Biochimica et Biophysica Acta, 771 (1984) 142–150
Elsevier

BBA 72057

THE EFFECT OF THE STEROL OXYGEN FUNCTION ON THE INTERACTION WITH PHOSPHOLIPIDS

R.A. DEMEL [a], A.K. LALA [b], S. NANDA KUMARI [b] and L.L.M. VAN DEENEN [a]

[a] *Laboratory of Biochemistry, State University of Utrecht, Transitorium 3, Padualaan 8, NL-3584 CH Utrecht (The Netherlands) and* [b] *Bioorganic Laboratory, Department of Chemistry, Indian Institute of Technology, Bombay, Powai, Bombay 400 076 (India)*

(Received November 17th, 1983)

Key words: Cholesterol; Cholesterol ether; Cholesterol ester; Phosphatidylcholine monolayer; Phase transition

The effect of cholesteryl ethers (namely cholesteryl methyl ether, cholesteryl ethyl ether, cholesteryl *n*-propyl ether, cholesteryl isopropyl ether, cholesteryl butyl ether, cholesteryl methoxymethyl ether, cholesteryl (2′-hydroxy)-3-ethyl ether) and cholesteryl ester (namely cholesteryl acetate) is tested on the interaction with phosphatidylcholines in liquid-crystalline and crystalline state. The interfacial properties of sterols are tested at the air-water interface. The cholesteryl ethers show a reduced interfacial stability with increasing hydrophobicity of the ether-linked moiety. The interaction between the sterol derivatives and phospholipids in mixed monolayers is indicated by measuring the deviation from the simple addivity rule (condensing effect). An interaction is found only for cholesteryl (2′-hydroxy)-3-ethyl ether, cholesteryl methyl ether and cholesteryl ethyl ether. These sterols also reduce the glucose permeability of liposomal membranes in this order. In this respect cholesteryl (2′-hydroxy)-3-ethyl ether is as effective as cholesterol. Cholesteryl methyl ether and cholesteryl ethyl ether show 62 and 33 percent of the effect observed with cholesterol. The effect of the sterol derivatives on the gel-to-liquid-crystalline phase transition of dipalmitoylphosphatidylcholine is measured by differential scanning calorimetry. Cholesteryl methyl ether, cholesteryl ethyl ether, and cholesteryl (2′-hydroxy)-3-ethyl ether reduce the energy content of the phase transition nearly as effective as cholesterol, cholesteryl *n*-propyl ether has only a small effect. Although cholesteryl acetate, and cholesteryl methoxymethyl ether have no condensing or permeability-reducing effect, they have a considerable effect on the gel-to-liquid-crystalline phase transition. Cholesteryl isopropyl ether and cholesteryl butyl ether have no effect. It is concluded that a free 3β-hydroxy group is not a prerequisite to observe a sterol-like effect in membranes. However, the interfacial stability and the orientation of the sterol and oxygen moiety at the sterol 3-position are important.

Introduction

Sterols are main constituents in many biological membranes. Many studies have demonstrated that sterols greatly affect the barrier properties of membranes. Monolayer studies have demonstrated a so-called condensing effect [1–3], this is a decrease in mean molecular area in mixtures of phospholipid and cholesterol. The increase in chain order in the liquid-crystalline state leads to a decreased permeability [4,5]. Differential scanning calorimetry measurements have shown that cholesterol has a liquifying effect, this means, decreases the chain order in the gel state [6]. These effects of cholesterol have led to the concept that cholesterol can introduce an intermediate gel state [7]. Both model membrane experiments and studies on biological membranes have revealed that the sterol structure is of critical importance for the sterol phospholipid interaction [8,9]. A planar ring system and a side chain at C_{17} of at least 4 carbon atoms [10], were found to be prerequisites both for

the condensing and liquifying effect. It was, however, found that cholesterol caused the greatest rigidifying effect and that analogues with shorter or longer side chains were less effective [11,12]. These requirements were also found for sterols which promote growth of the sterol-requiring mycoplasma [13]. Also the 3β-OH group was thought to be required, since ketoderivatives and epicholesterol (3α-hydroxycholesterol) did not introduce a condensing effect in monolayers [8], nor a change in permeability of liposomes [14] and biological membranes [15–17]. OH-blocked amphiphilic derivatives of cholesterol such as *O*-(methoxyethoxy ethoxyethyl)cholesterol and cholesteryl phosphorylcholine did not condense a phosphatidylcholine monolayer [18]. It was found, however, that a cholesterol requiring bacterium, *mycoplasma capricolum* could grow nearly as well on media supplemented with cholesteryl methyl ether or cholesteryl acetate as on free cholesterol [19]. Cholesteryl methyl ether could also be used as a sterol source by a yeast mutant, strain GL7, defective in 2,3-oxidosqualene-lanosterol [19]. However, microorganisms generally contain lower sterol concentrations than mammalian cells. It has been suggested also that in microorganisms sterols could fulfil additional functions to regulate membrane fluidity [39]. In order to determine whether a free sterol OH-group is a prerequisite for membrane function in sterol-rich membranes, as many mammalian membranes, the interfacial stability of 3β-OH-blocked cholesterol derivatives is measured. In mixtures of these derivatives with phosphatidylcholine the ability to reduce the mean molecular area, (condensation) at the air/water interface is measured. The changes in mean molecular area are correlated with the effects these sterols have on the glucose permeability. At the same time the ability of these 3β-OH-blocked cholesterol derivatives to liquify phospholipid membranes in the gel state, is studied.

Materials and Methods

Cholesteryl methyl ether, cholesteryl ethyl ether, cholesteryl *n*-propyl ether, cholesteryl isopropyl ether, cholesteryl butyl ether [20,21], cholesteryl methoxymethyl ether (Chol-O-CH_2-O-CH_3) [22] and cholesteryl (2'-hydroxy)-3-ethyl ether (Chol-O-CH_2-CH_2-OH) [23] were prepared by published procedures.

Cholesteryl acetate was obtained from Sigma (St. Louis, MO, U.S.A.). Dioleoylphosphatidylcholine and dipalmitoylphosphatidylcholine were synthesized according to established procedures [24] and purified by HPLC [25].

Determination of force-area curves

Force-area measurements were performed at the air/water interface in a teflon trough, 32.3 cm long × 17.2 cm wide. The trough was filled with 10 mM Tris-HCl (pH 7.0). Water was twice distilled. The surface pressure was determined with a recording Beckman LM500 electrobalance. The compression rate was 0.258 $nm^2 \cdot mol^{-1} \cdot min^{-1}$. The trough was placed in a thermostated box. All experiments were performed at 22°C. 50 nmol of lipid dissolved in chloroform were carefully released onto the air/water interface from an Agla micrometer syringe. For mixed films, the surface pressure was plotted against the mean area per molecule, that is, the total area divided by the total number of sterol and phospholipid molecules. For a quantitative evaluation of the interaction, the variation of the mean molecular area at a given constant surface pressure was plotted as a function of the mole fraction.

Differential scanning calorimetry

Differential scanning calorimetry measurements were performed on a Perkin-Elmer DSC 2B apparatus, with a heating and cooling rate of 5 deg. $C \cdot min^{-1}$. 5 μmol of dipalmitoylphosphatidylcholine, to which increasing amounts of sterol were added, was suspended in 40 μl Tris-acetate (40 mM) (pH 7.0), containing 100 mM NaCl. 15 μl of the suspension was transferred to the sample pan. The amount of phospholipid in the sample pan was determined by the method of Fiske and SubbaRow as described by Bartlett [26].

Measurement of glucose permeability

The release of glucose from dioleoylphosphatidylcholine liposomes containing 4 mol% phosphatidic acid and 12, 20, 33 and 50 mol% sterol after 1 h at 40°C was measured as described before [14].

Results

Reduction in polarity of the sterol 3β-OH group leads to a decrease in film stability and collaps pressure. Cholesteryl methyl ether (Fig. 1A, Table I) shows a collapse pressure of 27.4 $mN \cdot m^{-1}$, compared to 37.2 $mN \cdot m^{-1}$ for cholesterol [14]. The minimal surface area is 38 $Å^2 \cdot mol^{-1}$ which is equal to that of cholesterol. In mixed films of cholesteryl methyl ether and dioleoylphosphatidylcholine an interaction is observed at all mole fractions of the mixed film. A strong condensing effect is demonstrated, plotting the mole fraction of sterol against the mean molecular area, and a strong deviation from ideal behaviour is found at all surface pressures. At 50 mol% cholesteryl methyl ether and a surface pressure of 5 or 20 $mN \cdot ml^{-1}$, the reduction in mean molecular area is 10.5 and 9.5 $Å^2 \cdot mol^{-1}$, respectively (Table II), which is slightly less than obtained for cholesterol in a mixed film with oleoylstearoylphosphatidylcholine [1,14].

Due to the increased hydrophobicity of the ethyl group, cholesteryl ethyl ether shows a collapse pressure of only 21 $mN \cdot m^{-1}$ (Fig. 1B, Table I). The minimal surface area is slightly increased to 41 $Å^2 \cdot mol^{-1}$ (Fig. 1B). In mixed monolayers of cholesteryl ethyl ether and dioleoylphosphatidylcholine a reduction in mean molecular area can be measured. At 50 mol% cholesteryl ethyl ether and a surface pressure of 5 or 20 $mN \cdot m^{-1}$ the condensing effect is 9.5 and 7.5 $Å^2 \cdot mol^{-1}$, respectively (Table II). This is less than found for cholesteryl methyl ether. Cholesteryl *n*-propyl ether shows a further reduction in interfacial stability, having a collapse pressure of only 11 $mN \cdot m^{-1}$ (Fig. 1C, Table I). the minimal area has increased to 45 $Å^2 \cdot mol^{-1}$. Mixed films of cholesteryl *n*-propyl ether with dioleoyl-phosphatidylcholine show a small deviation from ideal behaviour only. The condensing effect is small, especially at low mole fractions of cholesteryl *n*-propyl ether. At 50 mol% and a surface pressure of 5 $mN \cdot m^{-1}$ the condensing effect is 4.5 $Å^2 \cdot mol^{-1}$ (Table II). At high surface pressures, even at low mole fractions of cholesteryl *n*-propyl ether, the mixed monomolecular films appear to be rather unstable. Cholesteryl isopropyl ether forms films with very low interfa-

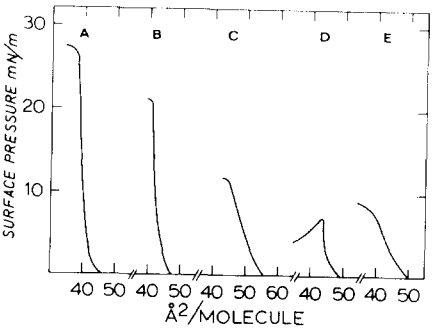

Fig. 1. Force-area characteristics of various cholesteryl ether derivatives at 22°C. A, cholesteryl methyl ether; B, cholesteryl ethyl ether; C, cholesteryl *n*-propyl ether; D, cholesterol isopropyl ether; E, cholesteryl butyl ether.

TABLE I

COLLAPSE PRESSURES OF CHOLESTERYL ETHERS AND ESTER AT 22°C AND THE AREA PER MOLECULE OF THESE STEROLS AT 5 AND 20 $mN \cdot m^{-1}$

Sterol	Collapse pressure ($mN \cdot m^{-1}$)	$Å^2 \cdot mol^{-1}$ at	
		5 $mN \cdot m^{-1}$	20 $mN \cdot m^{-1}$
Cholesteryl methyl ester	27.4	41.0	39.5
Cholesteryl ethyl ether	21.0	43.5	41.0
Cholesteryl *n*-propyl ether	11.0	49.5	
Cholesteryl isopropyl ether	7	44.5	
Cholesteryl butyl ether	9	42.5	
Cholesteryl acetate	14.4	43.5	
Cholesteryl methoxymethyl ether	31.8	42.5	38.0
Cholesteryl (2'-hydroxy)-3-ethyl ether	42.0	45.0	42.5

TABLE II

MEAN MOLECULAR AREA OF DIOLEOYLPHOSPHATIDYLCHOLINE/STEROL MIXTURES 1:1 MOLAR RATIO IN $Å^2$ AT 22°C AND THE DEVIATION FROM THE SIMPLE ADDITIVITY RULE IN $Å^2$ AT SURFACE PRESSURES OF 5 AND 20 $mN \cdot m^{-1}$

Sterol	Mean molecular area		Deviation from ideal behaviour ($Å^2$)	
	5 $mN \cdot m^{-1}$	20 $mN \cdot m^{-1}$	5 $mN \cdot m^{-1}$	20 $mN \cdot m^{-1}$
Cholesteryl methyl ether	54.5	44.0	10.5	9.5
Cholesteryl ethyl ether	57.1	47.5	9.5	7.5
Cholesteryl *n*-propyl ether	65.0		4.5	
Cholesteryl isopropyl ether	66.5		0	
Cholesteryl butyl ether	66.1		0	
Cholesteryl acetate	66.5		1.0	
Cholesteryl methoxymethyl ether	66.5	53.2	0	0
Cholesteryl (2'-hydroxy)-3-ethyl ether	55.0	47.5	10.1	9.0

cial stability (Fig. 1D, Table I). The maximal surface pressure for the pure sterol is 7 $mN \cdot m^{-1}$, but the pressure drops sharply after further compression of the monolayer. The interfacial instability of cholesterol isopropyl ether is also observed in mixed films with dioleoylphosphatidylcholine. The surface pressure drops sharply after further compression specially at mole fractions higher than 0.25. The drop in surface pressure indicates that cholesteryl isopropyl ether segregates from dioleoylphosphatidylcholine. No deviations from ideal behaviour are measured in mixed monolayers of cholesteryl isopropyl ether and dioleoylphosphatidylcholine at 5 or 2 $mN \cdot m^{-1}$ (Table II). Cholesteryl butyl ether forms a monolayer with a collapse pressure of about 9 $mN \cdot m^{-1}$ (Fig. 1E, Table I). The compressibility is higher than for cholesterol, which is due to the hydrophobicity of the butyl moiety attached to the hydroxyl groups. No condensing effect is measured in mixed films of cholesteryl butyl ether and dioleoylphosphatidylcholine at 5 $mN \cdot m^{-1}$ (Table II).

Cholesteryl acetate shows a collapse pressure of 14.4 $mN \cdot m^{-1}$ (Fig. 2A, Table I). This is lower than that measured for cholesteryl ethyl ether. The minimal area is 41.5 $Å^2 \cdot mol^{-1}$. In mixed monolayers of cholesteryl acetate and dioleoylphosphatidylcholine no significant reduction in mean molecular area can be measured (Table II).

Cholesterol ether derivatives having an additional oxygen function as an ether or hydroxyl moiety show an increased interfacial stability due to the increased polarity. Cholesteryl methoxymethyl ether shows a collapse pressure of 31.8 $mN \cdot m^{-1}$ (Fig. 2B, Table I) as limiting area 36 $Å^2 \cdot mol^{-1}$ is found. A remarkable transition is observed at 10 $mN \cdot m^{-1}$. Below 10 $mN \cdot m^{-1}$ the compressibility of the cholesteryl methoxymethyl ether monolayer is much higher than above this pressure. In mixed monolayers of cholesteryl methoxymethyl ether and dioleoylphosphatidylcholine the transition is also apparent. At a sterol mole fraction of 0.65 it is found at a pressure of 3.4 $mN \cdot m^{-1}$, at a mole fraction of 0.50 at a pressure of 9.6 $mN \cdot m^{-1}$ and at a mole fraction of 0.35 at a pressure of 17.4 $mN \cdot m^{-1}$. The mixed monomolecular films did not show any deviation from the ideal behaviour at 5 or at 20 $mN \cdot m^{-1}$ (Table II). The derivative having the second oxygen function present as a free hydroxylgroup, cholesteryl (2'-hydroxy)-3-ethyl ether demonstrates a very high interfacial stability. The collapse pressure is 42 $mN \cdot m^{-1}$ (Fig. 2C, Table I). This is higher than for cholesterol. The compressibility is similar to that found for cholesteryl methyl ether, as a limiting area 39 $Å^2 \cdot mol^{-1}$ is measured. Mixed monomolecular films of cholesteryl (2'-hydroxy)-3-ethyl ether and dioleoylphosphatidylcholine show a significant reduction in mean molecular area. At a surface pressure of 5 or 20 $mN \cdot m^{-1}$ the deviation from ideal behaviour is 10.1 and 9.0 $Å^2 \cdot mol^{-1}$, respectively (Table II).

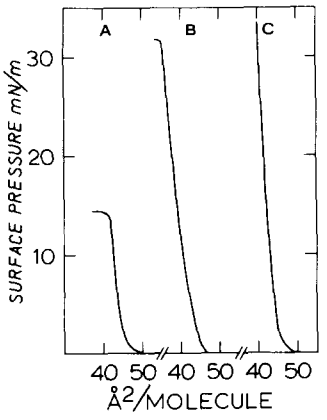

Fig. 2. Force-area characteristics of A, cholesteryl acetate; B, cholesteryl methoxymethyl ether (Chol-O-CH_2-O-CH_3); C, cholesteryl (2'-hydroxy)-3-ethyl ether (Chol-O-CH_2-CH_2-OH).

Measurement of the heat content of the phase transition of dipalmitoylphosphatidylcholine, in the absence of ethylene glycol, showed that it is 9.4 kcal · mol^{-1} [27]. Increasing amounts of cholesteryl methyl ether reduce the heat content of the phase transition linearly. At 30 mol% cholesteryl methyl ether the reduction in heat content of dipalmitoylphosphatidylcholine is 90%. The phase transition is no longer detectable at mole percentages cholesteryl methyl ether higher than 35 (Fig. 3A). Also cholesteryl ethyl ether reduces the heat content of the transition but less effectively than cholesteryl methyl ether. At 30 mol% cholesteryl ethyl ether the ΔH of the dipalmitoylphosphatidylcholine phase transition is still 3.5 kcal · mol^{-1} (Fig. 3B). This is a reduction with 64%. Cholesteryl *n*-propyl ether gives a very small decrease in ΔH (Fig. 3C). At 30 mol% cholesteryl propyl ether the reduction is 15%. With cholesteryl isopropyl ether and cholesteryl butyl ether no reduction in ΔH can be measured (Figs 3D, E). In the presence of cholesteryl acetate the heat content of the phase transition is found to be reduced (Fig. 3F). At 30 mol% cholesteryl acetate the reduction is 52%.

Cholesteryl methoxymethyl ether has an effect on the phase transition of dipalmitoylphosphatidylcholine (Fig. 3G), although it is much smaller than that observed for cholesteryl (2'-hydroxy)-3-ethyl ether (Fig. 3H). At 30 mol% cholesteryl methoxymethyl ether the reduction is 45%.

The effect of cholesteryl (2'-hydroxy)-3-ethyl ether on the heat content of the dipalmitoylphosphatidylcholine phase transition (Fig. 3H) is comparable to that found for cholesteryl methyl ether. At 30 mol% cholesteryl (2'-hydroxy)-3-ethyl ether the reduction is 81%.

The effect of cholesteryl ethers on the glucose release from dioleoylphosphatidylcholine liposomes is illustrated in Fig. 4. Only in the presence of cholesteryl methyl ether or cholesteryl ethyl ether a decrease in glucose permeability could be observed. At 50 mol% cholesteryl methyl ether the reduction is 62% of that obtained in the presence of cholesterol. Similar reductions in glucose permeability are also observed in mixtures of cholesteryl methyl ether and oleoylstearoylphosphatidylcholine or egg phosphatidylcholine. At 50 mol% cholesteryl ethyl ether the reduction is 38% of the effect obtainable with cholesterol. In

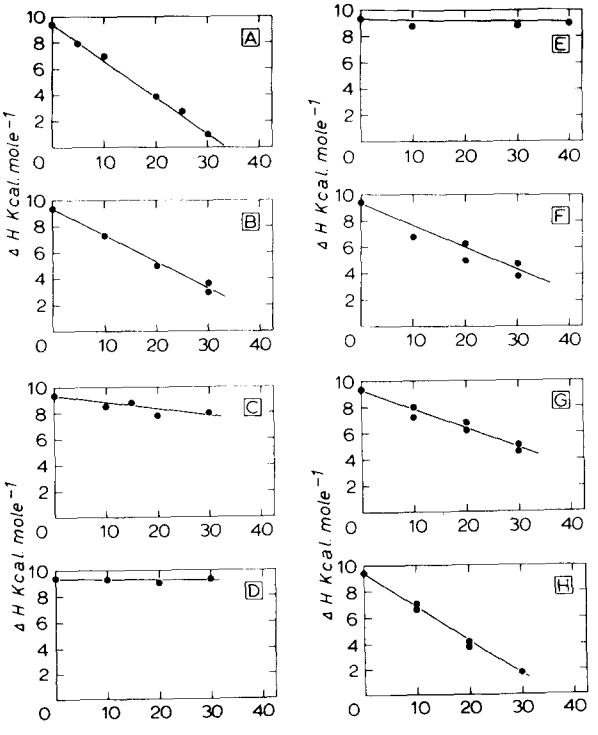

Fig. 3. Effect of cholesteryl ether and ester derivatives upon the energy content of the gel-to-liquid-crystalline phase transition of dipalmitoylphosphatidylcholine. Abscissa indicates mol%: (A) cholesteryl methyl ether; (B) cholesteryl ethyl ether; (C) cholesteryl *n*-propyl ether; (D) cholesteryl isopropyl ether; (E) cholesteryl butyl ether; (F) cholesteryl acetate; (G) cholesteryl methoxymethyl ether; (H) cholesteryl (2'-hydroxy)-3-ethyl ether.

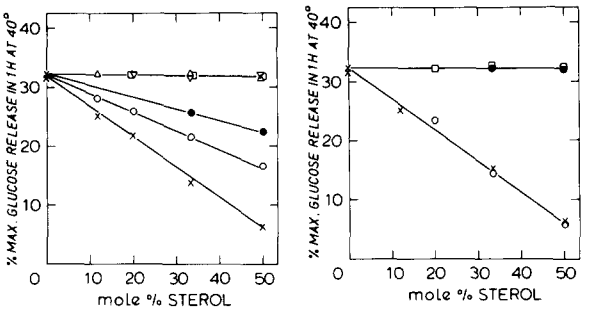

Fig. 4. Effect of cholesteryl ethers in liposomes prepared with dioleoylphosphatidylcholine on the relative amount of glucose released after 1 h at 40°C. Liposomes are prepared from dioleoylphosphatidylcholine and 4 mol% phosphatidic acid with varying amounts, respectively, of: (×) cholesterol; (○) cholesteryl methyl ether; (●) cholesteryl ethyl ether; (▽) cholesteryl *n*-propyl ether; (□) cholesteryl isopropyl ether; (△) cholesteryl butyl ether.

Fig. 5. Effect of different cholesterol derivatives in liposomes prepared with dioleoylphosphatidylcholine on the relative amount of glucose released after 1 h at 40°C. Liposomes are prepared from dioleoylphosphatidylcholine and 4 mol% phosphatidic acid with varying amounts, respectively, of: (×) cholesterol; (○) cholesteryl (2′-hydroxy)-3-ethyl ether; (●) cholesteryl methoxymethyl ether; (□) cholesteryl acetate.

the presence of cholesteryl *n*-propyl ether, cholesteryl isopropyl ether, and cholesteryl butyl ether, no measurable effect on the glucose permeability was observed. Also cholesteryl acetate did not alter the glucose permeability of dioleoylphosphatidylcholine liposomes (Fig. 5). The diether derivative, cholesteryl methoxymethyl ether, and the ether, hydroxy derivative, cholesteryl (2′-hydroxy)-3-ethyl ether had completely opposite effects on the glucose permeability. Cholesteryl methoxy methyl ether had no effect on the glucose permeability, whereas cholesteryl (2′-hydroxy)-3-ethyl ether had a permeability reducing effect similar to cholesterol (Fig. 5).

Discussion

A free hydroxyl group is found for all sterols in cell membranes. Sterol esters as found in serum lipoproteins are thought to be mainly present in the hydrophobic core and in a small percentage only in the interfacial layer. Cholesterol has a high interfacial stability. The collapse pressure of cholesterol at the air/water interface is 38 mN · m^{-1} [28]. 3α-Hydroxy derivatives show a markedly reduced collapse pressure; for epicholesterol (3α-hydroxycholesterol), a collapse pressure of 32 mN · m^{-1} is noted [8]. Keto derivatives show, due to a lower polarity, a lower interfacial stability and an increased molecular area [8]. Long chain cholesteryl ester derivatives as cholesteryl oleate from hardly monomolecular layers in a pure form [29]. These observations have led previously to the assumption that a 3β-hydroxyl group is essential for membrane sterols [7].

It has been proposed in the past that the sterol hydroxyl group would play an essential role in hydrogen bridge formation between sterol and phospholipid [30–32]. There is, however, no experimental support for a direct interaction between phospholipid and sterol. Studies with phospholipids lacking the ester linkage, as ether and alkyl derivatives, indicate that a direct hydrogen bonding is not required for the condensing effect of cholesterol [33–36]. Certain critical proportions of cholesterol to phospholipid have been described. They reflect the distribution of cholesterol and phospholipid in the membrane rather than a stoichiometric ratio of a complex. Starting from a hexagonal chain packing of posphatidylcholine, replacing two fatty acid chains for each cholesterol molecule added, a critical situation is reached at a cholesterol mole fraction of 0.2 [37]. Each molecule is surrounded by eight unshared fatty acid chains. At a cholesterol mole fraction of 0.33 each cholesterol molecule is surrounded by eight fatty acid chains. At a cholesterol mole fraction of 0.5 each cholesterol molecule is surrounded by six shared chains. This ratio represents the maximum solubility of cholesterol in phosphatidylcholine in a thermodynamic stable bulk system. At high mole fractions of cholesterol, cholesterol-cholesterol contacts take place. Modifications of the sterol OH-group effects highly the properties of the sterol. Keto derivatives were not able to induce a condensing effect or to reduce membrane permeability. On the contrary, cholest-4-en-3-one, cholest-4,6-dien-3-one and cholest-3,5-dien-7-one increased the permeability of liposomes [14] and erythrocyte membranes [17].

In this paper we show that cholesteryl methyl ether can interact with phosphatidylcholine in the gel and liquid-crystalline state. In mixed mono-

molecular layers of cholesteryl methyl ether and dioleoylphosphatidylcholine a reduction in mean molecular area is observed only slightly less than what is observed with cholesterol. As can be expected this condensing effect is also reflected in the permeability properties of liposomes. In mixtures of cholesteryl methyl ether and dioleoylphosphatidylcholine the glucose permeability was reduced by 62% compared to cholesterol. This is similar to the effect found in the presence of ergosterol [14]. Also the liquifying effect of cholesteryl methyl ether on dipalmitoylphosphatidylcholine in the gel phase is only slightly less than what is observed in the presence of cholesterol. Cholesteryl ethyl ether shows also a condensing effect in monomolecular layers, a reduction in glucose permeability in liposomes and a liquifying effect on the gel state as measured by differential scanning calorimetry. These effects are however already considerably smaller than found for cholesteryl methyl ether. It was demonstrated before that cholesterol requiring *Mycoplasma capricolum* and yeast mutant strain GL7 defective in 2,3-oxido squalene-lanosterol can grow on media containing cholesteryl methyl ether [19]. These results demonstrate that a free sterol hydroxyl group is not a prerequisite to affect membrane fluidity and permeability and that hydrogen bridge formation between phospholipid and sterol is not required.

Increasing the chain length of the ether linked moiety reduces the interfacial stability considerably. The collapse pressure is strongly reduced and little or no condensing effect is observed in the presence of cholesteryl *n*-propyl ether, cholesteryl isopropyl ether and cholesteryl butyl ether. It is worth noting that cholesteryl isopropyl ether has even a lower interfacial stability than cholesteryl *n*-propyl ether or cholesteryl butyl ether. These three sterol ethers had little or no influence on the liposomal glucose permeability and on the gel-to-liquid-crystalline phase transition. Cholesteryl acetate having a second oxygen function linked to the carbon atom attached to the 3-position of cholesterol, has still a low interfacial stability and neither a condensing effect on monomolecular layers nor a permeability reducing effect on liposomal membranes. The inability to affect the permeability was already described before [4]. Cholesteryl acetate has, however, a considerable effect on the gel-to-liquid-crystalline phase transition of dipalmitoylphosphatidylcholine. It has been observed that cholesteryl acetate has growth supporting properties for the sterol requiring *Mycoplasma capricolum* and yeast mutant strain GL7 [19]. The introduction of a second oxygen atom in the sterol derivative in the form of a diether, namely cholesteryl methoxymethyl ether, increases the interfacial stability considerably. This compound has, however, no condensing effect on monomolecular layers nor an effect on the permeability of liposomes. Cholesteryl methoxymethyl ether has, however, an effect on the gel-to-liquid-crystalline phase transition of dipalmitoylphosphatidylcholine like cholesteryl acetate. On the other hand, cholesteryl methoxymethyl ether barely supports the growth of yeast mutant strain GL7 (Nanda Kumari, S. and Lala, A.K., unpublished data). The derivative with both an oxygen ether function attached to the 3C atom of cholesterol and a free hydroxyl group, namely cholesteryl (2′-hydroxy)-3-ethyl ether has a very high interfacial stability. This compound performs properties very similar to cholesterol. It brings about a condensation in monomolecular layers only slightly less than cholesterol and reduces the glucose permeability of liposomal membranes as effectively as cholesterol. It also affects the gel-to-liquid-crystalline phase transition of dipalmitoylphosphatidylcholine very effectively. Although the phase transition is not completely eliminated in the presence of 30 mol% of this sterol derivative as it is in the presence of cholesterol.

The results demonstrate that although interfacial stability of sterols is important in order to perform a sterol like effect it is not the only prerequisite. Cholesteryl methyl ether and cholesteryl ethyl ether have a lower interfacial collapse pressure than cholesterol. The first compound and to some extent the latter compound have a membrane ordering effect and reduce membrane permeability. On the other hand, cholesteryl methoxymethyl ether which has a high interfacial stability has no membrane ordering effect or effect on membrane permeability.

The attachement of a large polar moiety to the 3β-OH group of cholesterol as *O*-(methoxyethoxy

ethoxyethyl)cholesterol and cholesteryl phosphorylcholine led to high interfacial stability. However, no condensing effect was found for these OH blocked sterol derivatives [18].

Although a free 3β-hydroxy group is not required, an oxygen function is apparently required. It was recently shown that thio cholesterol had a significantly weaker phospholipid ordering effect, as judged by a cholestane spin probe, than cholesterol [38].

Also the orientation of the cholesterol oxygen function at the 3-position is of critical importance. It has been shown before 3α-hydroxycholesterol (epicholesterol) is without effect [8,14]. A second oxygen atom close to the oxygen at the 3-position of cholesterol reduces the cholesterol-like effect and most likely effects the orientation of sterol. Cholesteryl acetate and cholesteryl methoxymethyl ether have no effect on phospholipid ordering or liposomes permeability. They do, however, influence the phospholipid phase transition to some extent. Possibly these compounds act merely as spacer molecules as suggested by Razin [39]. Cholesteryl acetate has a low interfacial stability. The transition in cholesteryl methoxymethyl ether monolayers at 10 $mN \cdot m^{-1}$ demonstrate that the second ether linkage affects the interfacial orientation of the sterol derivative. Cholesteryl (2'-hydroxy)-3-ethyl ether having a second oxygen function which is two carbon atoms separated from the ether oxygen at the cholesterol 3-position, has a high interacial stability and a cholesterol like effect both in the liquid-crystalline and gel state of the membrane. The results support the idea that no direct hydrogen bridge formation between phospholipid and sterol is required; hydrogen bonds with the bound water system still seem most plausible [7].

Acknowledgment

Part of the work reported in this paper which was carried out in Laboratory of A.K.L. was supported by a grant from DAE, Bombay.

References

1 Demel, R.A., Van Deenen, L.L.M. and Pethica, B.A. (1967) Biochim. Biophys. Acta 135, 11–19
2 Demel, R.A., Geurts van Kessel, W.S.M. and Van Deenen, L.L.M. (1972) Biochim. Biophys. Acta 266, 26–40
3 Ghosh, D. and Tinoco, J. (1972) Biochim. Biophys. Acta 266, 41–49
4 Demel, R.A., Kinsky, S.C., Kinsky, C.B. and Van Deenen, L.L.M. (1968) Biochim. Biophys. Acta 150, 655–665
5 De Gier, J., Mandersloot, J.G. and Van Deenen, L.L.M. (1968) Biochim. Biophys. Acta 150, 666–675
6 Ladbrooke, B.D., Williams, R.M. and Chapman, D. (1968) Biochim. Biophys. Acta 150, 333–340
7 Demel, R.A. and De Kruijff, B. (1976) Biochim. Biophys. Acta 457, 109–132
8 Demel, R.A., Bruckdorfer, K.R. and Van Deenen, L.L.M. (1972) Biochim. Biophys. Acta 255, 311–320
9 Hsia, J.C., Long, R.A., Hruska, F.E. and Gesser, H.D. (1972) Biochim. Biophys. Acta 290, 22–31
10 Nakamura, T., Nishikawa, M., Inoue, K., Nojima, S., Akiyama, T. and Samkawa, U. (1980) Chem. Phys. Lipids 26, 101–110
11 Sucking, K.E., Blair, H.A.F., Boyd, G.S., Craig, J.F. and Malcolm, B.R. (1979) Biochim. Biophys. Acta 551, 10–21
12 Bloch, K.E. (1983) CRC Crit. Rev. Biochem. 14, 47–92
13 Rothem, S., Pfend, E.A. and Hayflick, L. (1971) J. Bacteriol. 105, 323–330
14 Demel, R.A., Bruckdorfer, K.R. and Van Deenen, L.L.M. (1972) Biochim. Biophys. Acta 255, 321–330
15 De Kruijff, B., Demel, R.A. and Van Deenen, L.L.M. (1972) Biochim. Biophys. Acta 255, 331–347
16 De Kruijff, B., De Greef, W.J., van Eijk, R.V.W., Demel, R.A. and Van Deenen, L.L.M. (1973) Biochim. Biophys. Acta 298, 479–499
17 Bruckdorfer, K.R., Demel, R.A., De Gier, J. and Van Deenen, L.L.M. (1969) Biochim. Biophys. Acta 183, 334–345
18 Ayengar, N.K.N., Lipton, L.C. and Brockerhoff, H. (1979) Chem. Phys. Lipids 25, 203–208
19 Lala, A.K., Buttke, T.M. and Bloch, K. (1979) J. Biol. Chem. 254, 10582–10585
20 Narayanan, C.R. and Iyer, K.N. (1965) J. Org. Chem. 30, 1734–1736
21 Dusza, J.P., Joseph, J.P. and Bernstein, S. (1966) Steroids 8, 495–509
22 Gorey, E.J., Gross, J.L. and Ulrich, P. (1976) Tetrahedron Lett. 809–810
23 Amed, M.S. and Logani, S.C. (1971) Aust. J. Chem. 24, 143–151
24 Van Deenen, L.L.M. and De Haas, G.H. (1964) Adv. Lipid Res. 2, 167–234
25 Geurts van Kessel, W.S.M., Tieman, M. and Demel, R.A. (1981) Lipids 16, 58–63
26 Bartlett, G.R. (1959) J. Biol. Chem. 234, 466–468
27 Hinz, H.J. and Sturtevant, J.M. (1972) J. Biol. Chem. 247, 6071–6075
28 Pethica, B.A. (1955) Trans Faraday Soc. 50, 1402–1409
29 Harmony, J.A.K., Jackson, R.L., Ihm, J., Ellsworth, J.L. and Demel, R.A. (1982) Biochim. Biophys. Acta 690, 215–223
30 Brockerhoff, H. (1974) Lipids 9, 645–650

31 Huang, C. (1977) Lipids 12, 348–353
32 Fong, J.W., Tieri, L.G., Desmukh, D.S. and Brockerhoff, H. (1977) Lipids 12, 857–862
33 De Kruijff, B., Demel, R.A., Slotboom, A.J., Van Deenen, L.L.M. and Rosenthal, A.F. (1973) Biochim. Biophys. Acta 307, 1–19
34 Clejan, S., Bittman, R., Deroo, P.W., Isaacson, Y.A. and Rosenthal, A.F. (1979) Biochemistry 18, 2118–2125
35 Bartholow, L.C. and Geyer, R.P. (1982) Biochemistry 21, 1271–1273
36 Bittman, R., Clejan, S., Jain, M.K., Deroo, P.W. and Rosenthal, A.F. (1981) Biochemistry 20, 2790–2795
37 Lundberg, B. (1982) Chem. Phys. Lipids 31, 23–33
38 Parkes, J.G., Watson, H.R., Joyce, A., Phadke, R.S. and Smith, J.C.P. (1982) Biochim. Biophys. Acta 691, 24–29
39 Razin, S. (1982) Curr. Top. Membranes Transp. 17, 183–205