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STEROL-PHOSPHOLIPID INTERACTIONS IN MODEL MEMBRANES

EFFECT OF POLAR GROUP SUBSTITUTIONS IN THE CHOLESTEROL SIDE-CHAIN AT C_{20} AND C_{22}

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The interactions of phospholipids with four different cholesterol derivatives substituted with one OH or one keto group at position C_{20} or C_{22} of the side-chain were studied. The derivatives were the 22,*R*-hydroxy; 22,*S*-hydroxy; 22-keto- and 20,*S*-hydroxycholesterol. Two aspects of the interactions were investigated: (1) the effect of the cholesterol derivatives on the gel → liquid crystalline phase transition of dipalmitoylphosphatidylcholine (DPPC) and of dielaidoylphosphatidylethanolamine (DEPE) monitored by differential scanning calorimetry and (2) The effect on the lamellar → hexagonal H_{II} phase transition of DEPE monitored by DSC and by ^{31}P-NMR to determine structural changes. The gel → liquid crystalline phase transition was affected by the cholesterol derivatives to a much larger extent in the case of DPPC than of DEPE. In both cases, there was a differential effect of the four derivatives, the 22,*R*-hydroxycholesterol being the less effective. In DPPC-sterol 1:1 systems, 22,*R*-hydroxycholesterol does not suppress the melting transition, the ΔH values becomes 7.1 kcal·mol^{-1} as compared to 8.2 kcal·mol^{-1} for the pure lipid. 22,*S*-OH cholesterol has a much stronger effect ($\Delta H = 3.1$ kcal·mol^{-1}) and 22-ketocholesterol suppresses the transition completely. In DEPE mixtures of all these compounds, the melting transition of the phospholipid is still observable. The transition temperature was shifted to lower values (−13.5°C in the presence of 20,*S*-OH cholesterol). The ΔH of the transition was lowered by these compounds except in DEPE-22,*R*-OH cholesterol mixtures and the cooperativity of the transition (reflected by the width at half peak height) was reduced. The lamellar → hexagonal H_{II} phase transition was also affected by the presence of these cholesterol derivatives. The transition temperature value was depressed with all these compounds. 20,*S*-OH cholesterol was the most effective followed by 22,*R*-OH cholesterol. The ΔH of the transition was not strongly affected. The molecular interfacial properties of these derivatives were studied by the monomolecular film technique. It is most likely that 22,*R*-OH cholesterol due to the hydroxyl groups at the

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Abbreviations: DEPE, 1,2-dielaidoyl-*sn*-glycero-3-phosphoethanolamine; DOPE, 1,2-dioleoyl-*sn*-glycero-3-phosphoethanolamine; DPPC, 1,2-dipalmitoyl-*sn*-glycero-3-phosphocholine; DOPC, 1,2-dioleoyl-*sn*-glycero-3-phosphocholine; EDTA, ethylenediaminetetraacetic acid; 22,*R*-OH cholesterol, (22,*R*)-5-cholestene-(3β,22*R*)-diol; 22,*S*-OH cholesterol, (22,*S*)-5-cholestene-(3β,22*S*)-diol; 20,*S*-OH cholesterol, (20*S*)-5-cholestene-(3β,20*S*)-diol; 22-ketocholesterol, 5-cholestene-3β-ol-22-one; cytochrome *P*-450$_{scc}$, cytochrome *P*-450 specific for the side-chain cleavage of cholesterol.

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3β- and 22,*R*-positions orients with the sterol nucleus lying flat at the air/water interface, since the compression isotherm of either the pure sterol or the DOPC-sterol mixture (molar ratio, 1:1) monomolecular film exhibits a transition at approx. 103 Å^2, corresponding to the area of revolution of the sterol nucleus. This remarkable property, due probably to the existence of a kink between the side-chain and the long axis of the steroid nucleus, might explain the smaller effect of this sterol on the melting transition of either PC or PE systems.

Introduction

Cholesterol is a membrane building block and as such it interacts strongly with most of the phospholipids present in biological membranes [1]. As a consequence of this interaction, the membrane physical properties are altered in such a way that the gel state is less ordered (spacing effect) and the liquid state more ordered (condensing effect). Structural requirements, such as a 3β-OH group, a flexible side-chain and a plane nucleus, are prerequisite for maximizing these effects [1]. In addition, cholesterol and derivatives affect also the lipid polymorphism of non-bilayer-forming lipids [2–5]. This effect is related to their dynamical shape as estimated by their properties in monomolecular films [4].

However, cholesterol is not only a membrane structural component, but is also of metabolic importance. In steroidogenic tissues, cholesterol is the precursor of the steroid hormone biosynthesis. In this process, cholesterol is converted to the final hormone products via a series of enzymatic reactions which are mostly catalyzed by membrane-bound enzymes [6]. The initial steps of this conversion are occurring in the mitochondria and lead to the formation of pregnenolone [7]. It is generally accepted that these steps are rate-limiting in steroidogenesis [8]. Current evidence suggests that these initial steps of steroidogenesis are regulated in the adrenal cortex by ACTH [9]. The first reaction taking place during the conversion of cholesterol into pregnenolone is the hydroxylation at the C_{22} position and conversion to the *R* configuration [10]. The formation of this compound (22,*R*-OH cholesterol) catalyzed by the membrane-bound cytochrome *P*-450 specific for the side-chain cleavage of cholesterol exhibits the highest activation energy [11] as compared to the subsequent catalytic hydroxylation steps leading to the final removal of the isocaproic side-chain. Reconstitution of cytochrome $P\text{-}450_{scc}$ phospholipid have shown that, whereas the interaction of cholesterol with the cytochrome was strongly dependent upon the kind of phospholipid present, the interaction of the hydroxylated compounds, and especially of the 22,*R*-hydroxycholesterol, was not [11]. To explain this difference, it has been proposed (i) that phospholipids are part of the binding site and (ii) that the interaction between hydroxycholesterol and these phospholipids might not be strong enough to modulate the binding on the protein [11]. In agreement with this hypothesis, it has been shown recently by fluorescence anisotropy technique that the interaction of cholesterol derivatives hydroxylated in the side-chain with phospholipids was strongly dependent upon the substitution position of the polar group in the side-chain [12]. The C_{22} position appeared to be quite critical, since striking differences on lipid dynamics were observed between the two epimers 22,*R*-OH and 22,*S*-OH cholesterol.

In this paper we describe further investigations on the interactions of these biologically interesting derivatives with phospholipids. A comparative study was carried out with 22,*R*-OH, 22,*S*-OH, 20,*S*-OH and 22-ketocholesterol. Two aspects of the interaction were investigated: (i) the influence of these derivatives on the membrane lipid fluidity; and (ii) their effect on the membrane lipid structure. Therefore, the influence of these cholesterol derivatives on (i) the gel → liquid crystalline phase transition of DPPC and of DEPE was studied by differential scanning calorimetry and (ii) on the lamellar → H_{II} phase transition of DEPE was studied by DSC and ^{31}P-NMR. Additionally, the interfacial behavior of these derivatives was studied by the monomolecular film technique. A preliminary report of this work was already published [13].

Materials and Methods

Chemicals

$C_{18}:1_t/C_{18}:1_t$ phosphatidylethanolamine (DEPE), $C_{16}:0/C_{16}:0$ phospathidylcholine (DPPC) and $C_{18}:1_c/C_{18}:1_c$ phosphatidylcholine (DOPC) were synthesized as described previously [14,15].

22,*R*-OH, 22,*S*-OH, 20,*S*-OH and 22-ketocholesterol were purchased from Makor Chem. Ltd (Jerusalem, Israel) and were used as supplied. All the other chemicals were of the highest grade commercially available.

Monolayer experiments

Compression curves of pure or mixed sterol-DOPC films spread at the air/water interface were measured using a Teflon trough. The total capacity of the trough was 1200 ml. The trough was filled with 10 mM Tris buffer (pH 7.4). The aqueous surface was swept clean with a Teflon bar. Surface pressure was recorded using a recording electrobalance (Beckman LM500) with a sand-blasted platinum plate (Prolabo) 1.96 cm wide. 50 nmoles of either pure sterol, or pure DOPC or 1:1 mixture in chloroform were carefully spread. Recording of the isotherms was performed at room temperature during continuous compression of the film at a rate of 74.65 $cm^2 \cdot min^{-1}$. The initial area was 553.84 cm^2.

^{31}P-NMR spectroscopy

Proton-noise-decoupled ^{31}P-NMR spectra were recorded on a Brüker WH90 operating at 36.4 MHz. Operating conditions have been described previously [16]. 20000 scans were accumulated. The samples were prepared as follows: 50 μmol DEPE solution (in 1 ml chloroform) containing the appropriate amount of sterol was evaporated under nitrogen in a flat-bottom 10 mm NMR tube and removal of the remaining solvent was performed under high vacuum for at least 7 h. The dry lipids were then hydrated by adding 0.8 ml 0.01 M Tris-HCl buffer (pH 7.4)/0.1 M NaCl/2 mM EDTA and 0.2 ml of the same buffer in ^2H_2O. Dispersion was accomplished by agitation on a vortex mixer. The percentage of lipids organized in extended bilayer or in hexagonal H_{II} phase were determined by computation using the characteristic pure 'hexagonal' type of spectrum of ^{31}P-NMR line-shape as reference.

Differential scanning calorimetry measurements

Sterol-DPPC mixtures. Samples were prepared directly in the sample container by carefully weighing the phospholipid (approx. 1.5 mg). Subsequent addition of chloroform solutions of sterols, careful mixing and exhaustive drying were carried out. Hydration of the sample was carried out with 100 μl 10 mM Tris-HCl buffer (pH 7.4)/0.1 M NaCl/2 mM EDTA. The thermograms were recorded with a Setaram 111 differential scanning calorimeter at a rate of 2 C deg $\cdot$ min^{-1}. Integration of the peaks and computation of the transition enthalpy were performed by an interface Apple II computer using a program devised by Dr. Van Miltenburg (Laboratory of Chemical Thermodynamics, State University of Utrecht, The Netherlands). The transition temperatures were determined as the intercept between the baseline and the tangent of the rising slope.

Sterol-DEPE mixtures. The samples were prepared as for NMR experiments. The samples were centrifuged at 20000 rpm for 60 min in an SS34 Sorvall rotor, the lipids were carefully transferred to the sample pans of a DSC II Perkin Elmer differential calorimeter. Heating scans were performed at a rate of 5 C deg $\cdot$ min^{-1}. The transition temperature was determined as the intercept between the base line and the rising slope. Phosphorus determinations were performed according to Fiske and SubbaRow [17] after lipid extraction in chloroform/methanol (1:1) and destruction of the sample by perchloric acid. Calculation of the transition enthalpy was performed by weighing the thermograms. DPPC was taken as reference (ΔH = 8.2 kcal $\cdot$ mol^{-1} as determined with a Setaram DSC 111 calorimeter).

Results

Differential scanning calorimetry study of sterol-DPPC interaction

The influence of the cholesterol derivatives modified on the side-chain on the gel $\rightarrow$ liquid crystalline phase transition of DPPC was studied by differential scanning calorimetry. The thermograms of the different DPPC-sterol mixtures at a

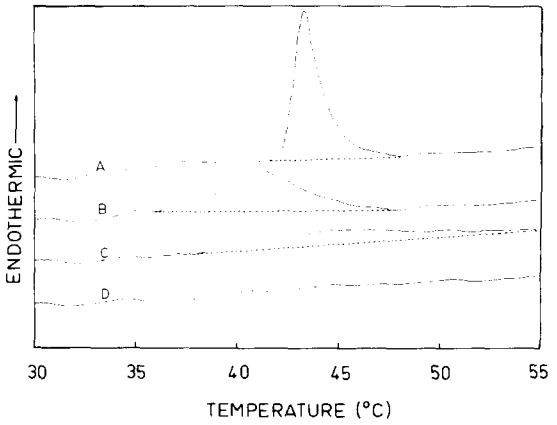

Fig. 1. Thermograms of DPPC and sterol-DPPC 1 : 1 mixtures. Heating rate, 2 C deg·min^{-1}. Trace A, pure DPPC; B, mixture of DPPC and 22,*R*-OH cholesterol (1 : 1); C, mixture of DPPC and 22,*S*-OH cholesterol (1 : 1); D, mixture of DPPC and 22-ketocholesterol (1 : 1).

1 : 1 molar ratio are presented in Fig. 1. It can be observed that the 22,*R*-OH derivative does not suppress the transition. It results only in a broadening (expressed as the width at half peak height, $\Delta T_{1/2}$) of the transition and a downward shift of the transition temperature of 4.2 C deg. The ΔH of the transition is decreased only by 13% (Table I). On the other hand, 22,*S*-OH cholesterol has a much stronger effect on the transition than its epimer. The transition is almost completely suppressed as the ΔH is decreased by 62% (Table I). 22-Ketocholesterol suppressed the transition completely.

DEPE-sterol interaction

DSC in combination with ^{31}P-NMR techniques were used to study the effect of these cholesterol derivatives on the gel → liquid crystalline phase transition and on the bilayer → hexagonal H_{II} phase transition of DEPE. The phase behavior of this lipid has been studied by X-ray diffraction and by ^{31}P-NMR (Ref. 2 and De Kruijff, B., unpublished data). The gel → liquid crystalline

TABLE I

THERMODYNAMIC PARAMETERS OF THE GEL → LIQUID CRYSTALLINE PHASE TRANSITION OF DPPC AS AFFECTED BY THE PRESENCE OF STEROLS

$\Delta T_{1/2}$ is defined as the width at half peak height.

	ΔH (kcal·mol^{-1})	$\Delta T_{1/2}$ (C deg.)	T_c (°C)
DPPC	8.2	1.3	42
DPPC + 22,*R*-OH cholesterol, 1 : 1	7.1	4.8	37.8
DPPC + 22,*S*-OH cholesterol, 1 : 1	3.1	> 10	37.5
DPPC + 22-ketocholesterol, 1 : 1	0	–	–

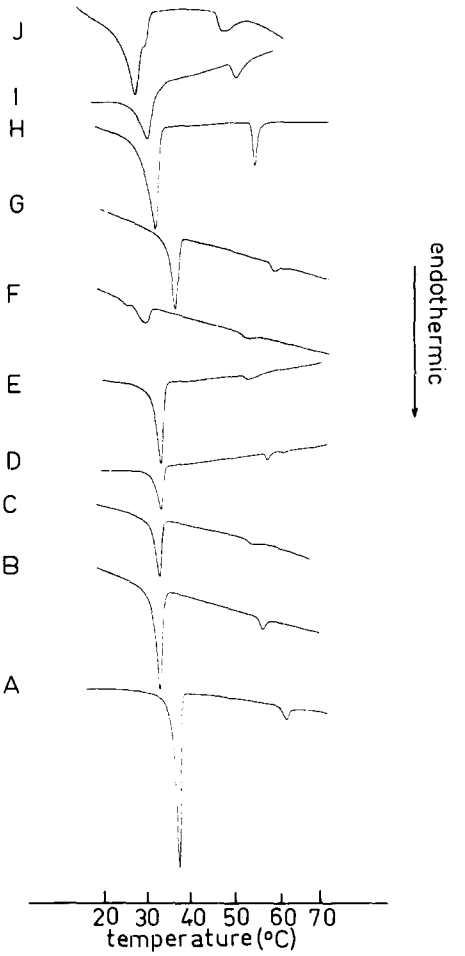

Fig. 2. Thermograms of DEPE and sterol-DEPE mixtures. (A) pure DEPE; (B) 22,*R*-OH cholesterol-DEPE mixture (sterol mole fraction, 0.19); (C) 22,*R*-OH cholesterol-DEPE mixture (sterol mole fraction, 0.5); (D) 22,*S*-OH cholesterol-DEPE mixture (sterol mole fraction, 0.2); (E) 22,*S*-OH cholesterol-DEPE mixture (sterol mole fraction, 0.33); (F) 22,*S*-OH cholesterol-DEPE mixture (sterol mole fraction, 0.5); (G) 22-ketocholesterol-DEPE mixture (sterol mole fraction, 0.2); (H) 22-ketocholesterol-DEPE mixture (sterol mole fraction, 0.5); (I) 20,*S*-OH cholesterol-DEPE mixture (sterol mole fraction, 0.2); (J) 20,*S*-OH cholesterol-DEPE mixture (sterol mole fraction, 0.5). Heating rate, 5 C deg·min^{-1}.

TABLE II

THERMODYNAMIC PARAMETERS OF THE GEL → LIQUID CRYSTALLINE PHASE AND OF THE LAMELLAR → HEXAGONAL H_{II} PHASE TRANSITION OF DEPE AS AFFECTED BY THE PRESENCE OF STEROLS

Sterol	Mole fraction (sterol/lipid)	Gel → liquid crystalline phase transition			Lamellar → hexagonal H_{II} phase transition		
		ΔH (kcal·mol^{-1})	transition temperature (°C)	width at half peak height (C deg.)	ΔH (kcal·mol^{-1})	transition temperature (°C)	width at half peak height (C deg.)
None		7.4±0.5	38.4	1	0.6 ±0.1	64.4	1.5
22,*R*-OH cholesterol	019	6.8±0.6	33.9	1.5	0.68±0.01	55.9	1.5
	0.50	6.7±0.0	33.9	1.8	0.70±0.07	53.9	2.5
22,*S*-OH cholesterol	0.20	7 ±1	33.9	2	0.7 ±0.1	61.9	2
	0.33	5.9±0.4	32.9	2	0.59±0.04	54	3
	0.50	3.3±0.3	29.9	4	1.01±0.04	54	2.5
22-Ketocholesterol	0,20	6.3±0.5	34.7	1.8	0.64±0.05	58.4	2
	0.50	4.4±0.4	28.9	3	0.33±0.1	58.9	1
20,*S*-OH cholesterol	0.20	4.4±0.3	27.9	4	0.54±0.06	51.9	2
	0.50	4.6±0.5	24.9	4	0.78±0.08	50.9	4

phase transition occurs at 38°C and the bilayer → hexagonal H_{II} phase transition around 60°C (see Ref. 18 for a review of the published values of transition temperatures).

In contrast to the results obtained with DPPC-sterol mixtures, the gel → liquid crystalline phase transition is not completely suppressed by any of the cholesterol derivatives (Fig. 2). The transition temperature is shifted to lower values and the width at half peak height (expressed as $\Delta T_{1/2}$) is increased, but only to a smaller extent as compared to DPPC (Table II). The ΔH values are reduced. 22,*R*-OH cholesterol exhibits the smallest effect, followed by the 22,*S*-OH cholesterol, whereas 20,*S*-OH cholesterol exhibits the strongest effect on the gel → liquid crystalline phase transition. At the highest concentration of 22,*S*- and 20,*S*-OH cholesterol, the thermograms exhibit a shoulder.

The bilayer → hexagonal H_{II} phase transition temperature is significantly affected by the presence of the cholesterol derivatives. Each derivative induces a downward shift of the transition temperature. The smallest effect is observed with 22-ketocholesterol (Fig. 2, Table II). The ΔH of the transition is not significantly affected by the presence of the sterols.

^{31}P-NMR allows monitoring of the bilayer to hexagonal H_{II} phase transition by following the change in the line-shape of the phosphorus spectrum [19]. These measurements are summarized in Fig. 3. No line-shape other than the typical 'bilayer' or 'hexagonal' line-shape was detectable. A qualitative agreement is found between the trends observed by DSC and by NMR.

Interfacial properties

The force-area curves of the pure sterols in monomolecular films at the air/water interface are presented in Fig. 4. Whereas 20,*S*-OH, 22,*S*-OH and 22-ketocholesterol exhibit interfacial properties with an orientation perpendicular to the interface comparable to cholesterol itself, 22,*R*-OH cholesterol exhibits a remarkable difference at low surface pressures. At a surface pressure of 1.9 mN·m^{-1} and a molecular area of 103 Å^2, a transition is observed. The monomolecular films of 22-keto- and 20,*S*-OH cholesterol are quite stable with a high collapse pressure whereas the monomolecular films of 22,*S*-OH and 22,*R*-OH cholesterol appear to be much less stable (Table III). The molecular areas at a surface presure of 12 mN·m^{-1} are listed in Table III. All these cholesterol derivatives exhibit higher molecular areas than cholesterol itself.

Mixed-film studies of DOPC-sterol were also

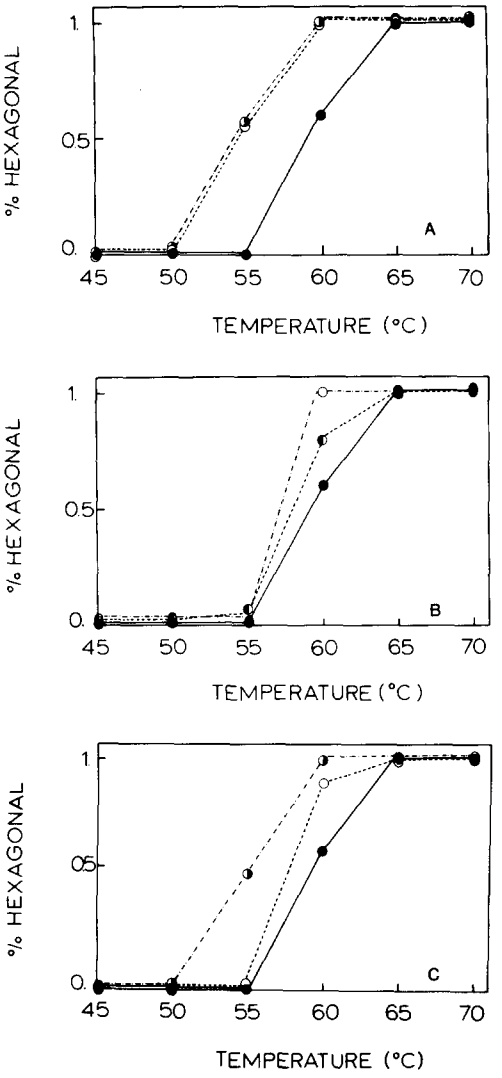

Fig. 3. The amount of phospholipid molecules organized in hexagonal H_{II} phase in DEPE and sterol-DEPE mixtures. (A) ●, pure DEPE; ◑, DEPE + 22,*R*-OH cholesterol (sterol mole fraction, 0.19); ○, DEPE + 22,*R*-OH cholesterol (sterol mole fraction, 0.5). (B) ●, pure DEPE; ◐, DEPE + 22,*S*-OH cholesterol (sterol mole fraction, 0.33); ○, DEPE + 22,*S*-OH cholesterol (sterol mole fraction, 0.5). (C) ●, pure DEPE; ○, DEPE + 20,*S*-OH cholesterol; and ◑, DEPE + 22-ketocholesterol (sterol mole fraction, 0.5).

examined. The force-area curves of the mixed monolayers are represented in Fig. 5. As in pure monomolecular films, 22,*R*-OH cholesterol exhibits the transition at low surface pressures. The values of the mean molecular areas calculated from these curves at a surface pressure of 12

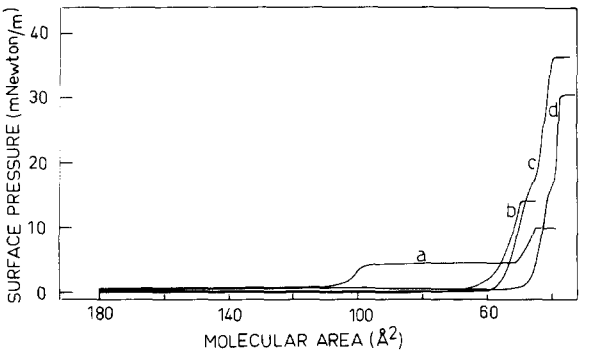

Fig. 4. Compression isotherms of pure monomolecular films of (a) 22,*R*-OH cholesterol; (b) 22,*S*-OH cholesterol; (c) 20,*S*-OH-cholesterol; and (d) 22-ketocholesterol. Temperature, 20°C.

TABLE III

AIR/WATER INTERFACIAL PROPERTIES OF THE CHOLESTEROL DERIVATIVES SUBSTITUTED BY A POLAR GROUP AT C_{20} OR C_{22}

Temperature, 25°C.

Sterol	Molecular area at 12 $mN \cdot m^{-1}$ ($Å^2$)	Collapse pressure ($mN \cdot m^{-1}$)
22,*R*-OH cholesterol	42.2 ± 1.8 [a]	10.0 ± 0.5
22,*S*-OH cholesterol	48.5 ± 2.7	14 ± 1
22-Ketocholesterol	41.4 ± 0.6	31.1 ± 0.1
20,*S*-OH cholesterol	47.5 ± 0.7	36.6 ± 0.2
Cholesterol [b]	39.0	37.2

[a] Extrapolated.
[b] From Ref. 26.

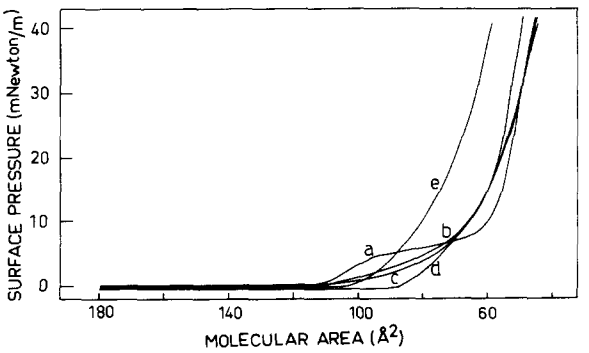

Fig. 5. Compression isotherms of sterol-DOPC 1:1 mixed films. (a) 22,*R*-OH cholesterol-DOPC; (b) 22,*S*-OH cholesterol-DOPC; (c) 20,*S*-OH cholesterol-DOPC; (d) 22-ketocholesterol-DOPC; and (e) pure DOPC. Temperature, 20°C.

TABLE IV

INTERFACIAL PROPERTIES OF MIXED MONOMOLECULAR FILMS OF STEROL-DOPC 1 : 1

Temperature, 25°C.

Sterol	Mean molecular area (Å^2) at 12 mN · m^{-1} for DOPC-sterol 1 : 1 mixtures	
	measured	calculated
22,*R*-OH cholesterol	57.0 ± 1.4	61.9
22,*S*-OH cholesterol	63.3 ± 1.3	65.0
22-Ketocholesterol	59.2 ± 3.4	61.5
20,*S*-OH cholesterol	64.6 ± 1.3	64.6
Cholesterol [a]	45.7	58.5

[a] From Ref. 26.

mN · m^{-1} are listed in Table IV. No significant condensation effect is observed for any of these compounds (Table IV).

Discussion

The magnitude of the cholesterol-phospholipid interaction is dependent on several structural features such as a 3β-OH group, a flat ring system and a hydrophobic side-chain. The role of this side-chain in the sterol-phospholipid interaction has been studied in several ways. It has been shown that the complete side-chain was required for maximizing the ordering effect of cholesterol, whereas either a longer or a shorter chain was less effective [20]. At least an isopropyl side-chain is needed to suppress the permeability increase at the phase transition [21]. On the other hand, plant sterols such as ergosterol and stigmasterol exhibit an ordering effect similar to that of cholesterol, despite the substitution by a methyl or ethyl group in the side-chain and the presence of a double bond [12,22].

In recent studies, it has been indicated that polar group substitutions in the side-chain were not equivalent with regard to the interaction of the modified compounds with phospholipids [12,23]. The fluorescence anisotropy technique using DPH as probe has demonstrated that substitution of an OH group at the 22-position was crucial for the interaction with DPPC. Whereas 22,*S*-OH cholesterol almost suppressed the melting transition, its epimer, 22,*R*-OH cholesterol, presented only a slight effect on the transition temperature (downward shift of approx. 1.2 C deg.). Almost no ordering effect was evoked by this compound. 22-Ketocholesterol suppressed the melting transition as well as 20,*S*-OH cholesterol.

The results obtained by DSC technique confirm completely these above-mentioned findings and are in agreement with other recent studies [23]. 22,*R*-OH cholesterol does not suppress the melting transition of DPPC, despite the fact that it broadens the transition and shifts the transition temperature to lower value. This kind of thermogram corresponds to a type A profile according to the studies of Jain and Wu [24]. In these studies, a number of biologically active lipid-soluble compounds have been tested for their effect on the melting transition of synthetic phosphatidylcholines. At least four different types of modified transition profile can be distinguished. The type A profile corresponds to a shift of T_m to lower temperatures, an increase in $\Delta T_{1/2}$ and an unaffected ΔH_m. The molecules presenting such behavior appear to be partially buried in the hydrocarbon core of the bilayer interacting primarily with the C_2-C_8 methylene region of the hydrocarbon chain.

Interaction of these cholesterol derivatives with phosphatidylethanolamines as exemplified by DEPE exhibits quite different features than cholesterol itself. Incorporation of cholesterol into saturated or unsaturated phosphatidylethanolamines broadened the melting transition, decreased the heat content of the transition and shifted the transition temperature to lower values [25]. At a concentration of 50 mol% the transition as detected by DSC was completely suppressed. These observations and especially the downward shift of the transition temperature can be interpreted as resulting from a modification of the polar group packing of the phosphatidylethanolamine.

The cholesterol derivatives substituted by a polar group at C_{20} and C_{22} shift the melting transition temperature of DEPE to lower temperature, as in the case of cholesterol. 22,*R*-OH cholesterol has the smallest effect, 20,*S*-OH cholesterol the strongest. The transition is also broadened by the presence of these compounds and 22,*R*-OH cholesterol exhibits the weakest effect. However, in contrast to cholesterol, the transition is still detectable at 50% sterol. This observation is in contrast

to our findings in DPPC-sterol mixtures where only the 22,*R*-OH cholesterol did not suppress the melting transition. The difference in behavior towards DPPC and DEPE may be due to the difference in packing of the polar headgroup or hydrophobic moiety between the two kinds of phospholipid, which in turn may modify the insertion mode of these sterols. In line with this idea, the existence of a shoulder on the DSC profiles of 20,*S*-OH and 22,*S*-OH cholesterol/DEPE 1 : 1 mixtures indicates that phase separation may occur. To test this hypothesis, more detailed DSC studies are needed.

All the sterol derivatives induced a shift of the bilayer → hexagonal H_{II} phase transition of DEPE to lower temperatures as observed by DSC and ^{31}P-NMR without modifying significantly the ΔH of the transition. This effect is much more pronounced than in the case of cholesterol itself [4]. The most effective sterol was the 20,*S*-OH derivative, followed by 22,*R*-OH cholesterol which in fact exhibited the largest shift at low concentrations. In contrast to what was found for a series of sterol derivatives modified on the polar group at the 3-position [4], there is no clear correlation between the bilayer-destabilizing effect of the sterols used in this study and their molecular surface area. The monolayer studies evidence that these sterols exhibit a larger molecular area than cholesterol at moderate surface pressure (12 mN · m^{-1}), indicating that these molecules are not completely perpendicular at the air/water interface as is cholesterol [26]. Accordingly, in mixed films of DOPC-sterols, no significant condensing effect of any of these derivatives was found. This is, however, in contrast to the suppression of the melting phase transition of DPPC exhibited by the 22-keto- and 22,*S*-OH cholesterol. The disagreement between the data may indicate an effect of the acyl chain unsaturation on the interaction of these derivatives.

Nevertheless, the interfacial behavior of the biological derivative 22,*R*-OH cholesterol is very interesting. The compression curves of either the pure sterol or of the DOPC-sterol 1 : 1 mixed film exhibit a transition which indicates that this compound is most likely not vertically but horizontally oriented, at least at low surface pressures, even in the presence of phospholipids. In the pure sterol film the value of 103 Å^2 obtained for the molecular area at the transition corresponds to the revolution area of the sterol nucleus (long axis ≈ 11 Å^2). This feature can be explained by the stereo configuration of the side-chain of this particular molecule. In contrast to the epimer 22,*S*-OH cholesterol and the other derivatives, the side-chain of 22,*R*-OH cholesterol has a blocked configuration as observed in molecular models and makes a kink with the long axis of the sterol nucleus as suggested by ^{1}H-NMR studies [27]. Such a peculiar configuration could explain the difference in interaction of this compound with phospholipids since, according to recent studies, the dynamics and/or orientation of the side-chain of cholesterol seems to be important for this interaction [28]. These findings can be of relevance for the functioning of the cytochrome *P*-450 in the inner mitochondrial membranes of steroidogenic tissues [29]. The striking modification of the orientation of 22,*R*-OH cholesterol as compared to cholesterol can explain the high activation energy of the 22,*R*-hydroxylation step [11]. Whether this orientation is maintained in the presence of the cytochrome and whether it facilitates the subsequent hydroxylations are appealing questions [30].

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