Biochimica et Biophysica Acta 1848 (2015) 1639–1645

Contents lists available at ScienceDirect

Biochimica et Biophysica Acta

journal homepage: www.elsevier.com/locate/bbamem

The influence of cholesterol precursor – desmosterol – on artificial lipid membranes

Katarzyna Hąc-Wydro [a,*], Karolina Węder [b], Marzena Mach [b], Michał Flasiński [a], Paweł Wydro [b]

[a] Department of Environmental Chemistry, Faculty of Chemistry, Jagiellonian University, Gronostajowa 3, 30-387 Kraków, Poland
[b] Department of Physical Chemistry and Electrochemistry, Faculty of Chemistry, Jagiellonian University, Ingardena 3, 30-060 Kraków, Poland

ARTICLE INFO

Article history:
Received 18 February 2015
Received in revised form 26 April 2015
Accepted 30 April 2015
Available online 7 May 2015

Keywords:
Cholesterol biosynthesis
Desmosterol
Lipid domain
Monolayer

ABSTRACT

The disorders in cholesterol biosynthesis pathway and various diseases manifest in the accumulation of cholesterol precursors in the human tissues and cellular membranes. In this paper the effect of desmosterol – one of cholesterol precursors – on model lipid membranes was studied. The investigations were performed for binary SM/desmo and POPC/desmo and ternary SM/POPC/desmo monolayers. Moreover, the experiments based on the gradual substitution of cholesterol by desmosterol in SM/POPC/chol = 1:1:1 system were done. The obtained results allowed one to conclude that desmosterol is of lower domains promoting and stabilizing properties and packs less tightly with the lipids in monolayers. Moreover, desmosterol probably could replace cholesterol in model membranes, but only at its low proportion in the system (2%), however, at a higher degree of cholesterol substitution a significant decrease of the monolayer stability and packing and alterations in the film morphology were detected. The results collected in this work together with those from previous experiments allowed one to analyze the effect of a double bond in the sterol side chain as well as its position in the ring system on membrane activity of the molecule and to verify Bloch hypothesis.

1. Introduction

Cholesterol is a dominating sterol in mammalian tissues and cells. In simple terms, biosynthesis of this compound is started from acetyl CoA via mevalonate to squalene. Then, in the cyclisation reaction squalene is transformed into lanosterol, which is the first sterol in cholesterol biosynthesis pathway [1,2]. Subsequent reactions lead to the formation of the next cholesterol intermediate products, one of them being zymosterol [2]. Further synthesis of cholesterol occurs via two alternative ways, proposed by Bloch and Kandutsch-Russell, respectively. According to Bloch pathway a direct precursor of cholesterol is desmosterol, while in Kandutsch-Russell conception cholesterol is produced from 7-dehydrocholesterol [2]. Cholesterol biosynthesis involves series of enzymatic reactions and today it is known that any disorders and errors in cholesterol synthesis generate serious malformation syndromes [3,4]. These are connected with the alterations in the level of cholesterol precursors in tissues and membranes. For example Smith–Lemli–Opitz Syndrome (SLOS) is associated with the increase of circulated 7-dehydrocholesterol, desmosterolosis is connected with the increase of desmosterol, while lathosterolosis is related to the accumulation of lathosterol [4–6]. The increase of non-cholesterol sterols concentration in mammalian membranes raises the question on the effect of the precursors on membrane organization. In the foregoing context it is of great importance to define a correlation between the structure of sterol molecule and its domains promoting properties. The latter stimulates the investigations on the effect of sterols on membranes. The membrane activity (that is condensing, ordering and domains promoting properties of sterols) of cholesterol versus its major precursors: lanosterol, zymosterol, desmosterol and 7-dehydrocholesterol was compared in the number of studies on artificial membranes. Despite the years of investigations some principles concerning the membrane activity of cholesterol forerunners are still under elucidation. For example the results obtained for lanosterol [7–12] evidenced that this steroid is of much lower condensing, ordering and domains promoting abilities as compared to cholesterol [7–12]. However, some of the results shown that this sterol is able to alter ordering of lipids, to reduce membrane permeability and to stabilize in some degree the lipids rafts, while the other indicated that its effect on domain formation is rather weak or even no domains were detected in model DOPC/DPPC/lanosterol membranes [7–12]. On the other hand, further precursors in cholesterol biosynthesis pathway, namely zymosterol, desmosterol and 7dehydrocholesterol reveal stronger membrane condensing and ordering effect than lanosterol; they are able to promote domain formation and stabilize rafts in model systems, however in a different extent as compared to cholesterol [7–10,13–16]. As it was evidenced in model bilayer and monolayer membranes, zymosterol membrane activity is weaker as compared to cholesterol [9,16], while 7-dehydrocholesterol is of greater raft-

* Corresponding author. Tel.: +48 12 664 67 97; fax: +48 12 634 05 15.
E-mail address: hac@chemia.uj.edu.pl (K. Hąc-Wydro).

http://dx.doi.org/10.1016/j.bbamem.2015.04.017
0005-2736/

forming properties than cholesterol [14]. As regards desmosterol, its behavior in membranes seems to be not fully clear. Namely, some of the studies proven that the influence of cholesterol and desmosterol on lipid packing in model membranes is very similar [8,14,17], and it was proposed that this sterol may replace cholesterol in membrane and rafts [8]. On the other hand, the other experiments evidenced that the stabilizing effect of cholesterol precursor (i.e. desmosterol) on lipid rafts is much weaker as compared to cholesterol [9] and it promotes domain formation in lower extent, which excluded possibility of the replacement of cholesterol by desmosterol in membrane domains [15]. Also the results on the influence of cholesterol substitution by desmosterol on signaling processes in cells did not provide clear conclusions whether these two sterols can replace each other and fulfill the same functions in membranes and cells. Namely, desmosterol similarly to cholesterol can support the ligand binding function of serotonin1A receptor [18]. However, the presence of desmosterol instead of cholesterol affects the pathway of the insulin signaling in human hepatoma cells [15]. The accumulation of desmosterol in the human organism is connected not only with malformation syndromes (desmosterolosis) but it was also detected in coronary artery disease or various liver diseases [3]. Moreover, the increase of desmosterol level occurs also in tissues and membranes of Alzheimer disease patients [19,20]. Thus, the identification of the similarities/differences in the properties of desmosterol vs cholesterol may be important also to understand the role of sterols in pathogenesis of various human disorders.

In this work we present the results of the monolayers studies aimed at analyzing the effect of desmosterol on membranes. The investigations involved the analysis of the effect of the sterol on sphingomyelin and POPC films as well as on SM:POPC = 1:1 mixture. Moreover, to verify, in what extent cholesterol could be replaced in the model membranes by desmosterol, the experiments based on the substitution of animal sterol (cholesterol) by its precursor (desmosterol) in SM/POPC/sterol = 1:1:1 mixture were performed. Based on the foregoing experiments the properties of desmosterol were analyzed and compared with the properties of cholesterol and zymosterol.

2. Experimental

2.1. Materials and methods

Synthetic sphingomyelin (N-palmitoyl-D-erythro-sphingosylphosphorylcholine, SM), 1palmitoyl-2-oleoyl-sn-glycero-3-phosphocholine (POPC) and desmosterol were purchased from Avanti Polar Lipids Inc., USA. Cholesterol was supplied by Sigma. All the compounds were the products of high purity (99%). Spreading solutions of SM and POPC were prepared in chloroform:methanol 9:1 v/v mixture, while the sterols were dissolved in chloroform. The solvents were purchased from Aldrich (HPLC grade, 99.9%). The concentration of the lipid solutions was of ca. 0.3 mg/ml. Mixtures were prepared from the respective stock solutions and deposited onto water subphase with the Hamilton microsyringe (±2.0 μl). After spreading the films were left for 10 min before the compression was initiated (barrier speed of 20 cm^2/min).

2.1.1. Langmuir trough experiments

The experiments were performed with NIMA (UK) Langmuir trough (total area = 300 cm^2) placed on an anti-vibration table. Surface pressure was measured (±0.1 mN/m) using the Wilhelmy plate made of filter paper (ashless Whatman Chr1) connected to an electrobalance. The subphase temperature (20 °C) was controlled thermostatically (±0.1 °C) by a circulating water system. For the experiments Ultrapure Milli-Q water was used.

2.1.2. Brewster angle microscopy experiments

Brewster angle microscopy experiments were performed with UltraBAM instrument (Accurion GmbH, Goettingen, Germany) equipped with a 50 mW laser emitting p-polarized light at a wavelength of 658 nm, a 10× magnification objective, polarizer, analyzer and a CCD camera. The spatial resolution of BAM was 2 μm. In order to notice anisotropy in the reflected images, the analyzer was rotated to specific angles in the directions opposite from p-polarization.

2.2. The studied monolayers

The experiments were done for desmosterol (desmo), sphingomyelin (SM)/desmosterol, phosphatidylcholine (POPC)/desmosterol mixtures differing in the lipid proportion. Then, the influence of desmosterol on binary SM/POPC = 1:1 films was studied at 33; 50 and 67% of the sterol in the systems (SM/POPC/desmo = 1:1:1; SM/POPC/desmo = 1:1:2 and SM/POPC/desmo = 1:1:4). Finally, in the SM/POPC/cholesterol = 1:1:1 monolayer the animal sterol was gradually substituted by desmosterol.

2.3. Data analysis

To analyze the condensation, miscibility and interactions between molecules in the studied monolayers the mean area per molecule values (A) were estimated from the isotherms as well as excess areas of mixing (A^{Exc}) values were calculated from Eq. (1) [21]

$$A^{Exc} = A - A^{id} \tag{1}$$

where A is the value of mean area per molecule derived from the isotherms at a given surface pressure, where A^{id} is the area corresponding to ideal mixing defined as (Eq. (2)):

$$A^{id} = \sum A_i X_i \tag{2}$$

where A_i is the mean area per molecule for the respective one component films, and X_i is the mole fraction of the respective component in the mixed monolayer. *Ai* calculated from the above equation means a linear combination of the areas of all the respective single components and their molar fractions in the mixtures. From the surface pressure–area (π–A) isotherms recorded upon compression of the monolayers the compression modulus values, were also calculated from Eq. (3) [22]:

$$C_S^{-1} = -A(d\pi/dA) \tag{3}$$

wherein A is the mean area per molecule value at a given surface pressure π.

3. Results

In Fig. 1a, b the surface pressure–area isotherms recorded for the mixtures of desmosterol and sphingomyelin or phosphatidylcholine, respectively, are shown. In the same figure (c, d) the mean area per molecule values taken from the isotherms at various surface pressures are presented. The dashed lines in these figures correspond to the areas resulting from the ideal mixing of the monolayer components (Eq. (2)). Finally in Fig. 2 the excess area per molecule values calculated for the investigated lipid/desmosterol systems at the surface pressure of 30 mN/m are shown. In the same figure, for the comparison, the values obtained for the monolayers composed of SM (or POPC) mixed with cholesterol at the same experimental conditions were presented [16].

The surface pressure–area isotherms for SM and POPC films (in Fig. 1) are in agreement with those published previously [16,23]. The curve for desmosterol is typical for the isotherms for sterols monolayers i.e. it is characterized by a rapid increase of the surface pressure with the monolayer compression and a steep course. This indicates on a high condensation and ordering of sterol film, which corroborates with small values of the mean area per molecule (38.2 A^2/molecule at π = 30 mN/m) and high values of the compression modulus ($C_S^{-1} \approx 700$ mN/m at π = 30 mN/m). As it can be seen, the addition of desmosterol to SM or POPC monolayer causes the shift of the curves to smaller areas and the

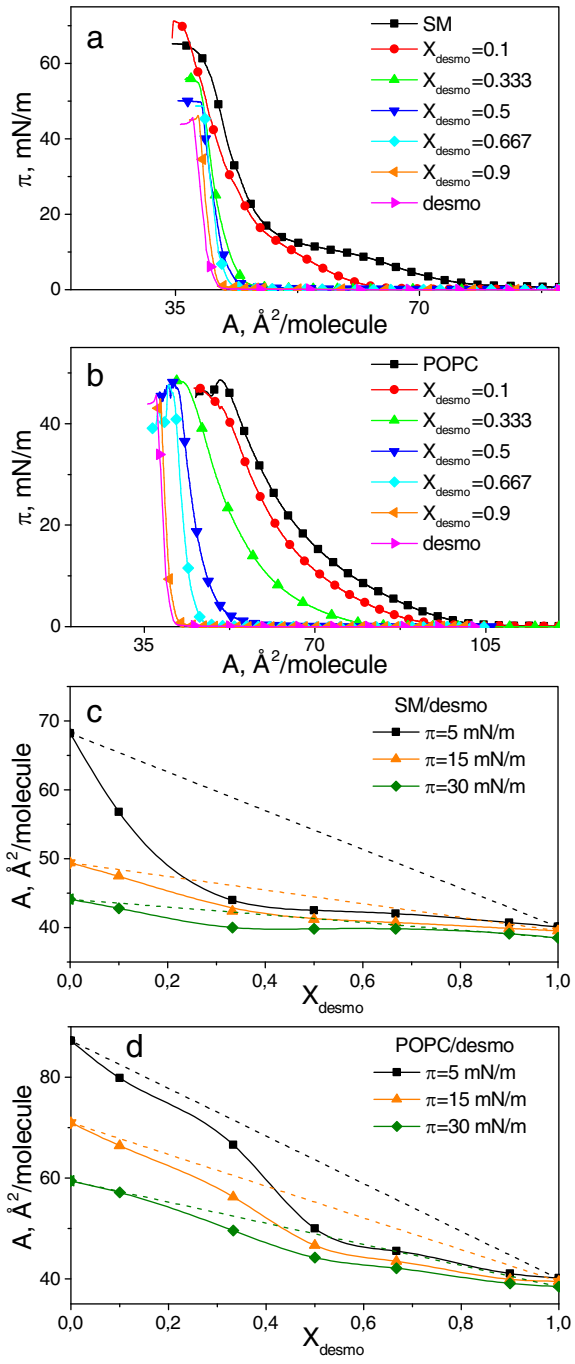

Fig. 1. The surface pressure area isotherms (π–A isotherms) for the mixed monolayers composed of desmosterol and SM and desmosterol and POPC differing in the lipid proportion defined by the molar fraction of sterol (1a, b) and the mean area per molecule values vs the composition plots for these films calculated at various surface pressures.

vanishing of the phase transition clearly observed in the isotherms for sphingolipid monolayer. To analyze the miscibility of desmosterol with the studied lipids the mean area per molecule values at various surface pressures were compared with the values resulting from additivity rule and corresponding to an ideal mixing of the monolayer components.

The obtained results indicate that (Fig. 1c, d) for both studied systems and for all the investigated surface pressures, the mean area per molecule values obtained from the experimental curves are lower than those resulting from additivity rule. The occurrence of these deviations evidences that the studied systems behave nonideally, while their negative character (the negative deviations from ideality) allows

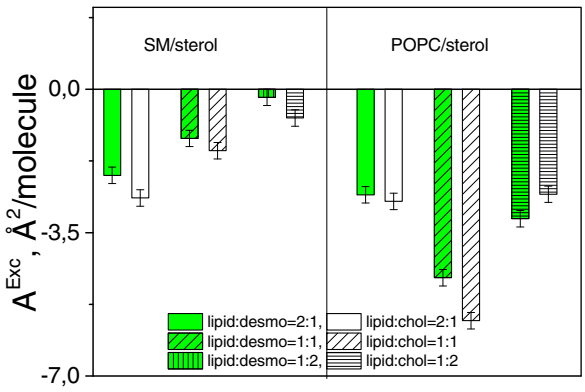

Fig. 2. The comparison of the excess area per molecule values for cholesterol vs desmosterol mixtures with SM and POPC (π = 30 mN/m). The data for SM/cholesterol and POPC/cholesterol mixed films were taken from Ref. [16].

one to conclude that the addition of desmosterol to SM or POPC film causes condensation of the area.

The deviations from ideality observed for the studied binary films (Fig. 1) achieve a minimum at 30% of sterol and 50% of sterol in the system for desmo/SM and desmo/POPC mixtures, respectively. A similar trend was observed previously for the systems containing cholesterol [23,24] and similar systems containing other membrane active sterols [24]. Further addition of sterol does not promote as strong decrease of the mean area per molecule, which is manifested in a decrease of the A^{Exc} values (the values of this parameter beyond the minimum become less negative for both mixed systems). The origin of this behavior is the differences in the structure of phospholipid molecules determining the properties of the respective one-component films. Namely, POPC has one monounsaturated chain with a cis double bond, which causes chain's bending. This limits a tight packing of the molecules and inhibits the effect of sterol as compared to the studied SM having two fully saturated chains. Therefore, POPC film is less condensed and less ordered than SM monolayer, which is confirmed by the larger mean area per molecule values at a given surface pressure and lower compression modulus values for POPC as compared to SM. The presence of sterol molecules causes systematic condensation and ordering of lipids, the changes in the tilt of the chains and reorientation of polar heads in respect to the surface normal, up to the maximum (minimum of A^{Exc}). Then the effect of sterol becomes weaker since the molecules in the monolayer are more tightly packed and ordered, which limits the effect of sterol. The differences in the effect of sterol on lipid films at low and high concentration of steroids were analyzed in details previously [23].

To compare the condensing effect of desmosterol and cholesterol, the excess area per molecule values were calculated (Fig. 2). As it was found, the values of this parameter are lower (more negative) for cholesterol-containing mixtures as compared to desmosterol containing films. During BAM experiments no substantial differences in the morphology of the lipid films containing desmosterol vs cholesterol were observed. As it can be seen in Fig. 3 cholesterol precursor studied herein forms the films, which are highly condensed even at very low surface pressure. A high condensation and ordering is also a characteristic feature of cholesterol monolayers [23,24]. However, based on BAM pictures taken for both sterols at the surface pressure of π = 0 mN/m (the images for cholesterol can be found in Ref. [23,24]) it can be concluded that in this region cholesterol films are more condensed than desmosterol monolayers. During compression of SM/desmo mixed system foam like structures typical for gaseous and fluid phase coexistence, appear initially in the pictures. However, with the increase of the surface pressure a condensed phase can be observed in the images and at ca. 10 mN/m (for SM/desmo = 2:1 mixture) this phase covers the whole interface. For the remaining SM/desmo mixtures the images were similar, however, the surface pressure at which the film becomes condensed

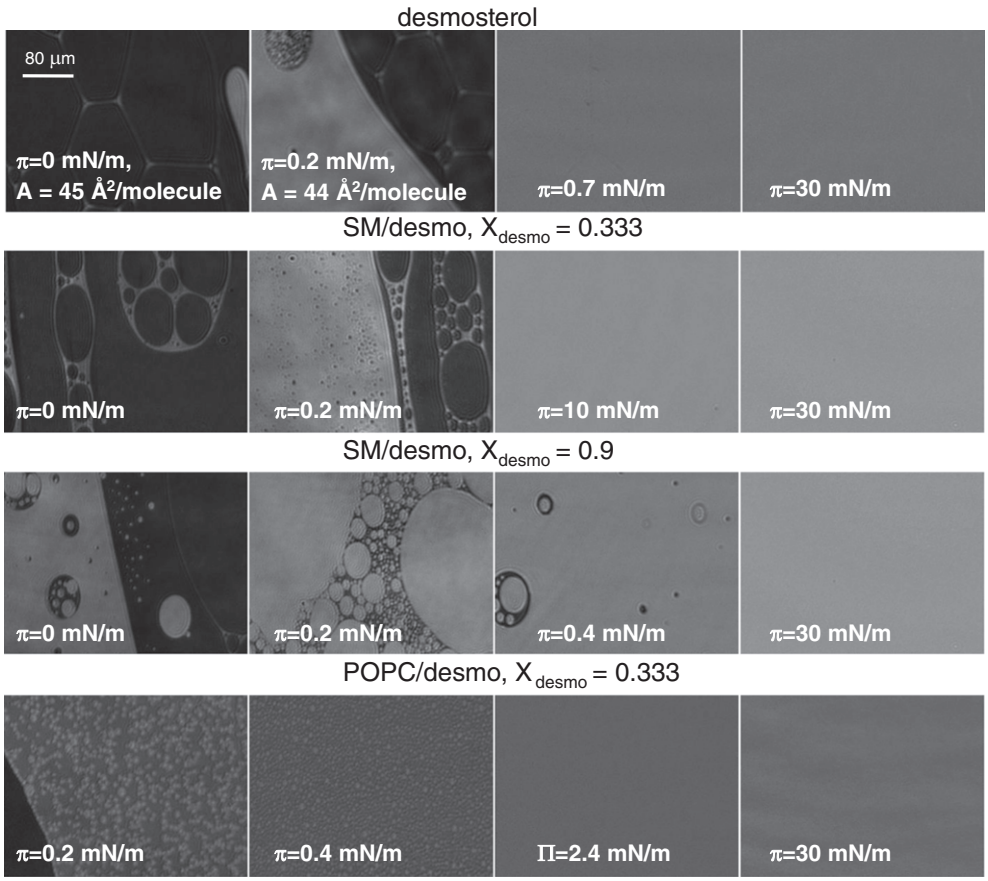

Fig. 3. BAM pictures for selected desmosterol/SM and desmosterol/POPC monolayers.

and fully homogenous decreased with the increase of the sterol fraction in the monolayer. The formation of the condensed phase was also observed after incorporation of desmosterol into POPC monolayer and also this effect with similar to that reported previously for cholesterol-containing films [23]. The addition of desmosterol to SM/POPC = 1:1 monolayer causes the shift of the isotherms to smaller areas, which is similar to the effect of cholesterol [16]. However, the comparison of the excess area per molecule values for SM/POPC/cholesterol and SM/POPC/desmosterol monolayers (Fig. 4b) evidences that cholesterol condenses SM/POPC film more strongly than desmosterol, which manifests in lower A^{Exc} values for the former monolayers. As can be observed in Fig. 4c, the addition of desmosterol to SM/POPC mixture causes also the increase of the compression modulus values. This indicates that the sterol molecules induce a systematic ordering of the lipids in the monolayers. Although the differences in the compression modulus values for SM/POPC/chol and SM/POPC/desmo monolayers are not significant, the values of this parameter are always higher for cholesterol-containing system. Therefore, it can be concluded that also the ordering effect of cholesterol is stronger than the effect of desmosterol. The condensing effect of desmosterol reflects also in BAM pictures recorded for the studied systems. Selected images are shown in Fig. 5, while the pictures for cholesterol-containing films can be found in Ref. [16].

At the initial step of the compression of SM/POPC/desmo = 1:1:1 monolayers the gaseous, fluid and liquid condensed phase coexist. Then, the gaseous phase vanishes and only domains of condensed phase dispersed within fluid matrix are visible. In the surface pressure ranging from 6 to 8 mN/m, the monolayer is homogenous, while at ca. 10 mN/m the small domains appears. These domains exist up to the collapse, however, with the increase of the surface pressure they become brighter. At a first glance these pictures are very similar to those presented previously for SM/POPC/chol = 1:1:1 films. However, some differences should be pointed out. Namely, at a given surface pressure the pictures for cholesterol-containing films are more condensed than those for the desmosterol-containing mixtures, which reflects in a faster (that is at lower surface pressure) formation of condensed phase and vanishing of gaseous and fluid phase. Moreover, condensed domains formed in SM/POPC/chol = 1:1:1 films are larger than those for SM/POPC/desmo = 1:1:1 monolayer.

In the final step of our investigations the effect of the gradual substitution of cholesterol in SM/POPC/chol = 1:1:1 mixture by desmosterol on the monolayer properties was verified. The isotherms recorded for the SM/POPC/sterols = 1:1:1 mixtures differing in the cholesterol to desmosterol ratio are shown in Fig. 6a. Based on the mentioned above results for SM/POPC/chol = 1:1:1 and SM/POPC/desmo = 1:1:1 it can be seen the area per molecule values for cholesterol-containing film are lower (that is the monolayer is more condensed) than those for the remaining system. On the other hand, the compression modulus values for both mixtures are similar in the range of error (ca 180 mN/m). To be able to quantitatively analyze the effect of cholesterol replacement by desmosterol the variations in the A^{Exc} values for these mixtures were calculated (Fig. 6b).

As it can be observed the substitution of cholesterol causes the increase of the A^{Exc} values, which means the decrease of the monolayer condensation. However, it is important to note that this effect is very weak when only 2% of cholesterol is replaced by desmosterol. Moreover, as evidenced in BAM images, at 2% of desmo the morphology of the mixed film does not differ from the film containing cholesterol as the sole sterol. However, further substitution of cholesterol by its precursor, induces much stronger fluidizing and destabilizing effect. This reflects both in a drastic increase of A^{Exc} values as well as in BAM pictures taken for these monolayers (Fig. 5).

Comparing the pictures taken for SM/POPC/chol = 1:1:1 mixture with those recorded for the systems, in which chol was partially (10% or 30%) or totally (SM/POPC/desmo = 1:1:1) substituted by desmosterol

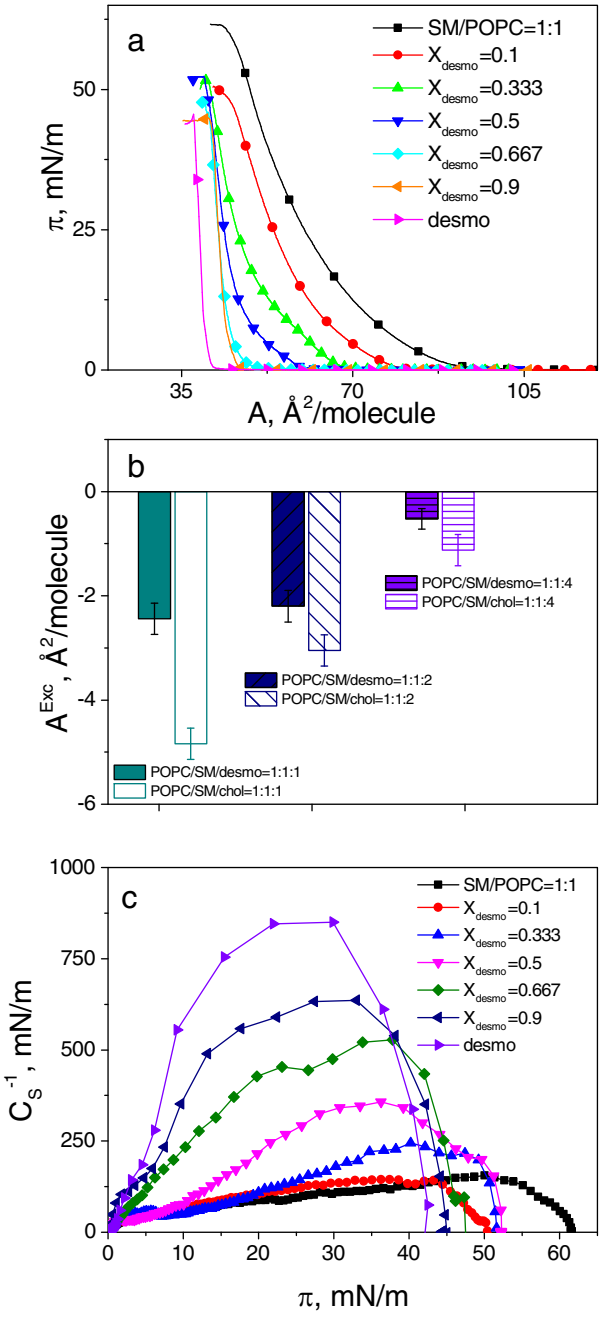

Fig. 4. The π–A isotherms (a), the excess area per molecule at π = 30 mN/m (b) and the compression modulus (c) values for SM/POPC = 1:1 mixed films differing in the content of desmosterol. The data for cholesterol-containing mixed films were taken from Ref. [16].

three characteristic changes caused by desmosterol addition should be noticed. Namely, at low surface pressures (up to 0.5 mN/m) the coexistence of gaseous, fluid and condensed phase can be observed in the presence of desmosterol in the films; the domains formed at higher surface pressures seem to become smaller with the increase of desmosterol in the monolayer, and finally the higher the level of desmo in the system the brighter the domains observed at π ≈ 30 mN/m. This is in contrast to SM/POPC/chol = 1:1:1 monolayer, which is more condensed even at low surface pressures than desmosterol-containing film.

4. Discussion

The results presented herein concerning the investigations on the miscibility and interactions between desmosterol and sphingomyelin or POPC in the mixed monolayers evidenced that the studied cholesterol precursors mix nonideally with both phospholipids. The addition of desmosterol to SM and POPC film causes monolayer condensation and ordering. The strongest contraction of the area occurs at 30% and 50% of sterol in the mixture with SM and POPC, respectively. Similar result, that is the lipid composition of the most stable monolayers found for desmosterol/lipid films, was observed previously for the systems containing cholesterol and other membrane active sterols [23–25]. However, the comparison of these results with those collected previously for cholesterol-containing systems allows one to conclude that desmosterol and cholesterol differ as regards the efficiency in modulating membrane properties. Although the recorded BAM images did not evidence significant differences in the effect of desmosterol vs cholesterol on SM and POPC film, the parameters calculated from the isotherms (that is more negative A^{Exc} values and higher C_S^{-1} values) indicate that cholesterol reveals stronger condensing and ordering properties than desmosterol. Similar conclusions (namely a lower ability of desmosterol to condense and order lipid monolayer) provided the results of our experiments on ternary SM/POPC/desmo systems. As it was found the incorporation of desmosterol into SM/POPC film causes its condensation, which is manifested in the negative values of the excess area per mixing values and this effect is, similar to cholesterol, the most pronounced for 1:1:1 mixture. However, the observed influence is weaker for desmosterol as compared to cholesterol. Also the morphology of the SM/POPC/desmo films analyzed based on BAM images taken at various stages of the compression suggests lower condensation of this monolayer as compared to SM/POPC/chol film. This proves that desmosterol is of weaker membrane activity than cholesterol and shows weaker domains-promoting properties. This is undoubtedly the prerequisite to conclude that desmosterol is not able to totally replace cholesterol in membranes and rafts. However, to verify the foregoing hypothesis, further experiments were performed, in which cholesterol was gradually replaced by desmosterol in SM/POPC/cholesterol = 1:1:1 films. As it was found the substitution of 2% of cholesterol in the foregoing system by desmosterol only slightly increases A^{Exc} values and does not alter the morphology of the studied film. It can be therefore proposed that the properties of these films are similar to the properties of the native monolayer. However, further substitution of cholesterol strongly modifies the properties of the system, that is decreases thermodynamical stability of the monolayer and makes the interactions between the molecules weaker as compared to those existed in SM/POPC/cholesterol mixture. This influence, which was found even at 5% substitution of cholesterol, is reflected in a strong increase of A^{Exc} values. Moreover, the replacement of cholesterol by desmosterol results in a decrease of the monolayer condensation, which was clearly observable in BAM pictures recorded for these systems. Therefore, we conclude that desmosterol can probably replace cholesterol in the model lipid membrane, however, in a very narrow range of concentrations and the replacement of even 5% of cholesterol by its precursor visibly modifies monolayer characteristic. The mentioned above Langmuir monolayer experiments provide the findings, which are in agreement with those published previously [15]. Namely, desmosterol is less effective as a promoter of domain formation and its stabilizer and packs with lipids less tightly than cholesterol. Similar conclusions, that are lower domains promoting abilities of desmosterol vs cholesterol were formulated in the studies by Megha and London [9]. These differences in packing abilities and rafts forming properties of desmosterol vs cholesterol were attributed to the differences in the tilt angles of both molecules [15]. The atomistic simulations evidenced [15] that the tilt of desmosterol in respect to DPPC bilayer membrane normal is 27° while for cholesterol it is of 20°. The differences in the tilt are the consequence of the presence of a double bond in the side chain of desmosterol molecules. In the same studies [15] 70% of cholesterol was depleted by desmosterol in human hepatoma cells (HuH7 cell) and the effect of this modification on insulin raft-dependent signaling was monitored. Based on these experiments it was concluded that desmosterol cannot fulfill the role of cholesterol in lipid rafts since it alters an insulin signaling pathway. On the other hand, the other results

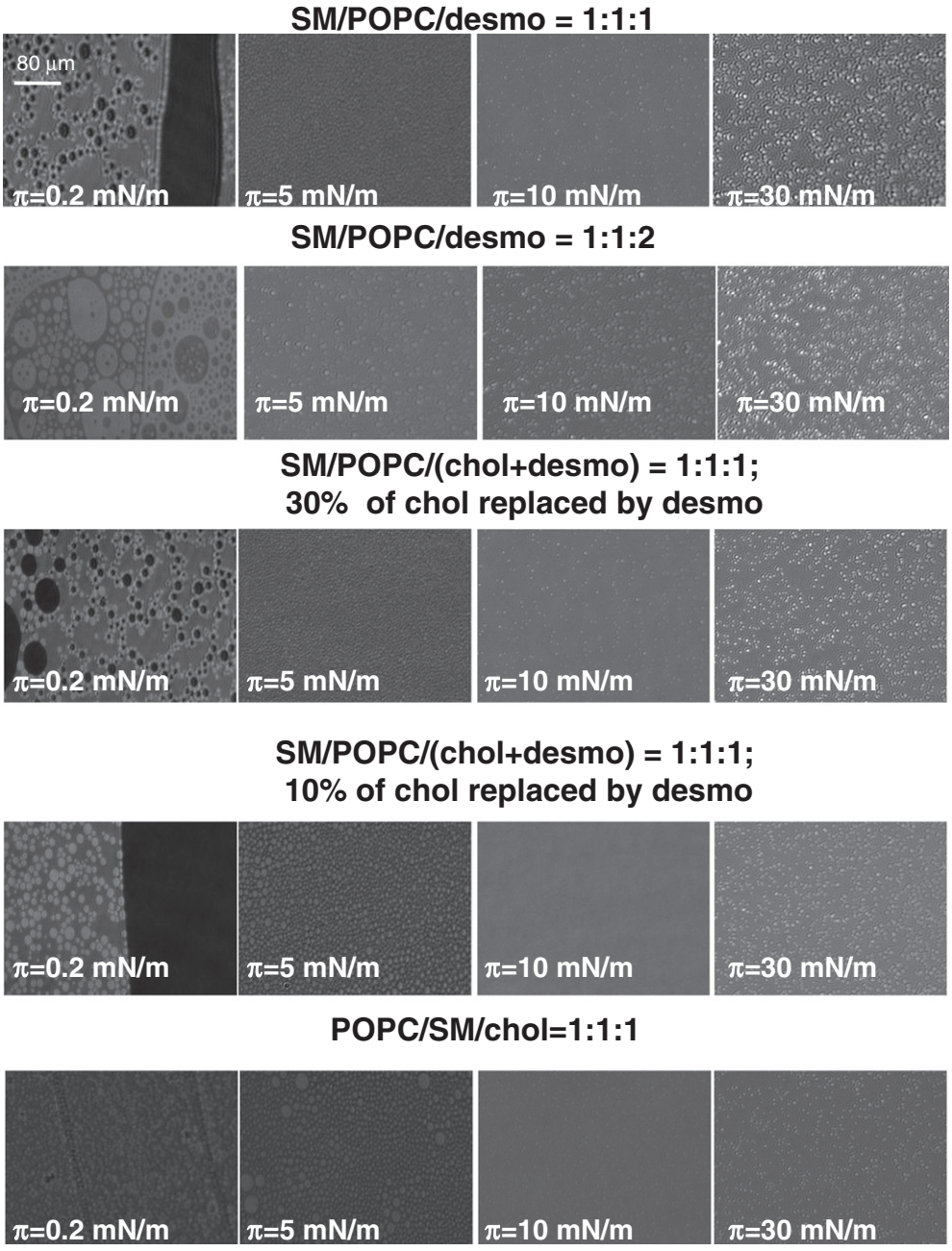

Fig. 5. BAM images for selected ternary POPC/SM/sterol monolayers. The pictures for cholesterol-containing films were taken from Ref. [16].

demonstrate that desmosterol can replace cholesterol for specific ligand binding function of serotonin1A receptor in solubilized membranes [18]. Taken together the results of our experiments and the results presented in the literature it can be only concluded that desmosterol can be in some degree (depending on the concentration and membrane type) able to mimic cholesterol in membranes. Finally, based on the experiments presented in this work as well as those reported previously [16] we are able to compare the effect of cholesterol and its two precursors, namely zymosterol and desmosterol on the mixed lipid films. In the Supplementary Materials (S1) we have collected the results for the respective SM/sterol, POPC/sterol and SM/POPC/sterol monolayers. These results evidenced that both mentioned above cholesterol precursors are able to condense the studied lipid films, which is reflected in the negative deviations from ideality. Interestingly, the condensation of these monolayers measured by the excess area and the mean area per molecule value (Fig. S1) changes progressively from zymosterol to cholesterol. The analysis of A^{Exc} values indicates that, among the investigated sterols, the strongest effect on the lipid monolayer condensation and packing as well as stability exerts cholesterol, while the weakest — zymosterol. Moreover, comparing the results in Fig. S1 a–b more similarities in the behavior of desmosterol to cholesterol than between zymosterol and cholesterol can be found. Namely, the minimum of A^{Exc}, which informs on the strongest area contraction and the stronger intermolecular forced appears at the same monolayer composition for desmosterol and cholesterol (50% for POPC/sterol and 30% for SM/sterol and SM/POPC/sterol monolayers). On the other hand, for zymosterol-containing mixtures this minimum is shifted to larger sterol proportion both for SM and POPC films and it is observed at 70% of sterol in the system. This enables to conclude on much lower condensing efficiency of zymosterol vs the remaining sterols. The foregoing results may prove the Bloch Hypothesis on gradual changes of the membrane properties of sterols in cholesterol biosynthetic pathway [26].

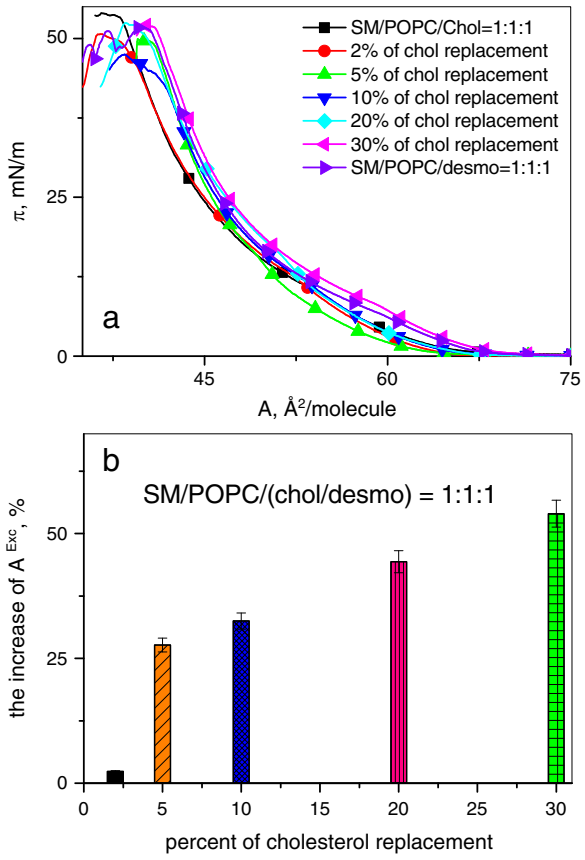

Fig. 6. The surface pressure–area isotherms for SM/POPC/sterols films (a) and the changes in the A^{Exc} values SM/POPC/Chol film caused by cholesterol replacement by desmosterol ($\pi = 30$ mN/m). The data for SM/cholesterol and POPC/cholesterol mixed films were taken from Ref. [16].

Moreover, the comparison of the results of similar experiments for zymosterol [16] and desmosterol enable us to verify the role of the position of a double bond in the steroid ring system. As it was found in the monolayer experiments zymosterol molecules pack less tightly with the lipids in the model membrane than desmosterol. Since these two sterols differ only as regards the position of a double bond in a ring B it can be proposed that the observed differences in the properties of zymosterol and cholesterol results from the foregoing differences in the double bond position. Moreover, the differences in the effect of zymosterol and desmosterol on the properties of SM and POPC monolayers are more pronounced than the differences between desmosterol and cholesterol. Therefore it can be postulated that the position of the mentioned above double bond more significantly affects the membrane activity of sterol than the structure of a side chain in desmosterol vs cholesterol molecule.

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.bbamem.2015.04.017.

Conflict of interest

The authors declare no conflict of interest.

Acknowledgement

This work was financed by grant Iuventus Plus (decision number: 0514/IP3/2013/72) from the Ministry of Science and Higher Education.

References

[1] M.I. Gurr, J.L. Harwood, K.N. Frayn, Lipid Biochemistry, 5th edition Wiley-Blackwell, 2002.
[2] D.E. Vance, J.E. Vance (Eds.), Biochemistry of Lipids, Lipoproteins and Membranes, 4th editionElsevier Science B.V., 2002
[3] J.A. Brown, E. Ikonen, V.M. Olkkonen, Cholesterol precursors: more than mere markers of biosynthesis, Curr. Opin. Lipidol. 25 (2014) 133–139.
[4] F.D. Porter, G.E. Herman, Malformation syndromes caused by disorders of cholesterol synthesis, J. Lipid Res. 52 (2011) 6–34.
[5] H.R. Waterham, J. Koster, G.J. Romeijn, R.C.M. Hennekam, P. Vreken, H.C. Andersson, D.R. FitzPatrick, R.I. Kelley, R.J.A. Wanders, Mutations in the 3β-hydroxysterol Δ24-reductase gene cause desmosterolosis, an autosomal recessive disorder of cholesterol biosynthesis, Am. J. Hum. Genet. 69 (2001) 685–694.
[6] P.A. Krakowiak, Ch.A. Wassif, L. Kratz, D. Cozma, M. Kovárova, G. Harris, A. Grinberg, Y. Yang, A.G.W. Hunter, M. Tsokos, R.I. Kelley, F.D. Porter, Lathosterolosis: an inborn error of human and murine cholesterol synthesis due to lathosterol 5-desaturase deficiency, Hum. Mol. Genet. 12 (2003) 1631–1641.
[7] M.E. Beattie, S.L. Veatch, B.L. Stottrup, S.L. Keller, Sterol structure determines miscibility versus melting transitions in lipid vesicles, Biophys. J. 89 (2005) 1760–1768.
[8] D. Huster, H.A. Scheidt, K. Arnold, A. Herrmann, P. Muller, Desmosterol may replace cholesterol in lipid membranes, Biophys. J. 88 (2005) 1838–1844.
[9] O. Megha Bakht, E. London, Cholesterol precursors stabilize ordinary and ceramide-rich ordered lipid domains (lipid rafts) to different degrees, J. Biol. Chem. 281 (2006) 21903–21913.
[10] B.L. Stottrup, S.L. Keller, Phase behavior of lipid monolayers containing DPPC and cholesterol analogs, Biophys. J. 90 (2006) 3176–3183.
[11] X. Xu, E. London, The effect of sterol structure on membrane lipid domains reveals how cholesterol can induce lipid domain formation, Biochemistry 39 (2000) 843–849.
[12] T. Róg, M. Pasenkiewicz-Gierula, I. Vattulainen, M. Karttunen, Ordering effects of cholesterol and its analogues, Biochim. Biophys. Acta 1788 (2009) 97–121.
[13] T.J. LaRocca, P. Pathak, S. Chiantia, J.R. Silvius, J.L. Benach, E. London, Proving lipid rafts exist: membrane domains in the prokaryote *Borrelia burgdorferi* have the same properties as eukaryotic lipid rafts, PLoS Pathog. 9 (5) (2013) e1003353.
[14] S. Shrivastava, Y. Devi Paila, A. Dutta, A. Chattopadhyay, Differential effects of cholesterol and its immediate biosynthetic precursors on membrane organization, Biochemistry 47 (2008) 5668–5677.
[15] S. Vainio, M. Jansen, M. Koivusalo, T. Róg, M. Karttunen, I. Vattulainen, E. Ikonen, Significance of sterol structural specificity, J. Biol. Chem. 281 (2006) 348–355.
[16] K. Hąc-Wydro, P. Wydro, M. Flasiński, The comparison of zymosterol vs cholesterol membrane properties—the effect of zymosterol on lipid monolayers, Colloids Surf. B: Biointerfaces 123 (2014) 524–532.
[17] A.B. Serfis, S. Brancato, S.J. Fliesler, Comparative behavior of sterols in phosphatidylcholine-sterol monolayer films, Biochim. Biophys. Acta 1511 (2001) 341–348.
[18] P. Singh, M. Jafurulla, Y.D. Paila, A. Chattopadhyay, Desmosterol replaces cholesterol for ligand binding function of the serotonin1A receptor in solubilized hippocampal membranes: support for nonannular binding sites for cholesterol? Biochim. Biophys. Acta 1808 (2011) 2428–2434.
[19] J. Drzewioska, Ł. Pułaski, M. Soszyński, G. Bartosz, Seladin-1/DHCR24: a key protein of cell homeostasis and cholesterol biosynthesis, Postepy Hig. Med. Dosw. (Online) 63 (2009) 318–330.
[20] N.B. Javitt, Alzheimer's disease: neuroprogesterone, epoxycholesterol, and ABC transporters as determinants of neurodesmosterol tissue levels and its role in amyloid protein processing, J. Alzheimers Dis. 35 (2013) 441–450.
[21] I.S. Costin, G.T. Barnes, Two-component monolayers. II. Surface pressure–area relations for the octadecanol—docosyl sulphate system, J. Colloid Interface Sci. 51 (1975) 106–121.
[22] J.T. Davies, E.K. Rideal, Interfacial Phenomena, Academic Press, New York and London, 1963.
[23] P. Wydro, S. Knapczyk, M. Łapczyńska, Variations in the condensing effect of cholesterol on saturated versus unsaturated phosphatidylcholines at low and high sterol concentration, Langmuir 27 (2011) 5433–5444.
[24] P. Wydro, Sphingomyelin/phosphatidylcholine/cholesterol monolayers—analysis of the interactions in model membranes and Brewster Angle Microscopy experiments, Colloids Surf. B 93 (2012) 174–179.
[25] K. Hąc-Wydro, P. Dynarowicz-Łątka, The impact of sterol structure on the interactions with sphingomyelin in mixed Langmuir monolayers, J. Phys. Chem. B 112 (2008) 11324–11332.
[26] K.E. Bloch, Sterol structure and membrane function, Crit. Rev. Biochem. 14 (1983) 47–92.