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Hydrophobic thickness, lipid surface area and polar region hydration in monounsaturated diacylphosphatidylcholine bilayers: SANS study of effects of cholesterol and β-sitosterol in unilamellar vesicles

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ABSTRACT

The influence of a mammalian sterol cholesterol and a plant sterol β-sitosterol on the structural parameters and hydration of bilayers in unilamellar vesicles made of monounsaturated diacylphosphatidylcholines (diCn:1PC, n=14–22 is the even number of acyl chain carbons) was studied at 30 °C using small-angle neutron scattering (SANS). Recently published advanced model of lipid bilayer as a three-strip structure was used with a triangular shape of polar head group probability distribution (Kučerka et al., Models to analyze small-angle neutron scattering from unilamellar lipid vesicles, Physical Review E 69 (2004) Art. No. 051903). It was found that 33 mol% of both sterols increased the thickness of diCn:1PC bilayers with n=18–22 similarly. β-sitosterol increased the thickness of diC14:1PC and diC16:1PC bilayers a little more than cholesterol. Both sterols increased the surface area per unit cell by cca 12 Å^2 and the number of water molecules located in the head group region by cca 4 molecules, irrespective to the acyl chain length of diCn:1PC. The structural difference in the side chain between cholesterol and β-sitosterol plays a negligible role in influencing the structural parameters of bilayers studied.

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1. Introduction

Sterols are ubiquitous components of biological membranes and influence their structural and functional properties [1–3]. Mammalian cells contain one major sterol – cholesterol. In higher plants, sterols are present as complex mixtures. The human body is able to take plant sterols from diet. It has been repoted that plant sterols effectively reduce the absorption of dietary cholesterol by the small intestine and thus protect from cardiovascular diseases [4]. Plant sterols reduce also common cancers including cancers of the colon, breast, and prostate [5,6]. Though the absorption of plant sterols in intestinal tract is very low in comparison with cholesterol (for review see [7]), the molecules of plant sterols incorporate into cellular membranes. Hence it is interesting to know whether the action of plant sterols in membrane is different from that of cholesterol.

One of the predominat plant sterols is β-sitosterol. It differs structurally from cholesterol by the presence of an additional ethyl group at C-24 in the side chain. Besides its anti-atherogenic and anti-cancer activity, β-sitosterol has been reported to have anti-inflammatory, anti-microbial, anti-bacterial and anti-fungal effects (for review see [8]). In the present study, the influence of cholesterol and β-sitosterol on the structure of phosphatidylcholine bilayers is compared. Bilayers of monounsaturated diacylphosphatidylcholines diCn:1PC (n=14–22 is the even number of acyl chain carbons) in unilamellar vesicles are used as a model of the lipid part of biological membranes.

Several authors studied the effect of cholesterol and plant sterols on model membranes. It was shown by means of different experimental methods that β-sitosterol, similarly to cholesterol, broadened the gel-liquid crystal phase transition (main phase transition) of saturated diacylphosphatidylcholines proportional to its bilayer concentration [9–13]. β-sitosterol as well as cholesterol induced sterol-rich and sterol-pure domain creation in diC16:0PC

Abbreviations: diC12:0PC, 1,2-dilauroyl-*sn*-glycero-3-phosphocholine; diC14:0PC, 1,2-dimyristoyl-*sn*-glycero-3-phosphocholine; diC16:0PC, 1,2-dipalmitoyl-*sn*-glycero-3-phosphocholine; diC18:0PC, 1,2-stearoyl-*sn*-glycero-3-phosphocholine; diC14:1PC, 1,2-dimyristoleoyl-*sn*-glycero-3-phosphocholine; diC16:1PC, 1,2-dipalmitoleoyl-*sn*-glycero-3-phosphocholine; diC18:1PC, 1,2-dioleoyl-*sn*-glycero-3-phosphocholine; diC20:1PC, 1,2-dieicosenoyl-*sn*-glycero-3-phosphocholine; diC22:1PC, 1,2-dierucoyl-*sn*-glycero-3-phosphocholine; diC24:1PC, 1,2-dinervonoyl-*sn*-glycero-3-phosphocholine; diC20:4PC, 1,2-diarachidonoyl-*sn*-glycero-3-phosphocholine; C16:0-C18:1PC, 1-palmitoyl-2-oleoyl-*sn*-glycero-3-phosphocholine; C18:0-C20:4PC, 1-stearoyl-2-arachidonoyl-*sn*-glycero-3-phosphocholine; EYPC, egg yolk phosphatidylcholine; SANS, small angle neutron scattering; SLD, scattering length density; TMA-DPH, 1-(4-trimethylammonium-phenyl)-6-phenyl-1,3,5-hexatriene; WALP, model peptides which consist of sequences of alternating alanine and leucine amino acids terminated by a pair of tryptophans at both ends

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bilayers. Both sterols caused a decrease of the main phase transition temperature in the sterol-pure domains where cholesterol was less effective than β-sitosterol. Both sterols increased the main phase transition temperature in the sterol-rich domains. In this case, cholesterol was more effective than β-sitosterol [9]. β-sitosterol with its bulkier side chain was less effective than cholesterol in ordering diC16:0PC acyl chains in fluid bilayers and disturbed the acyl chain packing of diC16:0PC bilayers in the gel state to a greater extent than cholesterol as shown using the fluorescence probe TMA-DPH [12]. The dynamic behavior of fluorescence probes located in different parts of the diC16:0PC bilayer was influenced similarly by cholesterol and β-sitosterol [14]. The lower ability of β-sitosterol to order the acyl chains of phosphatidylcholines in fluid bilayers was also shown for diC16:0PC [11] and for diC14:0PC/soybean phosphatidylcholine mixture [15]. On the other hand, β-sitosterol was more effective in reducing water permeability of soybean phosphatidylcholine bilayers than cholesterol [16]. Cholesterol [17] and β-sitosterol [7] interacted similarly with several different phosphatidylcholines in Langmuir monolayers at the air–water interface. Both sterols formed 1:1 complexes with diC16:0PC and diC18:1PC and 1:2 complexes with diC18:0PC, but complexes with cholesterol were more stable compared to β-sitosterol because the side chain of β-sitosterol was a steric hindrance decreasing the strength of the phytosterol–phosphatidylcholine interaction. Cholesterol is completely soluble in diC16:0PC bilayers at 50 mol% in contrary to β-sitosterol that created a crystalline structure within bilayer [13]. In Langmuir monolayers, both cholesterol [17] and β-sitosterol [7] were fully miscible with diC16:0PC, diC18:1PC and diC18:0PC up to 90 mol%.

A small-angle neutron scattering (SANS) study of cholesterol influence on the structural parameters of diCn:1PC bilayers ($n=14$, 18 and 22) was published recently [18]. In the present paper, we extend this study on the whole homologous series of diCn:1PC from $n=14$ to $n=22$ and compare the influence of cholesterol and β-sitosterol at sterol's content 33 mol%. Effects of sterols on the bilayer structure are characterised through the changes of transbilayer thickness (d_{TOT}) and the hydrophobic thickness (d_{HC}) of the bilayer, the lateral area (A_{UC}) of unit cell consisting of the phospholipid and a particular fraction of sterol, and the number of water molecules (N_W) per one phospholipid located inside the head-group region.

2. Materials and methods

Monounsaturated diacylphosphatidylcholines diCn:1PC were purchased from Avanti Polar Lipids (Alabaster, USA). Cholesterol and β-sitosterol were from Sigma-Aldrich (Germany) and heavy water (99.98% ^2H_2O) was obtained from Isotec (Matheson, USA). The other chemicals were obtained from Slavus (Bratislava, Slovakia). Organic solvents were redistilled before use. Weighted amounts of diCn:1PC, cholesterol and β-sitosterol were dissolved in chloroform. Appropriate volumes of diCn:1PC and sterol solutions were mixed in glass test tubes to achieve the molar ratio sterol:diCn:1PC = 0.5 (33 mol% of sterol). The solvent was evaporated to dryness under a stream of pure gaseous nitrogen, followed by evacuation in a vacuum chamber. Heavy water was added so that diCn:1PC + sterol concentration was 10 g/l. The tube was flushed with pure gaseous nitrogen and sealed with Parafilm M (American National Can, Greenwich, USA). The diCn:1PC + sterol mixtures in ^2H_2O were dispersed in nitrogen atmosphere by hand shaking, brief sonication in a bath sonicator and by vortexing. Unilamellar vesicles were prepared by the dispersion extrusion through polycarbonate filter (Nuclepore, Plesanton, USA) with pores of 50 nm diameter, using the LiposoFast Basic extruder (Avestin, Ottawa, Canada) fitted with two gas-tight Hamilton syringes (Hamilton, Reno, USA) as described in [19]. The samples were subjected to 51 passes through the filters at room temperature. An odd number of passes were performed to avoid contamination of the sample by large and oligolamellar vesicles, which might not have passed through the filter. The samples thus prepared were filled into 2 mm quartz cells (Hellma, Müllheim, Germany), closed and stored at room temperature. The maximum period between the sample preparation and its measurement was 5 h.

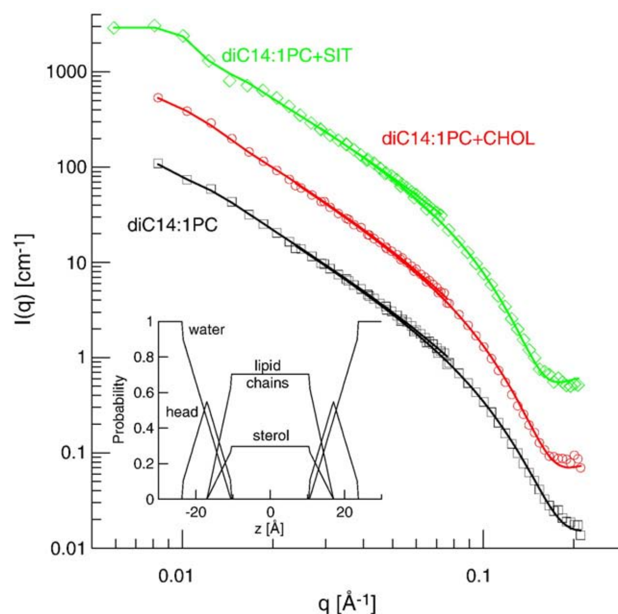

Fig. 1. Experimental SANS data obtained from unilamellar vesicles prepared from pure diC14:1PC bilayers □ and those containing 33 mol% of cholesterol (○) and of β-sitosterol (◊). Scattering curves are shifted vertically for clarity of presentation. Solid lines correspond to the best fits as obtained using the model described in Material and methods. The volume probability distributions is shown in the inset as a dependence on the distance from the bilayer centre *z*.

The SANS measurements were performed on the PAXE spectrometer located at the end of the G5 cold neutron guide on the Orphée reactor (Laboratoire Léon Brillouin, CEA Saclay, France). The experiments were performed with sample to detector distance of 1.77 and 5.07 m and the neutron wavelength of $\lambda=0.6$ nm. The sample temperature was set and controlled electronically at 30.0±0.1 °C. The acquisition time for one sample was 30 min.

The normalized SANS intensity $I_{\exp}(q)$ in cm^{-1} units as a function of the scattering vector modulus $q=4\pi\sin\theta/\lambda$, where 2θ is the scattering angle, was obtained as described in detail in [20]. An example of data is shown in Fig. 1, together with the best fits as obtained using the advanced evaluation model of lipid bilayer as a three-strip structure with a triangular shape of polar head group probability distribution [21]. In particular, scattering intensity for a polydisperse system of spherical vesicles has the form

$$I(q)=\int_R G(R)\cdot\left[4\pi\int_{R-d/2}^{R+d/2} r^2\Delta\rho(r)\frac{\sin(qr)}{qr}dr\right]^2 dR, \quad (1)$$

where r is the distance from the centre of a vesicle with radius R, $G(R)$ is the size distribution function (represented by the Schulz distribution), and $d=d_{TOT}$ is the bilayer thickness. The contrast of the scattering length density (SLD) between the bilayer and water is denoted by $\Delta\rho(r)$ and is expressed as a sum of SLD weighted probabilities

$$\Delta\rho(r)=\sum_i SLD_i\cdot P_i(r)-SLD_W. \quad (2)$$

In accordance to our model, the bilayer consists of three distinct components (see the inset to Fig 1). The probability distribution of water penetrating the lipid headgroup region is, inside this region, described by a linear function

$$P_W(r)=-kr+c_2. \quad (3)$$

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Table 1
Volumetric data used in the SANS

V_{CH}	V_{CH2}	V_{CH3}	V_{HEAD}	$V_{CHOLESTEROL}$	$V_{SITOSTEROL}$	V_{WATER}
22.3 Å^3	27.7 Å^3	53.5 Å^3	331 Å^3	633 Å^3	681 Å^3	30 Å^3

The headgroup itself is represented by the triangular shape formed by linear function

$$P_H(r) = kr + (1 - c_2) \quad (4)$$

throughout the outer part of headgroup region, and by

$$P_H(r) = -kr + (1 - c_2 - c_1) \quad (5)$$

throughout the inner part of this region. The remaining hydrocarbon part is modeled such that the total probability is equal to unity at each point across the entire bilayer. It is assumed that the sterol's probability profile is identical (scaled according to the particular concentration) to the hydrocarbon profile (Fig. 1, inset). The coefficients of probability distributions k, c_1, and c_2 then define the structural parameters characterised by the lateral area A_{UC} (unit cell comprised of the phospholipid and a particular fraction of sterol), number of water molecules N_W localized inside the unit cell, and volumetric information on water molecules V_W, headgroup region V_H, and hydrocarbon region V_{HC}

$$k = \frac{2A_{UC}N_W V_W}{(V_H + N_W V_W)^2} \quad (6)$$

$$c_1 = -2k\frac{2V_{HC} + V_H + N_W V_W}{2A_{UC}} \quad (7)$$

$$c_2 = k\frac{V_{HC}}{A_{UC}}. \quad (8)$$

Finally, due to the spatial conservation principle, these bilayer structural parameters are related through the headgroup region thickness d_H and hydrocarbon region thickness d_{HC}

$$A_{UC} = \frac{V_H + N_W V_W}{d_H} = \frac{V_{HC}}{d_{HC}}, \quad (9)$$

where the thicknesses are defined according to Gibb's dividing surface criterion (see reference [21] for more details).

The molecular volume of cholesterol was taken from [22] and that of ^2H_2O from [23]. β-sitosterol differs structurally from cholesterol in one additional ethyl group. The molecular volume of β-sitosterol was calculated as a sum of molecular volume of cholesterol plus 48 Å^3 which is the difference between the molecular volumes of 3-ethylnonane and *n*-nonane [24]. The fragmental volumes of diCn:1PC were taken from [25]. The volumetric data used in the analysis are summarized in Table 1. The model's mathematical description then allows to evaluate the lateral area A_{UC} and the number of water molecules in the head-group region N_W while the head-group region thickness is constrained to d_H = 10 Å [21]. The total thickness d_{TOT} as well as the hydrophobic thickness d_{HC} of the bilayer are obtained from A_{UC} and N_W using the volumetric data in Table 1. The method of SANS data evaluation is described extensively in [21].

3. Results and discussion

Structural parameters of phospholipid bilayers composed of diCn:1PC in unilamellar vesicles were determined. As expected, the total bilayer thickness of the sterol-free bilayers increased with increasing number of carbon atoms in the acyl chain (Fig. 2). The linear fit of the $d_{TOT}=f(n)$ dependence yields the equation d_{TOT} = (18.6 ± 1.8) + (1.7 ± 0.1)·n (in Å) which is in very good agreement with data in [26]. The hydrophobic thickness d_{HC} of diCn:1PC bilayers is summarized in Table 2. Good agreement between the present results and those of [18] and [27] can be seen.

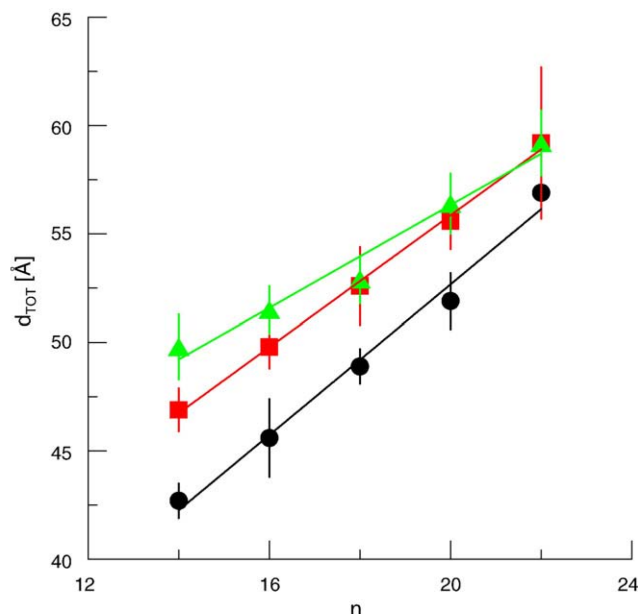

Fig. 2. The dependence of the bilayer thickness d_{TOT} on the number n of diCn:1PC acyl chain carbon atom. ● sterol-free diCn:1PC, ■ cholesterol:diCn:1PC = 0.5 mol/mol, ▲ β-sitosterol:Cn:1PC = 0.5 mol/mol.

The value of d_{HC} is important for the interaction between membrane proteins and lipid bilayer. Most of membrane proteins have one or more hydrophobic segments that span the membrane in an α-helix conformation. Studies of pore-forming proteins have shown that the hydrophobic mismatch between the thickness of hydrophobic part of the bilayer d_{HC} and the length of hydrophobic transmembrane segment of the protein is sufficiently energetic to give rise to alterations in the structure and function of the protein ([28] and references therein). Both positive and negative mismatch may drive oligomerization, especially in the case of small, single-span peptides or proteins ([29] and references therein). If the hydrophobic length of a helix is longer than the bilayer thickness, the helix may tilt to keep much of its hydrophobic part inside the bilayer [30,31]. On the other hand, the tilt of the peptide helix with respect to the bilayer normal is modulated not only by the hydrophobic mismatch. Molecular dynamics simulation study [32] on model peptides (WALP) with different length of transmembrane segment and terminated by a pair of tryptophans, incorporated into lipid bilayer, showed that the tryptophan arrangement around the helical axis plays important role. The conductance of a large conductance Ca^{2+}-activated K^+ channel in bilayers formed from diCn:1PC (*n* = 14–24) was maximal at a chain length of *n* = 18 and much reduced for both thinner (diC14:1PC) and

Table 2
Hydrophobic thickness d_{HC} in unilamellar vesicles of diCn:1PC

n	d_{HC} [Å][a]	d_{HC} [Å][b]	d_{HC} [Å][c]
14	11.4 ± 0.4	11.9 ± 0.5	11.5
16	12.8 ± 1.0		13.25
18	14.5 ± 0.4	14.7 ± 0.3	15
20	16.0 ± 0.7		16.75
22	18.3 ± 0.4	18.5 ± 0.7	

[a] Present study.
[b] Data taken from [18].
[c] Data taken from [27].

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thicker (diC24:1PC) bilayers, indicating that hydrophobic membrane thickness can modify the function of the channel [28]. The dependence of hydrolytic activity of Na,K-ATPase reconstituted into vesicles of diCn:1PC (n = 14–22) on the fatty acyl chain length was observed, with optimum activity at n = 22 [33]. Similar study of sarcoplasmic reticulum Ca-transporting ATPase reconstituted into vesicles of diCn:1PC (n = 14–22) displayed maximal specific activity at C18:1PC and decreased progressively for both shorter and longer acyl chains lengths (see [26] and references therein). The data in Table 2 can be used when studying the effects of hydrophobic bilayer thickness on membrane proteins and peptides quantitatively.

The influence of two sterols, cholesterol and β-sitosterol, on the structural parameters of phospholipid bilayers composed of diCn:1PC in unilamellar vesicles was studied at molar ratio sterol:diCn:1PC = 0.5. According to [7,13], we suppose that both sterols are fully miscible with phosphatidylcholines at this molar ratio. Bilayers composed of diCn:1PC form fluid disordered phase at 30 °C (for review see [25]) while the presence of 33 mol% of cholesterol induces liquid-ordered phase [34]. As can be seen from Fig. 2, 33 mol% of cholesterol causes an increase of d_{TOT} in all phosphatidylcholine bilayers studied. Cholesterol thickens the diC14:1PC bilayer by cca 4.2 Å and that of diC22:1PC by cca 2.3 Å. The linear fit of the $d_{TOT}=f(n)$ dependence yields the equation $d_{TOT}=(26.4\pm0.5)+(1.5\pm0.03)\cdot n$ (in Å). The increase in the bilayer thickness in the presence of cholesterol is slightly less steep than in sterol-free diCn:1PC bilayers. Similar influence of cholesterol on the diCn:1PC bilayers, where n = 14, 18 and 22, was observed in [18].

It was shown that the presence of cholesterol in lipid bilayer influences the function of transmembrane proteins. For example, the optimum of hydrolytic activity of Na,K-ATPase reconstituted into vesicles of diCn:1PC (n = 14–22) was shifted from acyl chain length 22 to 18 after inclusion of 40 mol% cholesterol [33]. This result was explained by the cholesterol induced increase in the hydrophobic thickness of diC18:1PC bilayers to the hydrophobic thickness of pure diC22:1PC bilayers. The ability of cholesterol to increase the bilayer thickness of "shorter" phosphatidylcholines in fluid phase is well known also from other studies. For diC14:0PC in the fluid phase, Pencer et al. [35] observed a concentration dependent thickening of lipid bilayer at 20 and 47 mol% of cholesterol. McIntosh [36] reported

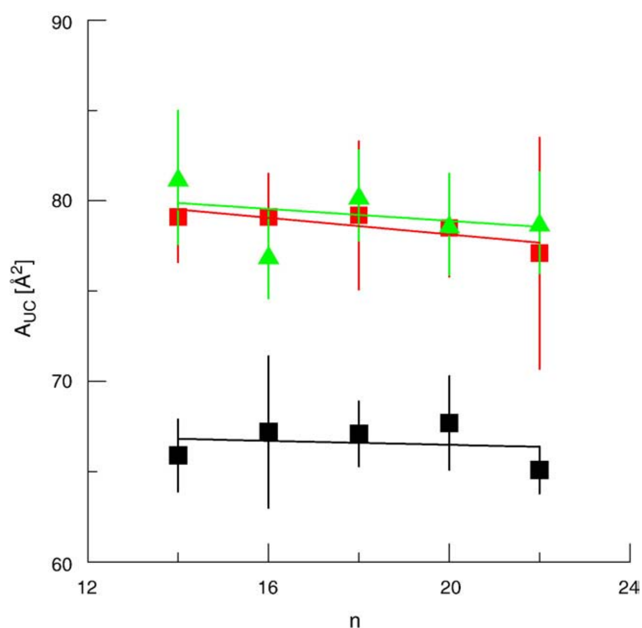

Fig. 3. The dependence of the surface area per unit cell A_{UC} on the number n of diCn:1PC acyl chain carbon atom. ● sterol-free diCn:1PC, ■ cholesterol:diCn: 1PC = 0.5 mol/mol, ▲ β-sitosterol:Cn:1PC = 0.5 mol/mol.

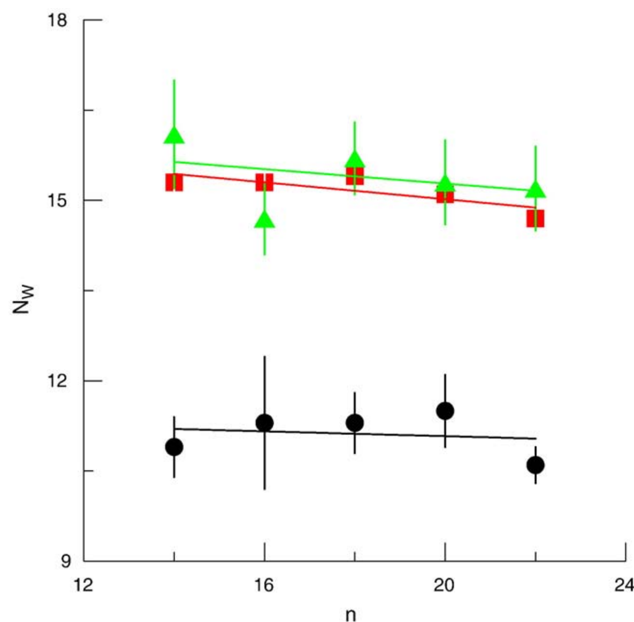

Fig. 4. The dependence of the number of water molecules N_W located in the head-group region per one diCn:1PC molecule on the number n of diCn:1PC acyl chain carbon atom. ● sterol-free diCn:1PC, ■ cholesterol:diCn:1PC = 0.5 mol/mol, ▲ β-sitosterol:Cn:1PC = 0.5 mol/mol.

that cholesterol increases the bilayer thickness for saturated phosphatidylcholines containing 12–16 carbons per chain, as it increases the *trans*-conformations of the chains in fluid phase. As published in our earlier papers, 44 mol% of cholesterol increased the thickness of diC12:0PC [37] and diC14:1PC [38] bilayer.

The increase in diC18:1PC bilayer thickness in the presence of 33 mol% cholesterol agrees well with 4 ± 1.4 Å [18] and 2.5 ± 1.1 Å [39]. In our earlier paper [37] we also observed a small increase in the thickness of diC18:1PC bilayer in the presence of 44 mol% cholesterol, but this effect was in the range of experimental error. Similar small increase in the range of experimental error was determined in the experiment with diC22:1PC in the presence of 44 mol% cholesterol [38]. However, Kratky–Porod approximation and a simple single-strip model of phospholipid bilayer were used to evaluate the bilayer thickness parameter in both our previous papers [37,38]. In the present paper, we use model with linear distribution of water molecules penetrating into the head group region for more realistic description of water-bilayer interface.

It is commonly accepted [40] that 3β-hydroxyl group of cholesterol locates just below the lipid-aqueous interface and the steroid moiety orients in the hydrophobic interior of membrane with its long molecular axis slightly tilted relative to the bilayer normal. The phospholipid acyl chain carbons in positions 2–10 have been estimated to lie in close proximity to the sterol tetracyclic ring structure. The close proximity of the planar sterol ring system orders the hydrocarbon chains and diminishes *trans*-gauche isomerization about their carbon–carbon bonds. As a consequence, the thickness of the bilayer increases which is confirmed by our results.

The interaction of "longer" monounsaturated diacylphosphatidylcholines with cholesterol has not received much attention yet. Similarly to our results, Kučerka et al. [18] observed an increase in the bilayer thickness of diC22:1PC in the presence of 40 mol% of cholesterol. Harroun et al. [41] confirmed the commonly accepted localization of cholesterol (10 mol%) in bilayers composed of C16:0–C18:1PC, diC18:1PC and C18:0–C20:4PC. Surprisingly, cholesterol hydroxyl group was found sequestered in the bilayer center in diC20:4PC membrane. This change of cholesterol location was attributed to the high disorder of polyunsaturated fatty acyl chains that is incompatible with close proximity of steroid moiety in its usual orientation. The

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"interior" location of cholesterol in diC20:4PC membrane was confirmed by coarse grain model simulation [42] at 10 mol% of cholesterol at 300 K. However, the probability of "interior" position of cholesterol was drastically decreased when phosphatidylcholines with low level of unsaturation, C16:0–C18:1PC or diC18:1PC, were used. The propensity of cholesterol to sit at the bilayer center was recently assigned to the higher disorder and permeability of these bilayers. A combined X-ray scattering and molecular dynamics simulation study [43] has suggested that a dynamic network of hydrogen bonds between cholesterol, lipids and water molecules enables cholesterol to exist in unusual orientations (i.e., bilayer center) in disordered bilayers. We do not suppose the interior location of cholesterol in the amount of 33 mol% in phosphatidylcholine bilayers used in this work.

According to the idea of hydrophobic mismatch between the length of cholesterol and diCn:1PC molecules, one expects opposite effect of cholesterol on the thickness of "shorter-" and "longer-chain" diCn:1PC bilayers. Our results indicate that also other factors, e.g. different position of double bond in different Cn:1PC molecules, may play important role. Róg and Pasenkiewicz-Gierula [44] reported in their molecular dynamics simulation study that cholesterol ring system reaches the same depth in the bilayer as the cis double bond between the C9–C10 carbons of C16:0–C18:1PC. On the other hand, the double bond in diC20:1PC and diC22:1PC used in our study is in the position C11 and C13, respectively. Moreover, according to [44], the localization of cholesterol in phosphatidylcholine bilayer may depend on the lipid's unsaturation — the cholesterol hydroxyl group in C16:0–C18:1PC is shifted by 3 Å closer to aqueous phase than in diC14:0PC bilayers. According to Ramstedt and Slotte [45], the interaction of cholesterol with sphingomyelin is not affected by the length of saturated *N*-linked acyl chain, n = 14–24. Cholesterol interacts favorably with spingomyelins with the long monounsaturated *N*-linked acyl chain (C22:1, C24:1), whereas its interaction (or miscibility) is weaker when the monounsaturated N-linked acyl chain becomes shorter.

The influence of β-sitosterol on the bilayers thickness is similar to that of cholesterol, but there are small differences (Fig. 2). Compared to cholesterol, β-sitosterol appears to induce a slightly greater bilayer thickening in shorter phospholipids, diC14:1PC and diC16:1PC. The result of the linear fit, d_{TOT}=(32.5±2.5)+(1.2±0.1)·n (in Å), suggests that the slope of the $d_{TOT}=f(n)$ dependence in the presence of β-sitosterol is less steep than in the presence of cholesterol.

We are not aware of other studies of the influence of β-sitosterol on the thickness of diacyl monounsaturated phosphatidylcholines. But using different lipid, C16:0–C18:1PC, Hodzic et al. [46] reported that 30 mol% of cholesterol increased the bilayer thickness at 25 °C by cca 7.2 Å while β-sitosterol only by cca 5.3 Å. This difference was less pronounced at 7 °C. Pencer et al. [35] have found that ergosterol and lanosterol increased the bilayer thickness of diC14:0PC in unilamellar vesicles similarly to cholesterol in both gel and fluid phases, but large differences in the influence of these sterols on the thermal area expansion coefficient were observed. Several authors showed that β-sitosterol is less effective than cholesterol in ordering acyl chains of different phosphatidylcholines [7,11,12,15,17]. It is known that the increase in acyl chain order is positively correlated with the bilayer thickness. However, our results, at least those with 'shorter' phospholipids, suggest that the effect of β-sitosterol on the thickness was a little higher than that of cholesterol.

The influence of cholesterol and β-sitosterol on the surface area per unit cell A_{UC} at the bilayer-aqueous interface is shown in the Fig. 3. Unfortunately, a rather large experimental error makes it impossible to resolve a maximum seen earlier at n = 18 for sterol-free bilayers [18,26], although the trend in data seems to be consistent. The effect of both sterols on the A_{UC} is the same in the range of experimental error. Both cholesterol and β-sitosterol increase A_{UC} by cca 12 Å^2 and this effect is not dependent on the acyl chain length. Kučerka et al. [18] observed that 29 mol% and 38 mol% of cholesterol increased A_{UC} of diC18:1PC by 9.9 Å^2 and 13 Å^2, respectively. This coincides with our results. Róg and Pasenkiewicz-Gierula [44] found in molecular dynamics simulation study that 22 mol% of cholesterol increased A_{UC} of C16:0–C18:1PC by cca 6.7 Å^2.

The separation of diCn:1PC molecules in the bilayer caused by sterol molecules is accompanied by increased bilayer hydration. Because the head-group region thickness is constrained, the number of water molecules N_W located in the bilayer polar region is fully determined by the unit cell area, A_{UC} (Eq. (9) in Material and Methods). The presence of cholesterol or β-sitosterol increases N_W from cca 11 found for sterol-free bilayer to 15 water molecules at sterol:diCn:1PC = 0.5 molar ratio (Fig. 4). The increase in the head-group hydration was observed for different phosphatidylcholines in the presence of cholesterol also by other authors [47–49].

In conclusion, we have compared the influence of the mammalian sterol cholesterol and the plant sterol β-sitosterol on the structural parameters of bilayers in unilamellar vesicles made of monounsaturated diacylphosphatidylcholines diCn:1PC. We have found that both sterols increase the bilayer thickness, where β-sitosterol seems to be more effective than cholesterol in the case of diC14:1PC and diC16:1PC bilayers. On the other hand, the structural difference in the side chain of cholesterol and β-sitosterol does not play an important role in influencing the area per diCn:1PC molecule at the water–bilayer interface and the head-group hydration. Further systematic investigation is needed, were the studied lipid bilayer model will be step by step closer to the complex composition of biological membranes, to explain the comprehensive action of different sterols.

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