3-keto sterol monolayers had collapse pressures of 33 and 27 mN/m (5-cholesten-3-one and 4-cholesten-3-one, respectively). The stability of the cholesteryl ether monolayers was similar to that observed with the 3-keto sterols, whereas the cholesteryl acetate monolayer displayed the lowest collapse pressure (16 mN/m; Table 1).

3.2. Surface dipole measurements

Measurement of the surface dipole of the pure sterol monolayers indicated that the 3-keto group was a significantly stronger dipole than the 3β-OH group, giving the 3-keto sterol monolayers a higher surface dipole potential

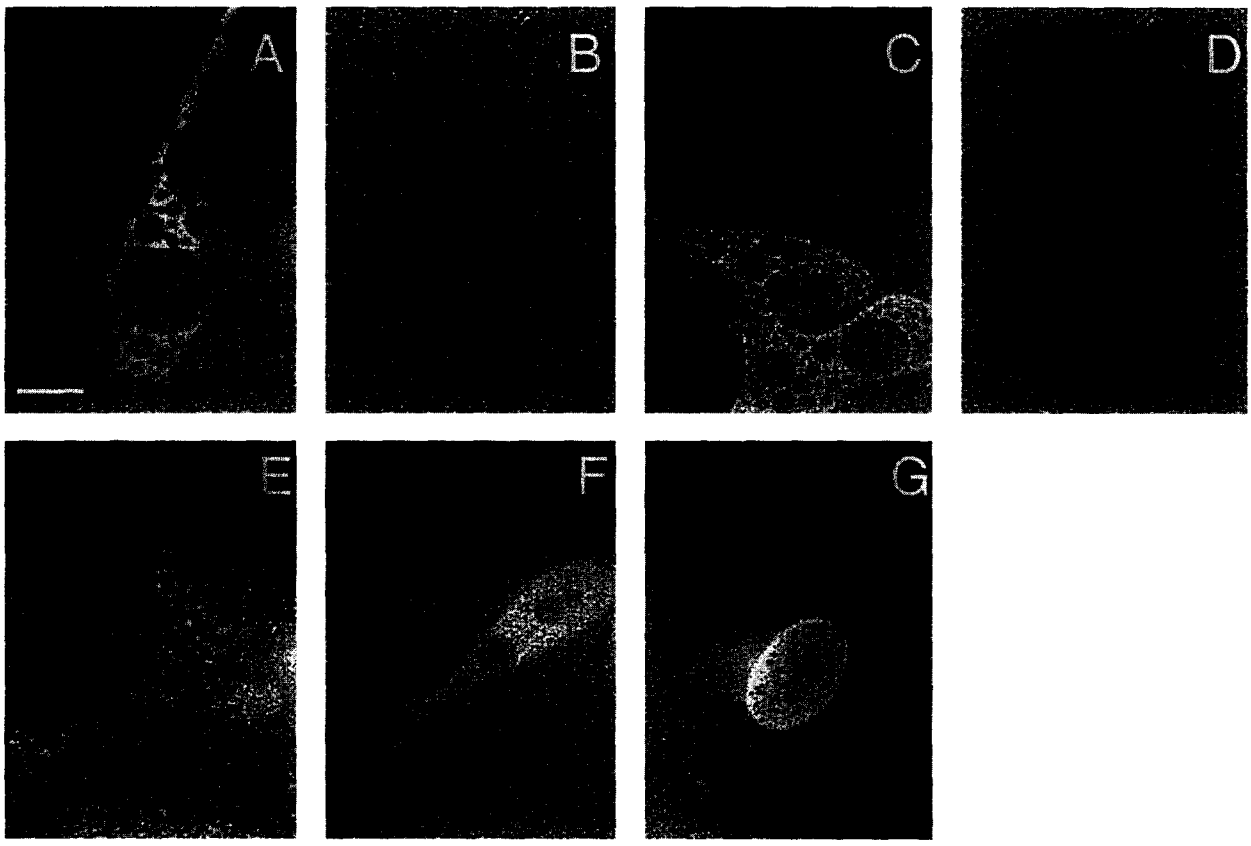

Fig. 1. Epifluorescence micrographs of pure sterol monolayers containing 0.5 mol% of NBD-cholesterol. The sterol monolayers were compressed with a speed not exceeding 3.4 Å^2/molecule, min. The lateral surface pressure is 1.5 mN/m for panels A–D, and 5 mN/m for E–G. Panel A is cholesterol (5-cholesten-3ß-ol), B is 5-cholesten-3-one, C is 4-cholesten-3ß-ol, D is 4-cholesten-3-one, E is cholesteryl acetate, F is cholesteryl methyl ether, and G is cholesteryl ethyl ether. The bar represents 100 μm. Surface texture was documented during the initial compression of the monolayers.

than the 3β-OH sterol monolayers (Table 1). It was also observed that with sterols having identical functional groups at the 3 position (i.e., 3β-OH of 3-keto), the Δ^4 sterols had a slightly higher surface dipole potential than the Δ^5 sterols (Table 1). Cholesteryl acetate, and the two cholesteryl ethers had surface dipole potentials which were intermediate to those of the 3β-OH sterols and the 3-keto sterols (Table 1). The interpretation of the surface dipole potentials of this study is complicated by the fact that we do not have information about the effects of the sterols on the orientation of interfacial water, a variable which also affects the measurable surface potential [16].

3.3. Sterol-induced condensation of DPPC packing in mixed monolayers at a low surface pressure

Mixed monolayers were prepared to contain 20 mol% of a sterol and 80 mol% of DPPC. The mean molecular area in such monolayers was determined at a surface pressure of 1.5 mN/m (this low surface pressure was selected because subsequent lateral domain visualizations were performed at low surface pressures), and the observed value was compared to the value expected based on simple additivity of the fractional areas of the two monolayer components. It was observed that all sterols condensed the packing of DPPC at this low surface pressure, the magnitude of the condensation being highest with cholesteryl acetate, followed by cholesterol, 5-cholesten-3-one, 4-cholesten-3β-ol, cholesteryl ethyl ether, and cholesteryl methyl ether, with 4-cholesten-3-one giving the lowest degree of condensation (Table 1).

3.4. Fluorescence microscopy of pure sterol monolayers

Next the distribution of NBD-cholesterol in pure sterol monolayers was examined by epifluorescence microscopy. We have shown that NBD-cholesterol preferentially partitions into loosely packed lateral domains and is excluded from more densely packed domains, and hence acts like an impurity in the monolayer [15]. In pure sterol monolayers, in which the mean molecular area of the sterols is similar (i.e., similar packing density), differences in the lateral solubility of NBD-cholesterol may be influenced by the extent of long-range ordering of the molecules present in

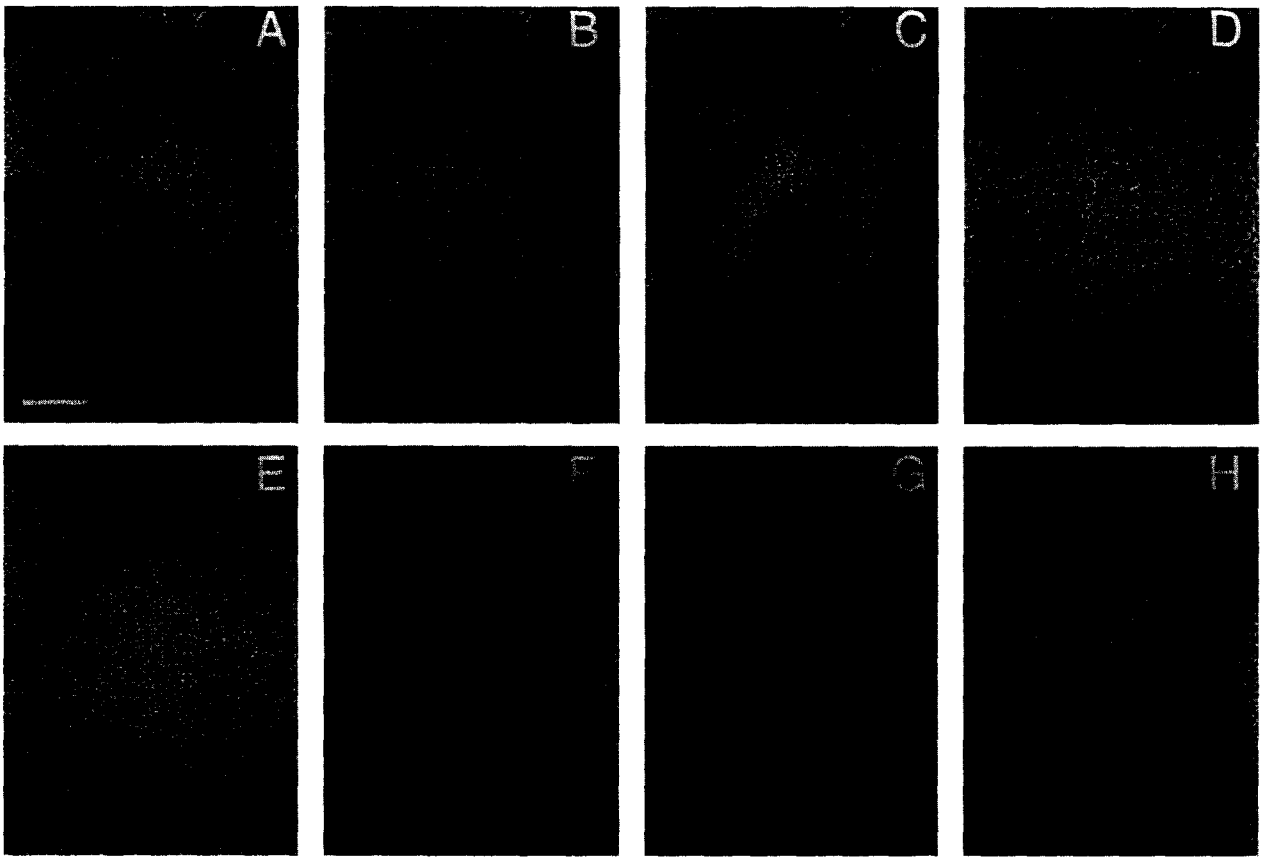

Fig. 2. Lateral domain formation in mixed sterol/phospholipid monolayers. Monolayers containing 20 mol% sterol in DPPC (with 0.5 mol% NBD-cholesterol) were compressed (at 3.4 Å^2/molecule, min) at 22° C, and micrographs taken at different intervals. The top row is for a cholesterol-containing monolayer, with panel A taken at a mean molecular area of 120 Å^2, panel B is at a lateral surface pressure of 1.5 mN/m (during the first compression), panel C at 4 mN/m (first compression), and panel D at 1.5 mN/m (during expansion). The bottom row represents monolayers with 4-cholesten-3ß-ol as the sterol, and the panels E, F, G, and H correspond to the same mean molecular area or surface pressure as the top row panels. The bar in panel A represents 100 μm, and depicts the magnification in all panels.

the monolayers. Since a pure cholesterol monolayer is densely packed and may have long-range order (although the monolayer still is fluid even at high compression), NBD-cholesterol appears to be excluded from the pure cholesterol phase, and is forced to partition into domains of its own (cf. [15]; and Fig. 1A). The partitioning of NBD-cholesterol in a 4-cholesten-3β-ol monolayer also resulted in the formation of small brightly fluorescent domains, indicating that the pure 4-cholesten-3β-ol phase effectively excluded the fluorophore due to its high packing density and/or long-range order (cf. Fig. 1C). However, the pure monolayers of the 3-keto sterols displayed a uniform distribution of NBD-cholesterol (Fig. 1B and D), indicating that the packing density in these pure monolayers was looser, or that the monolayer sterols did not have a significant long-range order which would affect the lateral solubility of NBD-cholesterol. Table 1 gives the mean molecular areas of these sterols at 1.5 mN/m, and only 4-cholesten-3-one has a markedly larger molecular area at this surface pressure. When monolayers containing cholesteryl acetate, cholesteryl methyl ether, and cholesteryl ethyl ether were examined, it was observed that NBD-cholesterol was not readily dissolved laterally, but accumulated in lateral domains of its own (Fig. 1E,F). The mean molecular area at 3 mN/m of cholesteryl acetate (Table 1) was slightly larger as compared to cholesteryl methyl-and ethyl ethers, and these in turn had similar molecular areas as the 3β-OH sterols. Since the mean molecular areas of cholesteryl acetate, and cholesteryl methyl-and ethyl ethers were similar to that of 4-cholesten-3-one, whereas the lateral solubility of NBD-cholesterol was not, it can be inferred that these sterol monolayers displayed a long-range order which was absent in monolayers of the 3-keto sterols.

3.5. Mixed monolayers of sterols and DPPC

To examine the interaction of the sterols with DPPC in mixed monolayers, we analyzed the formation of liquid-expanded and liquid-condensed lateral domains. For visualization of the lateral domains, the films were prepared to contain 0.5 mol% NBD-cholesterol, and were spread to a starting available mean molecular area of 133.5 Å^2, and then compressed with a barrier speed of 3.4 Å^2/molecule, min. At a mean molecular area of 120 Å^2 (at which point the lateral surface pressure was close to zero mN/m), one could observe the coexistence of gas and liquid-expanded phase in the cholesterol/DPPC monolayer (20 mol% sterol; Fig. 2A). The irregular form of the domain boundary line between the gas and liquid-expanded phase indicated that some specific interactions occurred between cholesterol and DPPC already in the liquid-expanded phase. When the monolayer was compressed to a surface pressure of 1.5 mN/m, circular liquid-condensed domains were formed (Fig. 2B). These domains were irregularly distributed and were not uniform in size. As the monolayer was compressed further, the domain boundary line between the liquid-expanded and the liquid-condensed phases dissipated at a surface pressure of about 2.5–3 mN/m (i.e., phase transformation pressure; [17,18]). Above this pressure (e.g., 4 mN/m, Fig. 2C), no lateral domains could be visualized. When the monolayer was expanded below the phase transformation pressure (to 1.5 mN/m), liquid-condensed domains reappeared in the bulk liquid-expanded phase (Fig. 2D).

Mixed monolayers containing 4-cholesten-3β-ol and DPPC (at 20 mol% sterol) also displayed coexistence of gas and liquid-expanded phases at 120 Å^2 (Fig. 2E). However, the line boundary between gas and liquid-ex-

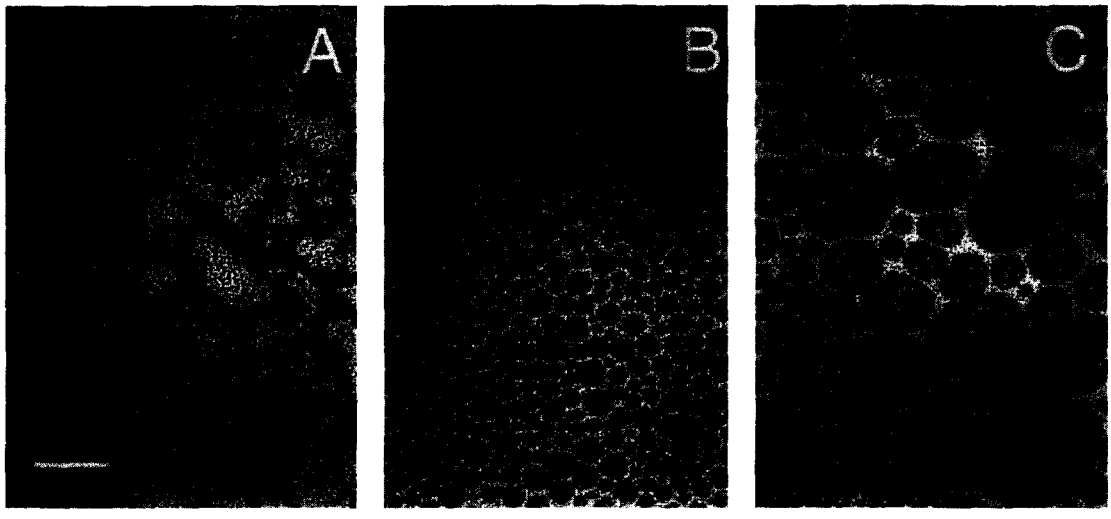

Fig. 3. Lateral domain formation in a mixed cholesteryl acetate/phospholipid monolayer. The monolayer with 20 mol% cholesteryl acetate in DPPC (with 0.5 mol% NBD-cholesterol) was compressed (at 3.4 Å^2/molecule, min) at 22° C, and micrographs were taken at different intervals. Panels A is at a mean molecular area of 110 Å^2, panel B at a lateral surface pressure of 3 mN/m (first compression), and panel C at a lateral surface pressure of 3 mN/m (during expansion from a higher surface pressure (i.e., 15 mN/m)). The length bar is 100 μm.

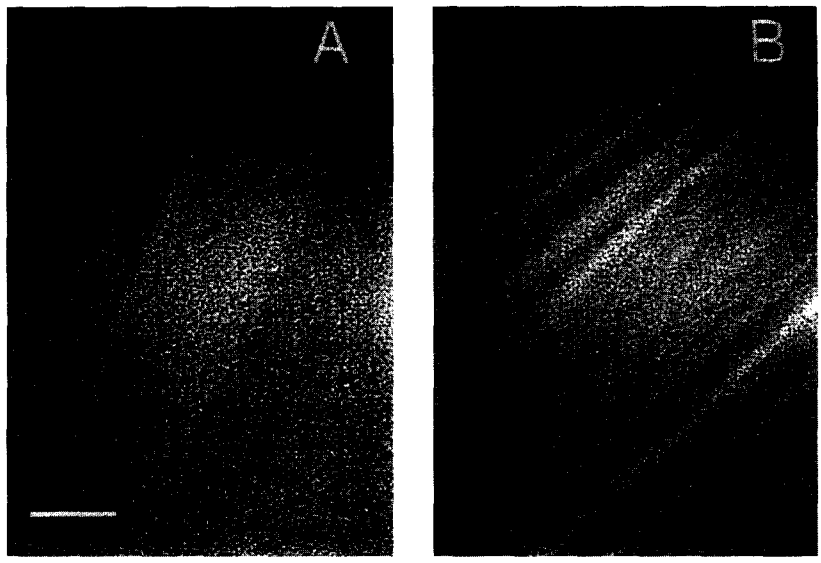

Fig. 4. Lateral domain formation in mixed sterol/phospholipid monolayers. Monolayers containing 20 mol% of cholesteryl methyl ether (A) or cholesteryl ethyl ether (B) in DPPC (with 0.5 mol% NBD-cholesterol) were compressed (at 3.4 Å^2/molecule, min) at 22° C, and micrographs were taken at a lateral surface pressure of 3 mN/m (during initial compression). The length bar is 100 μm.

panded phases was not irregular, in contrast to what was observed with cholesterol/DPPC mixed monolayers (Fig. 2A). Upon compression, the gas phase disappeared, and at 1.5 mN/m only a liquid-expanded phase could be seen (Fig. 2F). Since no liquid-condensed phases were present, no phase transformation pressure could be determined, and the monolayers were uniformly fluorescent (liquid-expanded) at the surface pressure examined (Fig. 2G is at 4 mN/m, and Fig. 2H at 1.5 mN/m during monolayer expansion). The two keto sterols of this study, 5-cholesten-3-one and 4-cholesten-3-one, also failed to form liquid-condensed domains when mixed with DPPC at 20 mol% sterol (micrographs not shown).

When cholesteryl acetate was mixed with DPPC (at 20 mol%), a coexistence pattern between gas and liquid-expanded phases was seen (Fig. 3A) which was very similar to that observed with cholesterol and DPPC (Fig. 2A). The highly irregular line boundary between gas and liquid-expanded phases suggest the presence of long-range order already in the liquid-expanded phase. When the cholesteryl acetate/DPPC monolayer was compressed to a surface pressure of 3 mN/m, the monolayer contained circular liquid-condensed domains in the bulk liquid-expanded phase (Fig. 3B). These liquid-condensed domains were larger in size that those seen in the cholesterol/DPPC monolayer. The phase transformation pressure, at which the line boundary between the liquid-condensed and the liquid-expanded phases dissolved was about 5.5 mN/m in the cholesteryl acetate/DPPC monolayer. When the monolayer was compressed to 15 mN/m, and then expanded back to a surface pressure of 3 mN/m, large liquid-condensed domains were formed, most of which contained clusters of small liquid-expanded domains (Fig. 3C). A similar domain pattern was not seen with the cholesterol/DPPC system.

Mixed monolayers of cholesteryl methyl ether or cholesteryl ethyl ether in DPPC failed to produce drop-like liquid-condensed domains, as seen with both cholesterol and cholesteryl acetate (Fig. 4A and B). Instead, the monolayer texture was characterized by a stripe-like pattern of lighter and darker zones.

4. Discussion

The molecular interaction between cholesterol and phospholipids is specific, and as a consequence of this, lateral cholesterol-rich domains are formed in mixed monolayers having a low-to-moderate concentration of cholesterol (10–33 mol%; [19]). In this study, the effect of substitutions at the 3 position of the cholesterol molecule have been examined with respect to the formation of lateral domains in monolayers.

If we first consider the packing properties of pure sterol monolayers as deduced from the partitioning of a fluorescent probe, NBD-cholesterol, it was observed that the lateral distribution of NBD-cholesterol was nonuniform in all sterol monolayers except those containing 3-keto sterols. Since the lateral distribution of NBD-cholesterol did not correlate with the lateral packing density of the corresponding sterols (Table 1), a nonuniform distribution of the probe most likely resulted because of long-range molecular interactions among the host sterols. Such interactions were present in cholesterol, 4-cholesten-3β-ol, cholesteryl acetate, and cholesteryl methyl and ethyl ether monolayers, but were absent from 3-keto sterol monolay-

ers. It is unclear at present what possible contribution hydrogen bonds at the water/lipid interface could make to these molecular interactions, in addition to the attractive van der Waals forces acting between the sterols in the hydrophobic region of the monolayer. Cholesterol, 4-cholesten-3β-ol, and cholesteryl acetate most likely form hydrogen bonds with water molecules [4]. In addition, due to their tight packing density, cholesterol and 4-cholesten-3β-ol monolayers probably are stabilized to a significant extent by van der Waals forces. The extent of van der Waals forces in stabilizing long-range order is apparently smaller with the 3-keto sterols and with cholesteryl acetate, since the monolayers of these sterols are much less densely packed as compared with cholesterol (Table 1; [6,10]).

The interaction of cholesterol with phosphatidylcholines in mixed monolayers is known to result in the formation of cholesterol-rich lateral domains [17–19], the fractional area of which appear to increase with increasing cholesterol concentration [19]. In the present study, sterol-rich, drop-like (liquid-condensed) domains were formed in cholesterol/DPPC and cholesteryl acetate/DPPC mixed monolayers, but not in mixed DPPC monolayers containing 5-cholesten-3-one, 4-cholesten-3β-ol, or 4-cholesten-3-one. Of these three latter sterols, 4-cholesten-3β-ol displayed long-range order in pure sterol monolayers, whereas the 3-keto sterols did not. The mixing of either of the two ether sterols with DPPC resulted in a monolayer in which the NBD-cholesterol partitioned heterogeneously in the lateral plane, although no distinct drop-like sterol-rich domains were formed. The surface texture of the mixed ether sterol/DPPC monolayers still revealed the occurrence of long-range order and nonuniform distribution of the monolayer components. The finding that 4-cholesten-3-one did not form lateral domains with DPPC, but rather dispersed homogeneously in the plane of the monolayer, is consistent with its suggested random distribution in DPPC bilayers [20].

The formation of sterol-rich lateral domains in the DPPC monolayers appeared not to correlate with the condensing effect (or lack thereof) that these sterols have on phosphatidylcholine packing in mixed monolayers. All the sterols were able to induce some condensation of phosphatidylcholine packing at a low surface pressure, the effect being smaller with 4-cholesten-3-one, whereas only a few sterols formed lateral domains with DPPC. Previous monolayer studies have also indicated that 3-keto sterols induce phospholipid condensation, although to a somewhat lesser extent than cholesterol [6,11]. Cholesteryl acetate and cholesteryl methyl and ethyl ethers have also been shown to induce condensation of phosphatidylcholine packing in mixed monolayers [10,21]. Although the 3-keto sterols may condense the lateral packing density of acyl-chain defined phosphatidylcholines in binary systems to some extent, they do not reduce the solute permeability of model membranes as cholesterol does [22], and they have even been shown to induce an increased membrane permeability to small solutes when incorporated into the membranes of red blood cells [23].

The formation of lateral domains in DPPC mixed monolayers by the sterols correlated to some extent with the presence of long-range order in the pure sterol monolayers, the only exception being 4-cholesten-3β-ol which had apparent long-range order in the pure monolayer but which failed to form lateral domains with DPPC. This discrepancy may be explained as follows: even though the A-ring of 4-cholesten-3β-ol is distorted as compared to the A-ring conformation of cholesterol (for a comparison, see [11]), the sterols would still be expected to pack efficiently (because they are all alike), and the monolayer would further be expected to be stabilized by hydrogen-bonded water to the 3β-OH function (as evidenced by the high collapse pressure of 4-cholesten-3β-ol monolayers). However, the distorted A-ring of 4-cholesten-3β-ol apparently makes its interaction with DPPC less favorable, at least as compared with cholesterol, and therefore condensed sterol/phospholipid domains could not form in this mixed monolayer. Supporting evidence for the less favorable interactions between 4-cholesten-3β-ol and phospholipids comes from permeability studies, in which it was shown that glucose-permeability in sterol/egg phosphatidylcholine vesicles (33 mol% sterol) was significantly increased when 4-cholesten-3β-ol replaced cholesterol [24]. 4-Cholesten-3β-ol was also incorporated to a lesser extent into vesicles as compared to the situation observed for cholesterol [24], suggesting that the sterol could not interact efficiently with the phospholipids, due to the distorted A-ring.

The formation of lateral domains in mixed DPPC/sterol monolayers also appeared to correlate with the growth-promoting capacity of the sterols in a *Mycoplasma capricolum* system. Cholesterol, cholesteryl acetate, and cholesteryl methyl ether, which all formed lateral domains with DPPC (although the domain properties differed for cholesteryl methyl ether, as compared to the two other sterols), have been shown to promote growth of a sterol-auxotroph strain of *Mycoplasma* [8,9]. The growth-promoting efficacy of cholesteryl ethyl ether is not known.

Theoretical models as well as experimental evidence suggest that the lateral domains formed in cholesterol/phospholipid mixed monolayers are stabilized by van der Waals forces acting at short distances between the sterols and the aliphatic chains of the phospholipids [25]. This interaction contributes significantly to the domain wall energy [25]. However, the attractive van der Waals forces are balanced by repulsive electrostatic interactions between the polar moieties of the lipids (the dipoles) [26,27]. Therefore, when mixed monolayers in the two-phase coexistence region are compressed isothermally, the dipolar repulsion starts to dominate, leading to a continually decreased domain-wall energy. When the dipolar repulsion forces are stronger than the van der Waals attractive forces, the domain-wall energy vanishes. This results

in the loss of the domain boundary line separating the sterol-rich domains from the bulk phospholipid domains. This phase transformation pressure [17,18] was lower for cholesterol/DPPC than it was for the cholesteryl acetate/DPPC system (2.5–3 versus 5–6 mN/m, respectively). Although neither the van der Waals attractive forces nor the dipole repulsion forces can easily be calculated for these two systems, the measurable surface dipole potential of the pure sterol monolayers indicated that the dipole potential was larger with cholesteryl acetate (+575 mV) than it was with cholesterol (+400 mV). Since the attractive van der Waals forces between cholesteryl acetate and DPPC are of a similar or smaller magnitude as compared to the cholesterol/DPPC system, and since the overall dipole of cholesteryl acetate is stronger, it appears possible that the higher phase transformation pressure in the cholesteryl acetate/DPPC system arises from a more efficiently stabilizing hydrogen-bond network between the cholesteryl acetate, water, and DPPC molecules.

In conclusion, we have utilized monolayer epifluorescence microscopy to study the formation of lateral domains in sterol/DPPC mixed monolayers, and correlated the domain-forming properties of the sterols with their structure and membrane characteristics. There appear to be a fairly good correlation between domain-forming sterols and growth-promoting sterols, suggesting the interesting hypothesis that growth-promotion is related to domain-formation in membranes. The monolayer epifluorescence microscopy technique is a powerful method by which it is possible to further test and refine this hypothesis.

Acknowledgements

I thank Peter Mattjus for comments on the manuscript. The technical assistance of Ms. Huifang Ge in some of the preliminary experiments is acknowledged. This study was supported by generous grants from the Sigrid Juselius Foundation, the Academy of Finland, and the Åbo Akademi University.

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