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Membrane properties of oxysterols. Interfacial orientation, influence on membrane permeability and redistribution between membranes

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The membrane properties of cholesterol auto-oxydation products, 7-ketocholesterol, 7β-hydroxycholesterol, 7α-hydroxycholesterol and 25-hydroxycholesterol were examined. Monolayer studies show that these oxysterols are perpendicularly orientated at the interphase. Only 7β-hydroxycholesterol and 7α-hydroxycholesterol are tilted at low surface pressures. In mixed monolayers with dioleoylphosphatidylcholine, 7-ketocholesterol, 7β-hydroxycholesterol and 7α-hydroxycholesterol show a condensing effect in this order, although to a lesser extent that that observed for cholesterol. In liposomes these oxysterols also reduce glucose permeability and in the same order as their condensing effect. On the other hand 25-hydroxycholesterol shows no condensing effect in monomolecular layers whereas glucose permeability in liposomes is enormously increased. The permeability increase is already maximal at 2.5 mol% 25-hydroxycholesterol. Differential scanning calorimetry experiments reveal that all four oxysterols tested reduce the heat content of the gel → liquid-crystalline phase transition. It is concluded that 7-ketocholesterol, 7β-hydroxycholesterol and 7α-hydroxycholesterol have a cholesterol like effect, although less efficient than cholesterol, whereas 25-hydroxycholesterol showing no condensing effect acts as a spacer molecule. Packing defects in the hydrophobic core of the bilayer due to the presence of the C-25 hydroxyl group are believed to cause the permeability increase. The transfer of radiolabelled (oxy)sterols from the monolayer to lipoproteins or vesicles in the subphase was studied. The transfer rate increases in the following order 7-ketocholesterol, 7β-hydroxycholesterol, 7α-hydroxycholesterol, 25-hydroxycholesterol. The difference in rate between 7-ketocholesterol and 25-hydroxycholesterol is 20-fold. A higher rate of transfer is observed in the presence of high density lipoproteins and small unilamellar vesicles. A transfer rate for cholesterol is hardly measurable under these conditions. The transfer measured is consistent with the involvement of a water-soluble intermediate.

Introduction

The presence of oxysterols in blood and tissues has been reported in several studies [1–4]. The major auto-oxidation products of cholesterol are 7-ketocholesterol, 7β-hydroxycholesterol, 7α-hydroxycholesterol and 25-hydroxycholesterol [5,6]. Other oxysterols, such as 26-hydroxycholesterol and lanosten-3β-ol-32-al, are formed by enzymatic reactions in situ [1,7,8]. Auto-oxidation of cholesterol occurs rapidly when the sterol is exposed to air, heat and radiation or a combination of these factors [6] and is of special significance in the

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Abbreviations: DOPC, dioleoylphosphatidylcholine; DPPC, dipalmitoylphosphatidylcholine; PA, phosphatidic acid; PE, phosphatidylethanolamine; PC, phosphatidylcholine; SUV, small unilamellar vesicles; LUV, large unilamellar vesicles.

production and storage of cholesterol containing foodstuffs like powdered milk or eggs [4].

The physiological consequence of oxysterols is of importance since it is known that they decrease cholesterol synthesis in mammalian cells by inhibiting 3-hydroxy-3-methylglutaryl coenzyme-A reductase (HMG-CoA reductase) [8–10]. They also inhibit cholesterol esterification [11]. The greater the distance between the 3β-hydroxyl group of the oxysterol and the second oxygen function, the greater the inhibitory effect [9] on cholesterol synthesis.

Oxysterols have also been shown to have profound effects on cell morphology and membrane-associated functions. Hsu et al. [12] reported echinocyte transformation of red blood cells by incorporation of certain oxysterols into the membrane. The echinocyte formation was explained by an expansion of the outer leaflet of the bilayer of the red blood cell membrane, by the presumed bulky oxysterols. At high concentrations, 25-hydroxycholesterol is cytotoxic towards aortic smooth muscle cells [13] and *Salmonella tiphimurium* [14]. The cytotoxicity of oxysterols is reduced by addition of lipoproteins [15], suggesting binding of oxysterols to lipoproteins.

In this paper, we report on the membrane properties of 7-ketocholesterol, 7β-hydroxycholesterol, 7α-hydroxycholesterol and 25-hydroxycholesterol, and compare their properties to the parent compound, cholesterol. Since it is well known that cholesterol can reduce the heat content of the DPPC phase transition [16], it was of interest to determine whether oxysterols can interact with DPPC and induce a so called liquefying effect or rather, function as a spacer at the interface. In addition, the interfacial orientation of the four oxysterols was determined from force-area curves at the air/water interface. Mixed monolayers of oxysterols and DOPC were used to establish their possible interaction with phospholipids.

It is an essential property of cholesterol to contribute to the barrier properties of phospholipids. For cholesterol it has been shown that it reduces the permeability of biomembranes and PC vesicles for small nonelectrolytes and induces a so called intermediate gel state [19]. To compare the effect of oxysterols with cholesterol in this respect, the egg-PC vesicle permeability for glucose was determined in the presence of increasing concentrations of oxysterols.

To understand the effect of oxysterols on the HMG-CoA reductase by possibly binding to LDL, their binding to lipoproteins was studied. For this purpose, radioactively labeled oxysterols were used and their redistribution between monolayer and lipoproteins or vesicles in the subphase was measured.

Materials and Methods

Oxysterols and phospholipids

5-Cholesten-3β,7α-diol (7α-hydroxycholesterol), 5-cholesten-3β,7β-diol (7β-hydroxycholesterol) and 5-cholesten-3β,25-diol (25-hydroxycholesterol) were purchased from Steraloids Inc. (Wilton, NH, U.S.A.). 5-Cholesten-3β-ol-7-one (7-ketocholesterol) was obtained from Sigma Chem. Co. (St. Louis, MO, U.S.A.). 25-Hydroxy[26,27-^{3}H]cholesterol and [4-^{14}C]cholesterol were purchased from New England Nuclear (Boston, MA, U.S.A.). 7α-Hydroxy[4-^{14}C]cholesterol, 7β-hydroxy[4-^{14}C]cholesterol and 7-keto[4-^{14}C]cholesterol were prepared according to a modified procedure of Smith et al. [6]. Liposomes of DOPC and 10 mol% [4-^{14}C]cholesterol were prepared in 10 mM Tris-HCl (pH 7.4), 0.9% NaCl 1 mM $CuSO_4$, total lipid concentration was 1 or 2 mM. The sample was incubated at 65°C in a sealed, 50 ml, conical flask. After 48 h incubation the solution was diluted with a large excess of ethanol and taken to dryness under reduced pressure. The lipids were dissolved in chloroform/methanol (1 : 3, v/v) and applied to a TLC plate (Merck, Silica 60); reference oxysterols and cholesterol were spotted in the outer lanes of the plate. After running twice in ethylacetate/benzene (3 : 2, v/v) [6] in order to increase the separation, the reference compounds only, were visualized with 0.01% rhodamine phosphate. The R_F values were for cholesterol 0.61; 7-ketocholesterol 0.45; 7β-hydroxycholesterol 0.34; and 7α-hydroxycholesterol 0.26. 15 to 20% of the [4-^{14}C]cholesterol was converted of which 50% was 7-keto[4-^{14}C]cholesterol, 35% was 7β-hydroxy[4-^{14}C]cholesterol, and 15% was 7α-hydroxy[4-^{14}C]cholesterol. Too little 25-hydroxycholesterol could be formed by this procedure to be recovered [5]. The purity of the labeled oxysterols

was established after isolation being more than 98%. DOPC, DPPC and egg-PC were prepared according to the established procedures [20]. All lipids used gave a single spot on HPTLC plates.

Differential scanning calometry

Measurements were performed on a Perkin-Elmer DSC-2B apparatus with a heating rate of 5 $K \cdot min^{-1}$ and a cooling rate of 10 $K \cdot min^{-1}$. Only heating scans were used for calculation. DPPC (5 μM), to which increasing amounts of oxysterols were added, was suspended in 40 μl Tris-acetate (40 mM, pH 7.4) containing 100 mM NaCl. 15 μl of the suspension were transferred to the sample pan. The amount of phospholipid in the sample pan was determined as phosphorus [21].

Determination of force-area curves

Force-area measurements were performed at the air/water interface in a teflon trough (32.3 × 17.2 cm) in a thermostatic box at 22°C. The trough was filled with 10 mM Tris-HCl (pH 7.4), 0.9% NaCl. Water was distilled trice. The surface pressure was determined with a recording Beckman LM 500 electrobalance equipped with a platinum plate 1.96 cm wide. The compression rate was 0.258 $nm^2 \cdot mol^{-1} \cdot min^{-1}$. 50 nmol of lipid dissolved in chloroform were carefully released onto the air/water interface from an Agla micrometer syringe. For all films, the surface pressure was plotted against the mean area per molecule, that is, the total area divided by the total number of oxysterols and/or phospholipid molecules. For a quantitative evaluation of the condensing effect of oxysterols the variation of the mean molecular area of a given constant surface pressure was plotted as a function of the mole fraction oxysterol to DOPC.

Glucose permeability

The release of glucose from egg-PC liposomes containing 4 mol% phosphatidic acid and up to 50 mol% oxysterol, after 1 h at 41°C, was measured as described before [22].

Oxysterol transfer from monolayers

Monolayer experiments were carried out at 37°C in a 20 ml teflon trough (5.9 × 5.4 × 0.5 cm) in a thermostatically controlled box. The subphase contained 20 ml of a standard buffer, 10 mM Tris-HCl (pH 7.4), 0.9% NaCl. Surface ^{14}C-radioactivity was monitored with a gas flow detector (Nuclear Chicago Model 8731); the gas was helium/1.3% butane. Surface pressure was detected as described above. Monolayers were formed of 95% DOPC and 5% oxysterol and spread from a chloroform/methanol (1 : 1, v/v) solution. After surface radioactivity and pressure had stabilized, lipid vesicles (1 μmol) or lipoproteins (25 μg protein) were added to the subphase through a hole of 0.5 cm^2 at an extended corner of the trough. The subphase was stirred with a magnetic bar [23]. For 25-hydroxy[26,27-^3H]cholesterol transfer from the monolayer was measured by taking 1 ml samples from the subphase at various time intervals and radioactivity was determined by scintillation counting.

Preparation of lipid vesicles and lipoproteins

Large unilamellar vesicles of DOPC containing 2% DOPA were prepared according to the method of Szoka et al. [23]. Small unilamellar vesicles of the same composition were made by sonication of a 1 mM lipid suspension in 10 mM Tris-HCl (pH 7.4), 0.9% NaCl as described previously [24].

Very low density lipoproteins (VLDL), low density lipoproteins (LDL) and high density lipoproteins (HDL) were isolated from plasma of normal fasting subjects by ultracentrifugation between $d < 1.006$ (VLDL), $d = 1.020–1.060$ (LDL), $d = 1.060–1.170$ (HDL_2), and $d = 1.15–1.25$ (HDL_3), as described in Ref. 25.

Results

The interfacial orientation and the occupied molecular area of the different oxysterols are determined from the pressure-area curves at the air/water interface (Fig. 1). Compared to the parent compound, cholesterol, the presence of a keto moiety at the 7 position, as in 7-ketocholesterol, leads to a small increase in the molecular area at low surface pressures (Fig. 1, curve A). The minimal surface area of 38.6 $Å^2$/molecule is very close to that of cholesterol.

A remarkable difference is noted for the 7α- and 7β-hydroxycholesterol isomers. Due to the

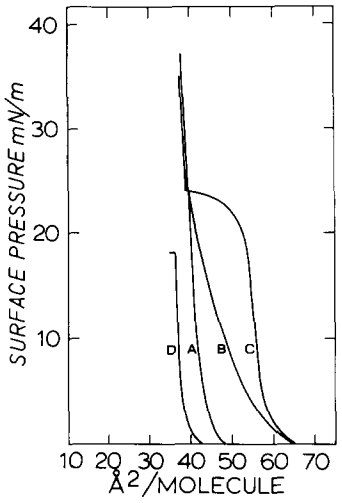

Fig. 1. Force-area curves of pure 7-ketocholesterol (A); 7β-hydroxycholesterol (B); 7α-hydroxycholesterol (C); and 25-hydroxycholesterol (D) at 22°C.

presence of the hydroxyl group at the B ring, at the 7 position, the molecular area is increased at low surface pressures. For 7β-hydroxycholesterol the molecular area is 53 $Å^2$/molecule at 5 mN · m^{-1} and decreases gradually to 38 $Å^2$/molecule at 30 mN · m^{-1} (Fig. 1, curve B). For 7α-hydroxycholesterol the molecular area is 57 $Å^2$/molecule at 5 mN · m^{-1} and there is a very steep incline in surface pressure from 5 to 18 nM · m^{-1}. A transition to a condensed state starts at 18 mN · m^{-1} and is completed at 24 mN · m^{-1} (Fig. 1, curve C). The molecular area at surface pressure higher than 24 mN · m^{-1} is comparable to that of 7β-hydroxycholesterol. 7-Ketocholesterol, 7α-hydroxycholesterol and 7β-hydroxycholesterol, show a high interfacial stability up to surface pressures of more than 30 mN · m^{-1}. 25-Hydroxycholesterol, having a hydroxyl group at the 3 position and an additional hydroxyl group at the side chain, does not show an increased molecular area. In contrast, the molecular area is even less than that of 7-ketocholesterol (Fig. 1, Curve D). The limiting area of 25-hydroxycholesterol is 36.5 $Å^2$/molecule, indicating a perpendicular orientation at the interface. The orientation is consistent with a lower collapse pressure of 18 mN · m^{-1}.

The interaction of oxysterols with phospholipids was next examined in mixed monomolecular films of DOPC at three different DOPC-oxysterol ratios. The variation of the mean molecular area was plotted as a function of the mole fraction oxysterol at surface pressures of 10 to 30 mN · m^{-1}. In mixed monolayers of DOPC and 7-ketocholesterol, a reduction in mean molecular area was observed at all mole fractions of oxysterol, at both 10 and 30 mN · m^{-1}. At 50 mol% 7-ketocholesterol, the reduction in mean molecular area is 8.5 $Å^2$, at 30 mN · m^{-1} (Fig. 2A). Also, for 7α-hydroxycholesterol and 7β-hydroxycholesterol an interaction with DOPC can be measured. However, the reduction in mean molecular area is less than that found for the 7-keto derivative. This condensing effect is 7 and 6 $Å^2$ for 7β-hydroxycholesterol and 7α-hydroxycholesterol, respectively, at 30 mN · m^{-1} (Figs. 2B, 2C). The transition found for pure 7α-hydroxycholesterol (Fig. 1, curve C) is not seen in mixtures with DOPC having 25 or 50 mol% 7α-hydroxycholesterol. At a concentration of 75 mol% 7α-hydroxycholesterol a smooth transition is observed between 14 and 22.6 mN · m^{-1}. Mixed films of 25-hydroxycholesterol and DOPC show no measurable deviation from ideal behaviour (Fig. 2D). At a mol fraction of 0.25 25-hydroxycholesterol, the collapse pressure of the

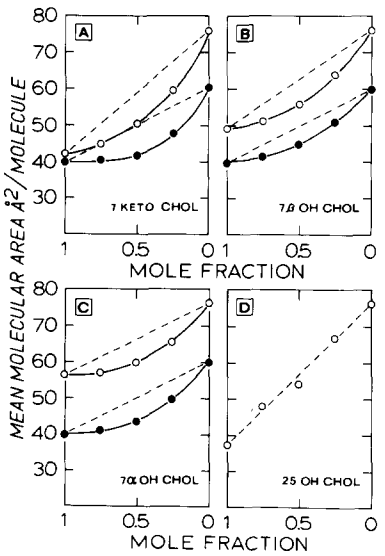

Fig. 2. Variation of the mean molecular area of sterol and phospholipid molecules as a function of the composition for mixed monolayers of DOPC and 7-ketocholesterol (A); 7β-hydroxycholesterol (B); 7α-hydroxycholesterol (C); 25-hydroxycholesterol (D) at surface pressures of 30 mN · m^{-1} (●) and 10 mN · m^{-1} (○) and 22°C.

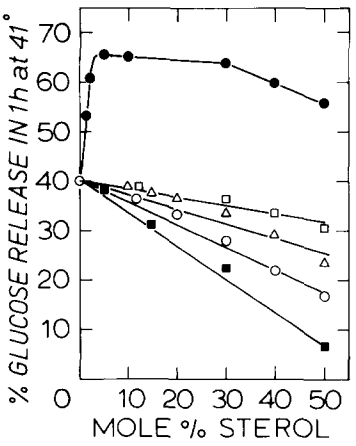

Fig. 3. Effect of oxysterol incorporation in egg-phosphatidylcholine liposomes on the relative amount of glucose released after 1 h at 41°C. Liposomes are prepared from egg-phosphatidylcholine and 4 mol% dioleoylphosphatidic acid with varying amounts of cholesterol (■); 7-ketocholesterol (○); 7β-hydroxycholesterol (△); 7α-hydroxycholesterol (□); 25-hydroxycholesterol (●).

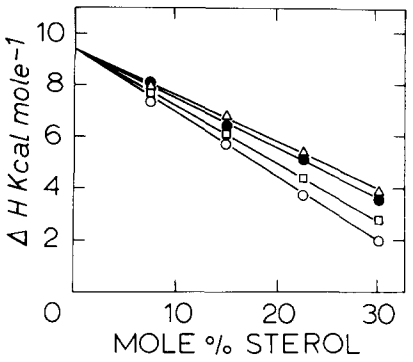

Fig. 4. Effect of oxysterols on the gel → liquid-crystalline phase transition of dipalmitoylphosphatidylcholine. Abscissa indicates mol%: 7-ketocholesterol (○); 7β-hydroxycholesterol (△); 7α-hydroxycholesterol (□); 25-hydroxycholesterol (●).

mixed film is identical to that of DOPC. At mol fractions of 0.50 and 0.75 25-hydroxycholesterol, the collapse pressure is 29.4 and 26.3 $mN \cdot m^{-1}$, respectively.

The effect of oxysterols on the permeability properties of membranes was tested by measuring glucose release from egg-PC liposomes after 1 h at 41°C. It is well established that the condensing effect of cholesterol is reflected in a reduced membrane permeability as illustrated in Fig. 3. The incorporation of 7-ketocholesterol, 7β-hydroxycholesterol or 7α-hydroxycholesterol into egg-PC liposomes. reduces the glucose permeability, in that order. These oxysterols are, however, far less effective than cholesterol itself.

At a concentration of 50 mol%, 7-ketocholesterol, 7β-hydroxycholesterol and 7α-hydroxycholesterol show a reduction of 68, 41 and 25 percent, respectively, relative to that obtained in the presence of cholesterol (Fig. 3). 25-Hydroxycholesterol, however, brings about a sharp permeability increase at very low concentrations already: concentrations between 2.5 and 5 mol% give a release of 60 to 65 percent of the trapped glucose (Fig. 3) and is approximately the amount of glucose entrapped in the outer shell of multilamellar vesicles. This maximal release is found between 2.5 and 30 mol% 25-hydroxycholesterol. At higher concentrations the effect is somewhat reduced.

It is well known that cholesterol decreases the heat absorbed at the crystalline → liquid-crystalline phase transition [16]. At concentrations greater than 33 mol% cholesterol, no apparent phase transition is found. As is shown in Fig. 4, all four oxysterols, namely 7-ketocholesterol, 7α-hydroxycholesterol, 25-hydroxycholesterol and 7β-hydroxycholesterol have a significant effect on the heat content of the phase transition of DPPC, in that order. At 30 mol% 7-ketocholesterol, the reduction in heat content of DPPC is 79%. For 7β-hydroxycholesterol this reduction is 59% showing that all these oxysterols are less effective than the parent compound cholesterol.

The pretransition of DPPC was eliminated in the presence of 7.5 mol% 7α-hydroxycholesterol, 7β-hydroxycholesterol and 7-ketocholesterol. However, the pretransition was still present with 7.5 mol% 25-hydroxycholesterol but no longer detectable at 15 mol%. 7α-Hydroxycholesterol and 7-ketocholesterol give rise to symmetric transition peaks at all molar concentrations. For 7β-hydroxycholesterol a shoulder in the transition peak is apparent on the lower side of the heating curve at concentrations higher than 15 mol%. A significant asymmetry for 25-hydroxycholesterol at the high temperature side of the heating curve starts at 22 mol% and increases at higher concentrations. 25-Hydroxycholesterol did not affect the bilayer → hexagonal H_{II} phase transition of dielaidoyl PE.

To measure a possible redistribution of oxysterols between membranes, and binding to the serum lipoproteins, radiolabeled oxysterols were incorporated in phospholipid monolayers and their rate of release from the interface was determined. The decrease in interfacial oxysterol concentration was followed both by measuring the reduction in surface radioactivity and by the increase in radioactivity in the subphase. The latter method to determine very low rates of oxy[^{14}C] sterol release and of 25-hydroxy[^{3}H]cholesterol. Incorporation of 5 mol% cholesterol or oxysterol (420 pmol) in a DOPC monolayer at a pressure of 22 mN · m^{-1}, gave very stable monolayers which show no measurable release of these sterols from the interface, even after 2 h.

In a 5 mol% cholesterol monolayer there is a hardly-measurable decrease in surface radioactivity even in the presence of a large excess of phosphatidylcholine vesicles or lipoproteins in subphase (Table I); higher concentrations of cholesterol in the monolayer or lower surface pressures did not give higher transfer rates.

In the presence of HDL_3, 7-ketocholesterol is transferred from the monolayer at a rate of 83 pmol/h (Table I). 7β-Hydroxycholesterol and 7α-hydroxycholesterol are transferred at rates approximately 3-times higher. For 25-hydroxycholesterol, the rate of 1.68 nmol/h is 20-times higher than that for 7-ketocholesterol. This means that the $t_{1/2}$ is reached within 7 min. (Fig. 5). The transfer of all four oxysterols to HDL_2, LDL or VLDL is approximately half the rate measured in the presence of HDL_3 (Table I). The rate at which cholesterol, 7-ketocholesterol, 7β-hydroxycholesterol, 7α-hydroxycholesterol and 25-hydroxycholesterol are released from the interphase in the presence of HDL_3 are compared in Fig. 5. The addition of lipid-free apolipoprotein A-I or E had no effect on the release of oxysterols from the interface. The rate of transfer of oxysterols to phospholipid vesicles is given in Table I. Small unilamellar vesicles of egg-PC-2% PA are as effective as HDL_3, except for 7β-hydroxycholesterol, which was about half that for HDL_3 (Table I). To determine if the curvature and molecular packing of the receptor particle is rate determining, large

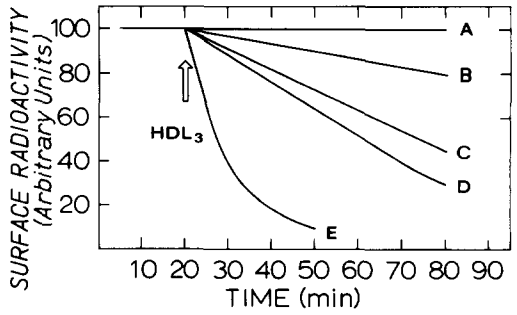

Fig. 5. Transfer of labelled cholesterol and oxysterols from a monomolecular layer to HDL_3. The monolayer consisted of dioleoylphosphatidylcholine containing 5 mol% (oxy)cholesterol (420 pmol) at an initial pressure of 22 nM·m^{-1}. (A) cholesterol; (B) 7-ketocholesterol; (C) 7β-hydroxycholesterol; (D) 7α-hydroxycholesterol; (E) 25-hydroxycholesterol. At the indicated time HDL_3 (25 μg protein) was injected into the subphase.

TABLE I

EFFECT OF LIPOPROTEINS AND PHOSPHOLIPID VESICLES ON THE INITIAL RATE OF (OXY)CHOLESTEROL TRANSFER FROM MONOMOLECULAR LAYERS

The monolayer consisted of dioleoylphosphatidylcholine containing 5 mol% of the indicated sterol (420 pmol) at an initial pressure of 22 mN·m^{-1}. Lipoproteins (25 μg protein) or dioleoylphosphatidylcholine-2% dioleoylphosphatidic acid (1 μmol) small unilamellar vesicles (SUV) or large unilamellar vesicles (LUV) were injected into the subphase as indicated. n.d., not detectable.

Sterol	Initial rate of (oxy)cholesterol release (pmol/h)					
	HDL_3	HDL_2	LDL	VLDL	SUV	LUV
Cholesterol	2	n.d.	n.d.	n.d.	3	n.d.
7-Ketocholesterol	84	36	44	40	72	40
7β-Hydroxycholesterol	220	100	52	88	112	43
7α-Hydroxycholesterol	284	132	104	152	324	120
25-Hydroxycholesterol	1680	600	648	912	1632	–

unilamellar vesicles were tested. The rate induced by LUV is much lower than by SUV (Table I).

Discussion

The major products of air oxidation of cholesterol are 7-ketocholesterol, 7α-hydroxycholesterol, 7β-hydroxycholesterol and 25-hydroxycholesterol. The monolayer experiments described in this paper show that 7-ketocholesterol, 7α-hydroxycholesterol and 7β-hydroxycholesterol have a high interfacial stability. The molecular area of 7-ketocholesterol is, compared to cholesterol slightly increased at low-surface pressures but at higher pressures the two sterols are identical. A more polar hydroxyl group at the 7 position will cause a tilt and increases the molecular area at low-surface pressures. 7β-Hydroxycholesterol having an equatorial hydroxyl group is gradually forced into a perpendicular orientation at higher surface pressures. The axial orientation of the 7α-hydroxyl group leads to a stronger interaction with the aqueous phase and the tiled orientation is maintained to pressures of 18 $mN \cdot m^{-1}$ where a sudden transition to a perpendicular orientation occurs. 25-Hydroxycholesterol with a hydroxyl group at both ends of the molecule, namely at position 3 and 25, has a perpendicular orientation, and is not folded as has previously been suggested [9,18]. Only with 22*R*-hydroxycholesterol it has been shown that the molecule is oriented initially parallel to the interface [27]. The interfacial stability of 25-hydroxycholesterol is reduced as can be concluded from the lower collapse pressure.

Mixed monomolecular layers of oxysterols and DOPC show that 7-ketocholesterol, 7β-hydroxycholesterol and 7α-hydroxycholesterol interact with the phospholipid in that order and induce a condensing effect. However, the effect is less than that for mixtures of DOPC and cholesterol [28]. 25-Hydroxycholesterol on the other hand shows no interaction with DOPC. The effect of 7-ketocholesterol, 7β-hydroxycholesterol and 7α-hydroxycholesterol on the glucose permeability are in accordance with the condensing effects measured in mixed monomolecular layers. That is, the sterols reduce the permeability in that order, albeit to a lower extent than cholesterol. The large increase in permeability of glucose at low 25-hydroxycholesterol concentrations is in agreement with that found for Ca^{2+} [29]. As a result of the perpendicular orientation of 25-hydroxycholesterol, the hydroxyl group at C25 is present in the hydrophobic core of the bilayer. We suggest that the perpendicular shutteling of 25-hydroxycholesterol between the inner and outer monolayer of the membrane, causes a local disordering of the membrane lipids leading to the observed high permeability.

At concentrations higher than 25 mol% 25 hydroxycholesterol, permeability is reduced [29] and is probably due to aggregation of the oxysterol. We previously reported that some other oxidation products of cholesterol auto-oxidation [13] namely: 4-cholesten-3-one and 3,5-cholestedien-7-one also increase the glucose permeability [30]. Although to a lesser extent than 25-hydroxycholesterol. We reason that these ketosterols which are less polar than cholesterol and occupy a higher molecular area, will be less strongly bound to the interface and thus cause a local disordering of the membrane lipids leading to increased permeability.

The oxygenated sterols 7-ketocholesterol, 7α-hydroxycholesterol, 7β-hydroxycholesterol and 25-hydroxycholesterol have a marked effect on the gel → liquid-crystalline phase transition which is in agreement with the recent results of Egli et al. [17]. Similarly we find that 7 to 10 mol% 25-hydroxycholesterol, the enthalphy of the phase transition is reduced comparable to the other oxysterols. On the other hand the pretransition of the lipid is still preserved. Only at higher concentrations of 25-hydroxycholesterol the pretransition is eliminated. Laser Raman studies [18] showed that at 20 mol% 25-hydroxycholesterol the main transition of DPPC is considerably reduced and broadened. Our results show that at 30 mol% 25-hydroxycholesterol the enthalphy of the DPPC phase transition is reduced similar to 7β-hydroxycholesterol. However, the effect of 25-hydroxycholesterol is different from 7-ketocholesterol, 7α-hydroxycholesterol and 7β-hydroxycholesterol since it shows no condensing effect, contrary to the other oxysterols. Although there is a reduction of the enthalpy of the gel → liquid-crystalline phase transition, it cannot simply be concluded that the behaviour of the oxysterol is comparable to cholesterol. 25-Hydroxycholesterol does not act as a

liquefyer introducing an intermediate gel state [19] but rather as a 'spacer' [31], thus separating the phospholipids without interacting with the phospholipids. A similar behaviour in this respect was noted for cholesteryl acetate and cholesteroyl methoxy methyl ether [32]. A perpendicular orientation of 25-hydroxycholesterol is in agreement with the observation of Gallay and De Kruijff [34] that sterols with a molecular area higher than cholesterol affect the bilayer → hexagonal H_{II} phase transition of dielaidoylphosphatidylethanolamine, 25-hydroxycholesterol does not affect this transition.

Streuli et al. [33] showed that even the more polar oxysterols could not be removed from the red blood cells despite repeated washing of the cells in buffer. Experiments with mixed monomolecular layers containing 5 mol% radiolabelled cholesterol or oxysterol show that there is no measurable decrease of the surface radioactivity even after 5 h. Samples from the subphase confirm this showing an insignificant number of counts to be present. The addition of HDL_3 (Fig. 5) or small unilamellar vesicles (SUV) to the subphase brings about a release of cholesterol from the interface, albeit at very low rate. 2–3 pmoles/h correspond to 0.5 to 0.7% of the amount present in the monolayer (Table I). Much higher rates of transfer were found for the oxysterols, specially in the presence of lipoproteins. A 2–3-times higher transfer rate was found for HDL_3 than for HDL_2, LDL or VLDL. 25-Hydroxycholesterol showed an extremely high rate of redistribution in the presence of HDL_3 and may have physiological consequences. It is possible that in situ after esterification by lecithin cholesterol acyl transferase that the oxysterol ester is transferred to LDL. In this regard oxydized cholesteryl esters have been demonstrated in plasma [3]. The axial hydroxyl group at C-7α produces a sterol which is more membrane disturbing and, therefore, redistributes faster than the one having an equatorial hydroxyl group at C-7β. (Compare Figs. 1, 2 and Table I.) The order of the transfer rates of oxysterols is similar to the order of their interaction with DOPC. That is, the most cholesterol like oxysterol, 7-ketocholesterol, shows the lowest transfer rate.

The particle size, and consequently, the molecular packing of the acceptor particle, largely influence the transfer rate. Very similar rates were obtained in the presence of HDL_3 and SUV of DOPC-2% PA whereas LUV gave 2–3-times lower rates and were comparable to that obtained with the other lipoprotein fractions. In the vesicle-vesicle exchange system it has been shown [35] that the rate of cholesterol exchange is almost inversely proportional to the radius of the vesicle. For 40 nm radius vesicles a $t_{1/2}$ of 10.2 h was found. Although it is not possible to compare directly those data with the monolayer system an extremely low rate of cholesterol desorption can be expected. The difference between cholesterol and 25-hydroxycholesterol measured in this study is about 800-fold (Table I). This is in fair agreement with the difference predicted by McLean and Philips [36] based on the transfer by a water-soluble intermediate. The insignificant rate of cholesterol transport in absence of protein can hardly have a physiological bearing. The non-specific lipid transferprotein (ns-PL-TP) can mediate the transfer of different phospholipids and also of cholesterol [26]. In our system ns-PL-TP increases the rate of cholesterol transfer from monolayer to SUV to 70 pmol/h. The transfer rate of 7-ketocholesterol and 7α-hydroxycholesterol increased in the presence of ns-PL-TP and SUV 10- and 3-fold, respectively (Demel, R.A., to be published).

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