ELSEVIER

Biochimica et Biophysica Acta 1372 (1998) 331–338

BIOCHIMICA ET BIOPHYSICA ACTA
BBA

The influence of hydrophobic mismatch on androsterol/phosphatidylcholine interactions in model membranes

Henna Ohvo-Rekilä, Peter Mattjus [1], J. Peter Slotte *

Department of Biochemistry and Pharmacy, Åbo Akademi University, P.O. Box 66, FIN 20521 Turku, Finland

Received 15 January 1998; revised 6 April 1998; accepted 16 April 1998

Abstract

We have examined the association of 5-androsten-3β-ol (androsterol) with saturated phosphatidylcholines (PCs), having symmetric acyl chains from 10 to 16 carbons in length, in both mono- and bilayer membranes. The emphasis of the study was to measure how hydrophobic mismatch (i.e. the difference in hydrophobic length of the interacting molecules) affected androsterol/PC interactions in model membranes. With monolayer membranes (33 mol% sterol, 20 mN/m, 25°C), androsterol was found to be macroscopically miscible with all the tested PCs. Androsterol was observed to condense the lateral packing of di14 and di15 PCs (by 6 and 4.5 $Å^2$ per molecule, respectively), but failed to condense shorter (di10, di11, di12 and di13 PCs) or the longer chain di16PC. The rate of androsterol desorption from mixed monolayers to β-cyclodextrin acceptors in the subphase was a clear function of the host PC acyl chain length. The slowest rate of androsterol desorption (i.e. best androsterol/PC interaction) was seen from a di14PC monolayer, whereas the desorption rate increased when the host PC had shorter or longer chains. When the cholesterol oxidase susceptibility of androsterol was determined in small unilamellar vesicles (SUV) containing PCs of different chain lengths (33 mol% androsterol), the slowest rate of oxidation was seen in di14PC vesicles, whereas higher rates were measured for shorter or longer chain PC vesicles, again suggesting that androsterol interacted more favorably with di14PC than with the other PCs. In conclusion, the hydrophobic mismatch between androsterol and different PCs appeared to greatly affect the intermolecular interactions, as determined from the condensation effect, from sterol desorption rates, and the oxidation susceptibility of androsterol. Although androsterol is not a physiological membrane component, the present model system clearly shows that hydrophobic mismatch has a great influence on how sterols and phosphatidylcholines interact in membranes.

Keywords: Model membrane; Hydrophobic mismatch; Lipid interaction; Desorption; Monolayer; Vesicle

1. Introduction

Membrane lipids and proteins interact mainly through hydrophobic interactions. The interaction between, e.g., phospholipids and an integral protein appears to be stabilized in situations where the hydrophobic length of the phospholipid acyl chains match the hydrophobic length of the transmembrane segment of the membrane protein [1]. Hydrophobic mismatch, on the other hand, may lead to lateral

Abbreviations: di10PC, didecanoyl phosphatidylcholine; di11PC, diundecanoyl phosphatidylcholine; di12PC, didodecanoyl phosphatidylcholine; di13PC, ditridecanoyl phosphatidylcholine; di14PC, ditetradecanoyl phosphatidylcholine; di15PC, dipentadecanoyl phosphatidylcholine; di16PC, dihexadecanoyl phosphatidylcholine; PC, phosphatidylcholine; SUV, small unilamellar vesicles

* Corresponding author. Fax: +358 (2) 2154-745; E-mail: jpslotte@abo.fi

[1] Present address: The Hormel Institute, University of Minnesota, 801 16th Avenue N.E., Austin, MN 55912, USA.

0005-2736/98/$19.00
PII: S0005-2736(98)00069-8

segregation and even aggregation of proteins in the plane of the membrane, as was recently shown for the Ca^{2+}-ATPase reconstituted into bilayer membranes [2]. However, some membrane-bound enzymes (e.g., Na^+,K^+-ATPase and bacteriorhodopsin) appear to function properly in membranes of different hydrophobic thicknesses [3].

Hydrophobic mismatch among different saturated PC species has also been shown to affect their mutual miscibility in model membrane systems [4–7]. The interaction between cholesterol and phospholipids also appear to be influenced by the hydrophobic length of the two molecular species. Using high-sensitivity differential scanning calorimetry (DSC), McMullen and coworkers [8] showed that cholesterol increased the bilayer transition temperature of the broad component of the DSC endotherms for saturated, equal-length PCs with less than 17 carbons per chain, whereas cholesterol decreased the transition temperature for PCs with acyl chains longer than 17. The hydrophobic length of cholesterol (17.5 Å) was calculated to match the mean hydrophobic thickness of a 17:0 PC bilayer [8]. When the interaction of cholesterol with PCs was measured in monolayer membranes using cholesterol oxidase as a probe, the oxidation susceptibility of cholesterol was observed to be lowest in PC monolayers having acyl chain lengths between 14:0 and 17:0, and higher in membranes with PCs having more than 17 or less than 14 carbons per chain [9]. Since a lower oxidation susceptibility (protection to oxidation) results from better interaction between the sterol and the phospholipid, it was concluded that cholesterol interacted more favorably with PCs having chain lengths between 14 and 17 carbons than with longer or shorter chain PCs. The above-mentioned study was, however, complicated by the fact that the PCs used (di10:0 to di20:0 PC) displayed different phase states at the temperatures examined.

In addition to having a model system with one sterol and varying phospholipid acyl chain lengths, one can achieve hydrophobic mismatch using one type of phospholipid and different length sterol molecules. Using a series of synthetic sterols with different length side chains at the C17 position, McMullen and coworkers [10] demonstrated a sterol chain-length-dependent effect on the phase transition temperature and cooperativity in sterol/DPPC bilayer membranes. This effect was ascribed to the hydrophobic mismatch between the sterols and DPPC. Using the same synthetic sterol analogs in monolayer membranes, it was demonstrated that the sterol side chain length had marked effects on the capacity of the sterols to form liquid-condensed macrodomains with DPPC in the mixed monolayers [11].

5-Androsten-3β-ol, or androsterol, is a cholesterol analog which lacks the iso-octyl side chain at C17, but has the same ring structure and stereochemistry as cholesterol. This sterol has very little influence on the physical properties of biological and model membranes, as compared to cholesterol. The condensing effect of androsterol on the lateral packing density of phospholipid monolayers is much smaller or non-existent as compared to the effect of cholesterol [12,13]. Using low-sensitivity DSC, it was shown that androsterol had a much smaller effect than cholesterol on the cooperativity and enthalpy of the gel to liquid–crystalline phase transition in sterol/egg PC or sterol/SOPC bilayer membranes [14,15]. In addition, androsterol was not as effective as cholesterol in reducing the conformational disorder of the liquid–crystalline phase of DPPC bilayers [16,17]. These differences between androsterol and cholesterol most likely derive from the fact that androsterol has a shorter hydrophobic length as compared to cholesterol. Due to hydrophobic mismatch, androsterol is not as readily miscible in medium or long chain phosphatidylcholine bilayers [18].

In this study we have examined the interaction of androsterol with saturated phosphatidylcholines in monolayers as a function of the PC acyl chain length (i.e. hydrophobic mismatch). We measured the condensation effect of androsterol on PC packing, the relative sterol desorption rate from the mixed PC monolayers, and the oxidation susceptibility of androsterol in small unilamellar vesicles (SUV). Based on the obtained results, we find that androsterol interacts most favorably with di14:0 PC, and less favorably with longer or shorter chain PCs. The observed results can be largely explained to arise from varying degrees of hydrophobic mismatch between the sterol and the PC in the membrane systems.

2. Materials and methods

2.1. Materials

The saturated PCs with symmetric acyl chains, ranging from 10 to 16 carbons per chain, were obtained from Sigma (USA). 5-Androsten-3β-ol was obtained from Steraloids (USA). Cholesterol oxidase (*Streptomyces sp.*) was purchased from Calbiochem (USA). β-Cyclodextrin, horseradish peroxidase and *p*-hydroxyphenyl acetic acid were from Sigma. The water used was purified by reverse osmosis followed by passage through a Millipore UF Plus water purification system, to yield a product with a resistivity of 18.2 MΩ cm. Buffers were made of pro analysis grade salts.

2.2. Preparation of monolayers and determination of lateral packing properties

Monolayers containing either pure PCs, pure androsterol, or 67 mol% PC and 33 mol% androsterol, were prepared at the clean air/water interface in a KSV 3000 surface barostat (KSV Instruments, Helsinki). The temperature was 25°C. When force–area isotherms were collected, the barrier speed during monolayer compression did not exceed 5 $Å^2$/molecule/min. Data were analyzed using proprietary KSV software. From the force–area isotherms the molecular area for a given surface pressure was obtained, and used for calculations of lipid desorption rates [19], or for calculation of the condensation of the lateral packing density, as described previously [20]. Monolayer collapse data were also derived from the force–area isotherms.

2.3. Cyclodextrin-induced androsterol desorption from mixed monolayers

Monolayers containing 33 mol% androsterol together with one of the PCs were prepared at the air/water interface. The trough used was of a zero-order type [21], with a reaction chamber (28 ml volume, 28.3 cm^2 area) separated by a glass bridge from the lipid reservoir [20]. The reaction chamber was thermostated to give a temperature of 25°C. The β-cyclodextrin was injected into the stirred reaction chamber without penetrating the monolayer (in a volume not exceeding 0.2 ml), to give a final β-cyclodextrin concentration of 0.33 mM. The removal of monolayer androsterol to the subphase was determined from the area decrease of the monolayer at constant surface pressure (20 mN/m). Knowing the mean molecular area at a given surface pressure and temperature, the amount of sterol removed could be calculated as a time function. The rate of PC removal by β-cyclodextrin was negligible compared to the rate recorded for androsterol (see also [19]).

2.4. Cholesterol oxidase-catalyzed oxidation of androsterol in small unilamellar vesicles

Small unilamellar vesicles containing either 33 or 50 mol% of androsterol and one of the PCs, were prepared by injection of ethanol-dissolved lipids into Dulbecco's phosphate-buffered saline (pH 7.4) at 25°C, using a Hamilton spring-loaded syringe. The oxidation buffer contained 0.15 mg/ml of *p*-hydroxyphenyl acetic acid and 5 U/ml of horseradish peroxidase, in addition to the SUV substrate. The final vesicle concentration was 1 mM of total lipids. The vesicles were incubated at 25°C for 30 min prior to exposure to cholesterol oxidase (375 mU/ml at 37°C). The time needed for the completion of 50% oxidation was determined with fluorescence spectroscopy, as described previously [22].

3. Results

3.1. Monolayers of androsterol and PCs as a function of PC chain length

Androsterol, although it lacks the iso-octyl chain of cholesterol, can form stable monolayers at the gas/aqueous interface. The androsterol monolayer collapsed around 35 mN/m (equilibrium) whereas a cholesterol monolayer has a corresponding collapse pressure of 43 mN/m (data not shown, but see also [12,23]). The di-saturated PCs also formed stable monolayers. The short-chain PCs (10–13 carbons per chain) were all completely liquid-expanded at the experimental temperature (25°C), whereas longer PCs (those with 14–16 carbons per chain) displayed liquid-expanded to liquid-condensed transitions at different surface pressure intervals (49–53 mN/m,

23–26 mN/m, and 6–9 mN/m for di14:0, di15:0 and di16:0 PCs, respectively; isotherms not shown). In mixed monolayers containing 33 mol% androsterol and 67 mol% PCs, the presence of androsterol appeared to destabilize di16:0 PC monolayers, as evidenced from the change in collapse pressure for the pure and mixed systems (Fig. 1), whereas the stability of the other PC monolayers appeared to be unaffected by androsterol. The molecular areas and compressibilities of the pure PC monolayers (di10–di20 PCs) have previously been reported [9]).

It is well known that cholesterol can condense the lateral packing of many classes of phospholipids in model membranes [24,25]. It is, however, mainly phospholipids in their liquid-expanded state that are condensed by cholesterol [26]. In addition to cholesterol, some other sterols have also been found to induce condensation of the lateral packing in monolayers [12,27]. In this study, we wanted to examine the condensation potential of androsterol in mixed monolayers containing different-length PCs. As shown in Fig. 2, only two of the PCs in their LE state were condensed in the presence of androsterol, namely di14PC and di15PC. The lateral packing density of the shorter PC was unaffected by androsterol, as was that of di16PC. However, di16PC was not liquid expanded at 20 mN/m and 25°C. These results clearly show that the condensing effect of a sterol on a phospholipid membrane is affected by the hydrophobic mismatch of the interacting molecules.

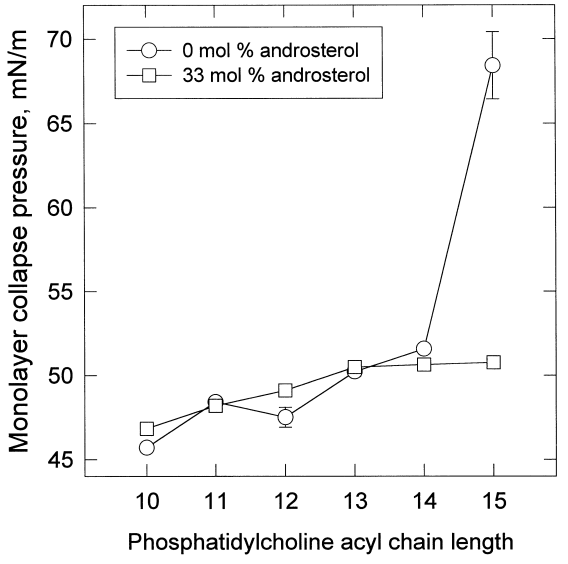

Fig. 1. Collapse stability of pure and mixed monolayers. Pure PC monolayers or mixed monolayers containing 33 mol% androsterol and one of the PCs were prepared at the air/water interface. The monolayers were compressed with a speed not exceeding 5 Å^2/molecule/min until monolayer collapse was reached. Each value is the average ± S.E. from three separate experiments.

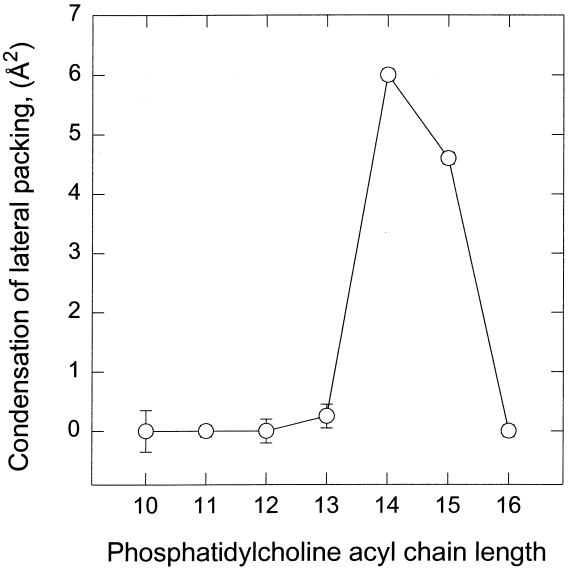

Fig. 2. Condensation of PC lateral packing by androsterol. Force–area isotherms of monolayers containing 33 mol% androsterol and 67 mol% PC were obtained at 25°C. The extent of condensation of PC packing (mean molecular area decrease, given in Å^2) at 20 mN/m is plotted as a function of PC acyl chain length. Each value is the average ± S.E. from three separate experiments.

3.2. *Desorption of monolayer androsterol to β-cyclodextrin as a function of PC chain length*

The effect of androsterol on the lateral condensation of di14PC and di15PC may suggest that the hydrophobic match between androsterol and a PC was optimal when the PC acyl chain length was about 14–15 carbons. To further examine this possibility, we decided to measure androsterol desorption rates from mixed monolayers containing 33 mol% sterol and one of the PCs, since the desorption rate is known to be influenced by the sterol/co-lipid interaction in the membrane [19,28]). Cyclodextrins can be used as tools to trap the desorbed sterol in the aqueous phase [29,30], and the monolayer system is especially suited for this assay, because the loss of

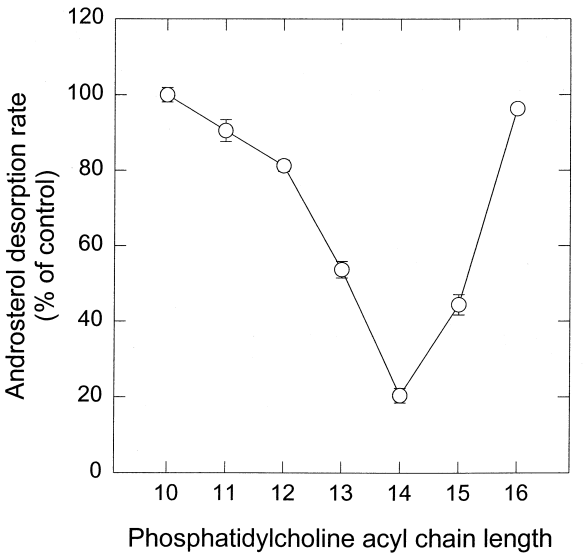

Fig. 3. Desorption of androsterol from mixed monolayers to β-cyclodextrin as a function of PC acyl chain length. The monolayers contained 33 mol% androsterol and 67 mol% PC, the temperature was 25°C and the monolayer lateral surface pressure was 20 mN/m. The final concentration of β-cyclodextrin in the subphase was 0.33 mM. Desorption rate of androsterol from a monolayer of PC 10:0 was arbitrarily set to 100% (corresponding to an androsterol desorption rate of 2.8 nmol/cm^2/min). Values are averages ± S.E. from 3–5 separate experiments with each monolayer composition.

sterol from the monolayer to cyclodextrin in the subphase can readily be detected (on-line) and measured quantitatively [19].

When rates of androsterol desorption was determined at 20 mN/m (25°C), the fastest desorption rate was observed from di10PC mixed monolayers, corresponding to about 2.8 nmol/cm^2/min (Fig. 3). As the acyl chain length of the PC increased, the rate of androsterol desorption decreased, and reached the slowest rate in a di14PC mixed monolayer (about 0.6 nmol/cm^2/min). As the chain length increased to 15 and 16 carbons per chain in the PC monolayer, the androsterol desorption rate again increased (Fig. 3). The slow rate observed for androsterol desorption from a di14PC monolayer suggest that the sterol interacted best with this PC, and less favorably with PCs of other chain lengths.

3.3. *Oxidation susceptibility of androsterol in SUVs as a function of PC chain length*

To verify that the apparent hydrophobic match that was observed between androsterol and di14PC in monolayers also existed in bilayer membranes, we prepared small unilamellar vesicles by ethanol injection (to contain 33 or 50 mol% androsterol), and

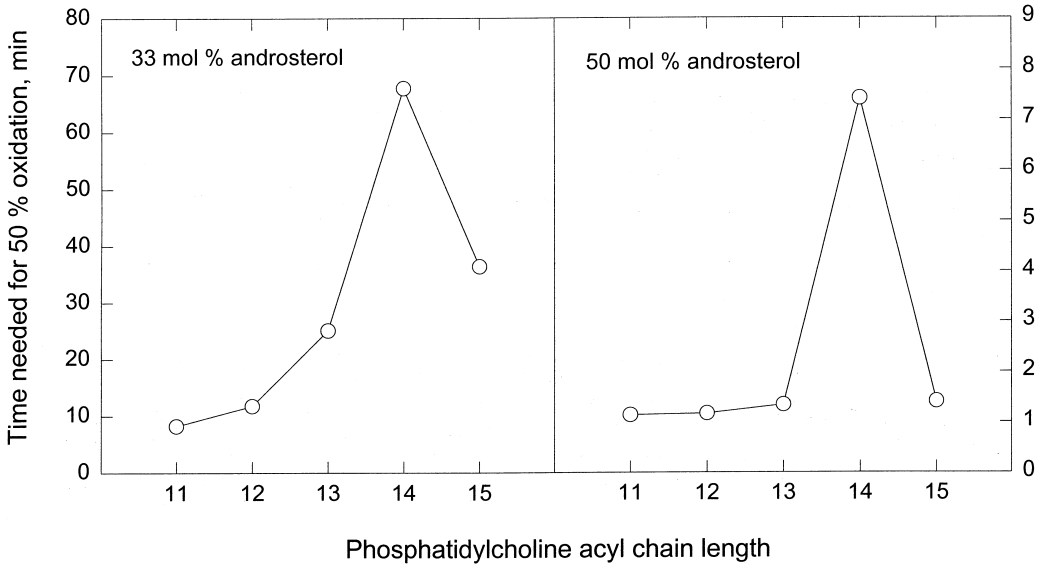

Fig. 4. Oxidation of androsterol by cholesterol oxidase in small unilamellar vesicles as a function of the PC acyl chain length. SUVs containing 33 mol% (left panel) or 50 mol% androsterol (right panel) and one of the PCs were prepared by ethanol injection into phosphate-buffered saline (final lipid concentration 1 mM). The oxidation of androsterol was followed kinetically using fluorescence spectroscopy. Values show the time needed for 50% oxidation, and are averages ± S.E. from three different batches of vesicles at each composition.

measured the rate of androsterol oxidation as catalyzed by cholesterol oxidase. We have previously shown that oxidation rate (or susceptibility) is proportional to the strength of interaction between a 3β-hydroxy sterol and a co-lipid in a model membrane [20,22]. As shown in Fig. 4, the average time needed to accomplish 50% oxidation of androsterol present in the SUVs was longest for di14PC vesicles and significantly shorter for androsterol in vesicles prepared from other PC species. This trend was evident both in vesicles containing 33 and 50 mol% androsterol, although the reaction times were shorter with equimolar vesicles. One can therefore conclude that qualitatively similar results were observed both in monolayer and in bilayer model membrane systems, and further that androsterol apparently interacted most favorably with di14PC compared to other chain length PCs.

4. Discussion

The results presented in this paper clearly show that a match of the hydrophobic length of a sterol and a phosphatidylcholine is important for optimal interaction between the molecular species in model membranes, and that hydrophobic mismatch makes the interaction less favorable. We show for example that androsterol is fully capable of condensing the lateral packing density of di14 and di15 PCs, whereas it is more or less incapable to condense phosphatidylcholines with shorter or longer acyl chains. Previously, neither androsten-3β-ol nor androsten-3α-ol was observed to condense the lateral packing of 1-oleoyl-2-stearoyl-*sn*-glycero-3-phosphocholine [12], whereas a small condensing effect of androsterol on 1-palmitoyl-2-oleoyl-*sn*-glycero-3-phosphocholine monolayers has been reported [13]. The finding that androsterol condenses liquid-expanded PCs selectively (di14 and di15 PCs, but not di10 and di12 PCs or di16PC) suggests that a hydrophobic match is important for the molecular interaction which eventually results in the condensing effect.

As a measure of androsterol/phosphatidylcholine interaction in monolayer membranes, the rate of androsterol desorption was determined. The rate of sterol desorption from model membranes (bilayers or monolayers) is markedly affected by the extent of van der Waals attractive interactions between cholesterol and the acyl chains of the phospholipids. In addition, sterol desorption from model membranes is affected by the phospholipid head group composition [31] and by the aqueous solubility of the desorbing sterol [32,33]. Spontaneous sterol desorption from model membranes is a fairly slow process, with measured half-times being in the range of hours rather than minutes [28,34]. The relatively slow desorption rate for, e.g., cholesterol is related to the fairly high activation energy needed for cholesterol desorption from the donor membranes to an acceptor membrane (20 kcal/mol), which results from the fact that cholesterol (being practically water-insoluble) has to pass trough the water phase on its way from a donor membrane to an acceptor particle [34]. Even though the spontaneous rate of androsterol desorption is significantly faster than the rate measurable for cholesterol, since it lacks cholesterol's iso-octyl side chain, the rate is still very slow. However, the rate of sterol desorption can be increased substantially by using β-cyclodextrins as acceptors, since these apparently can pick up the sterol from the membrane interface, and thus decrease the necessary activation energy for the desorption [35]. We have previously successfully used β-cyclodextrin to effect both cholesterol [19] and fatty acid [36] desorption from monolayers to the subphase, and correlated the desorption rates with intermolecular interactions in the monolayer membranes.

We show here that the rate of androsterol desorption from a mixed monolayer to β-cyclodextrin is markedly dependent on the phosphatidylcholine acyl chain length, with the rate being slowest from di14PC monolayers and faster from shorter or longer chain PCs. This difference in desorption rate is not due to differences in the physical state of the phosphatidylcholine monolayers, since di10PC to di15PC were all liquid-expanded at the surface pressure and temperature of the experiment. Instead, we believe that androsterol interacted most favorably with di14PC and therefore its rate of desorption was slowest from that monolayer. As the phosphatidylcholine chain length decreased below or increased above 14 carbons, the hydrophobic mismatch became more pronounced, and started to affect the strength of androsterol/phosphatidylcholine interaction (weaken-

ing it). This finding is consistent with the DSC results of McMullen and coworkers, who demonstrated that androsterol/di14PC interaction was most favorable (most effective decrease in main transition cooperativity and enthalpy), whereas in longer chain PC bilayers androsterol was less effective at decreasing the cooperativity and enthalpy of the main transition [18].

In addition to measuring androsterol desorption rates from mixed monolayers, we also determined the cholesterol oxidase susceptibility of androsterol in SUVs prepared from different phosphatidylcholines (at 33 mol% sterol). We have previously shown that androsterol is a good substrate of cholesterol oxidase, provided that it is presented to the enzyme in a membraneous form [13]. If androsterol is presented to cholesterol oxidase in a non-membraneous form, it is a much poorer substrate [37]. The oxidation susceptibility of a sterol in a phospholipid membrane is, in part, affected by the strength of intermolecular interactions in the membrane. Cholesterol is oxidized more readily in unsaturated than saturated phosphatidylcholine monolayers, and is also more readily oxidized in phosphatidylcholine monolayers than in acyl-chain matched sphingomyelin monolayers. These differences in oxidation susceptibility has been interpreted to show that cholesterol interacts more weakly with unsaturated than with saturated phospholipids, and more avidly with sphingomyelins than with phosphatidylcholines [20]. This study shows that androsterol is most resistant to oxidation by cholesterol oxidase when it is present in a di14PC SUV. Its oxidation susceptibility is greater in SUVs prepared from shorter or longer phosphatidylcholines. These results match exactly (qualitatively) the desorption results obtained with mixed monolayers, and strongly suggest that androsterol's best match is di14PC of the phosphatidylcholine tested in this study. Recently, we published a report in which we measured the oxidation susceptibility of cholesterol in monolayers prepared from disaturated phosphatidylcholines with chain lengths ranging from 12 to 20 carbons per chain [9]. In that study, cholesterol was most resistant to oxidation in phosphatidylcholine monolayers having chain lengths between 14 and 17 carbons. However, the physical state of the monolayers in that study differed for different phosphatidylcholines, so the interpretation of the results from that study is not straightforward [9].

In conclusion, our condensation results, desorption data, and the oxidation susceptibility data all suggest that androsterol interacted best with di14PC, and that the interaction was weaker with longer or shorter chain phosphatidylcholines. These novel findings, together with previous studies on the effect of the sterol side chain length on sterol/phosphatidylcholine interactions in monolayers [11] and bilayers [8,10,18,38], clearly show that sterol/phosphatidylcholine interactions are greatly influenced by hydrophobic mismatch.

Acknowledgements

This study was supported by grants from the Sigrid Juselius Foundation, the Academy of Finland, the Oskar Öflund Foundation, the Ella and Georg Ehrnrooth Foundation, the Magnus Ehrnrooth Foundation, and the Åbo Akademi University.

References

[1] O.G. Mouritsen, M. Bloom, Biophys. J. 46 (1984) 141–153.
[2] R.C. Cornea, D.D. Thomas, Biochemistry 33 (1994) 2912–2920.
[3] A. Johansson, A.G. Smith, J.C. Metcalfe, Biochim. Biophys. Acta 641 (1981) 416–421.
[4] S.J. Mabrey, J.M. Sturtevant, Proc. Natl. Acad. Sci. USA 73 (1976) 3862–3866.
[5] P.W.M. van Dijck, A.J. Kaper, H.J. Oonk, J. de Gier, Biochim. Biophys. Acta 470 (1977) 58–69.
[6] A.G. Lee, Biochim. Biophys. Acta 472 (1977) 285–344.
[7] J.R. Silvius, D. del Giudice, M. Lafleur, Biochemistry 35 (1996) 15198–15208.
[8] T.P.W. McMullen, R.N.A.H. Lewis, R.N. McElhaney, Biochemistry 32 (1993) 516–522.
[9] P. Mattjus, G. Hedström, J.P. Slotte, Chem. Phys. Lipids 74 (1994) 195–203.
[10] T.P.W. McMullen, C. Vilchèze, R.N. McElhaney, R. Bittman, Biophys. J. 69 (1995) 169–176.
[11] P. Mattjus, R. Bittman, C. Vilchèze, J.P. Slotte, Biochim. Biophys. Acta 1240 (1995) 237–247.
[12] R.A. Demel, K.R. Bruckdorfer, L.L.M. van Deenen, Biochim. Biophys. Acta 255 (1972) 311–320.
[13] J.P. Slotte, Biochim. Biophys. Acta 1124 (1992) 23–28.
[14] B.R. Ladbrooke, D. Chapman, Chem. Phys. Lipids. 8 (1969) 127–133.

[15] B. De Kruyff, R.A. Demel, L.L.M. van Deenen, Biochim. Biophys. Acta 255 (1972) 331–347.
[16] M. Vincent, J. Gallay, Biochem. Biophys. Res. Commun. 113 (1983) 799–810.
[17] L. Senak, D. Moore, R. Mendehlson, J. Phys. Chem. 96 (1992) 2749–2754.
[18] T.P.W. McMullen, R.N.A.H. Lewis, R.N. McElhaney, Biophys. J. 66 (1994) 741–752.
[19] H. Ohvo, J.P. Slotte, Biochemistry 35 (1996) 8018–8024.
[20] J.P. Slotte, Biochemistry 31 (1992) 5472–5476.
[21] R. Verger, G.H. De Haas, Chem. Phys. Lipids 10 (1973) 127–136.
[22] J.P. Slotte, M. Jungner, C. Vilchèze, R. Bittman, Biochim. Biophys. Acta 1190 (1994) 435–443.
[23] J.P. Slotte, A.-L. Östman, Biochim. Biophys. Acta 1145 (1993) 243–249.
[24] L. De Bernard, Bull. Soc. Chim. Biol. 40 (1958) 161.
[25] D. Chapman, N.F. Owens, M.C. Phillips, D. Walker, Biochim. Biophys. Acta 183 (1969) 458–465.
[26] J.M. Smaby, H.L. Brockman, R.E. Brown, Biochemistry 33 (1994) 9135–9142.
[27] R.A. Demel, A.K. Lala, S.N. Kumari, L.L.M. van Deenen, Biochim. Biophys. Acta 771 (1984) 142–150.
[28] R. Bittman, in: L. Finegold (Ed.), Cholesterol in Membrane Models, CRC Press, Boca Raton, FL, 1993, pp. 45–65.
[29] T. Irie, K. Fukunaga, J. Pitha, J. Pharm. Sci. 81 (1992) 521–523.
[30] E.P.C. Kilsdonk, P.G. Yancey, G.W. Stoudt, F.W. Bangerter, W.J. Johnson, M.C. Phillips, G.H. Rothblat, J. Biol. Chem. 270 (1995) 17250–17256.
[31] S. Lund Katz, H.M. Laboda, L.R. McLean, M.C. Phillips, Biochemistry 27 (1988) 3416–3423.
[32] K.R. Bruckdorfer, M.K. Sherry, Biochim. Biophys. Acta 769 (1984) 187–196.
[33] K.R. Bruckdorfer, J. Crowe, M.K. Sherry, Biochim. Biophys. Acta 778 (1984) 489–496.
[34] M.C. Phillips, W.J. Johnson, G.H. Rothblat, Biochim. Biophys. Acta 906 (1987) 223–276.
[35] P.G. Yancey, W.V. Rodrigueza, E.P.C. Kilsdonk, G.W. Stoudt, W.J. Johnson, M.C. Phillips, G.H. Rothblat, J. Biol. Chem. 271 (1996) 16026–16034.
[36] J.P. Slotte, S. Illman, Langmuir 12 (1996) 5664–5668.
[37] A.G. Smith, C.J.W. Brooks, J. Steroid Biochem. 7 (1976) 705–713.
[38] C. Vilchèze, T.P.W. McMullen, R.N. McElhaney, R. Bittman, Biochim. Biophys. Acta 1279 (1996) 235–242.