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Sterol affinity for phospholipid bilayers is influenced by hydrophobic matching between lipids and transmembrane peptides

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ABSTRACT

Lipid self-organization is believed to be essential for shaping the lateral structure of membranes, but it is becoming increasingly clear that also membrane proteins can be involved in the maintenance of membrane architecture. Cholesterol is thought to be important for the lateral organization of eukaryotic cell membranes and has also been implicated to take part in the sorting of cellular transmembrane proteins. Hence, a good starting point for studying the influence of lipid–protein interactions on membrane trafficking is to find out how transmembrane proteins influence the lateral sorting of cholesterol in phospholipid bilayers. By measuring equilibrium partitioning of the fluorescent cholesterol analog cholestatrienol between large unilamellar vesicles and methyl-β-cyclodextrin the effect of hydrophobic matching on the affinity of sterols for phospholipid bilayers was determined. Sterol partitioning was measured in 1,2-dilauroyl-sn-glycero-3-phosphocholine (DLPC), 1,2-dimyristoyl-sn-glycero-3-phosphocholine (DMPC) and 1,2-dipalmitoyl-sn-glycero-3-phosphocholine (DPPC) bilayers with and without WALP19, WALP23 or WALP27 peptides. The results showed that the affinity of the sterol for the bilayers was affected by hydrophobic matching. An increasing positive hydrophobic mismatch led to stronger sterol binding to the bilayers (except in extreme situations), and a large negative hydrophobic mismatch decreased the affinity of the sterol for the bilayer. In addition, peptide insertion into the phospholipid bilayers was observed to depend on hydrophobic matching. In conclusion, the results showed that hydrophobic matching can affect lipid–protein interactions in a way that may facilitate the formation of lateral domains in cell membranes. This could be of importance in membrane trafficking.

1. Introduction

Hydrophobic matching between lipid bilayers and protein transmembrane segments has been proposed to influence protein–lipid interactions in cell membranes [1]. This model predicts that the insertions of proteins with transmembrane segments that have shorter or longer hydrophobic lengths than the hydrophobic thickness of the lipid bilayer will distort the lipid bilayer. Indeed, such defects have been observed in model systems (reviewed in [2]). These defects can be minimized by the lateral sorting of lipids and proteins in the bilayer plane. Hence, it is possible that hydrophobic matching may facilitate the formation of lateral lipid–protein clusters in membranes. Support for this comes from studies on peptide incorporation into lipid bilayers, where both the hydrophobic length of the peptide transmembrane helices and the acyl chain length of the bilayer lipids were varied [3,4]. In these studies the incorporation of peptides was observed to be optimal in situations of hydrophobic matching, and no peptide incorporated in situations of extreme hydrophobic mismatch.

Hydrophobic matching between lipid bilayers and protein transmembrane segments may have a modulating role in a number of cellular events, but it seems to be an especially important factor in the trafficking of lipids and proteins from Golgi membranes to the plasma membrane. It is well known that the plasma membrane, due to its lipid composition, is thicker than the Golgi membranes. Correspondingly, the protein transmembrane helices of plasma membrane proteins are on average longer than those in Golgi and endoplasmic reticulum (ER) proteins [5]. Hence, the Golgi membranes are made up of lipids that form bilayers of different thickness, as well as proteins with transmembrane helices of different hydrophobic lengths, i.e. a lipid–protein mixture in which lateral segregation could occur. Such segregation would explain the observed role of transmembrane helical length in membrane protein trafficking [6,7].

Besides the acyl chain length of lipids, also the cholesterol content affects the hydrophobic thickness of phospholipid bilayers. It has been proposed that the increasing amount of cholesterol in the membranes ER<Golgi<plasma membrane could function as a guide when transmembrane proteins are sorted between different cellular membrane compartments [8]. Cholesterol is also an important component of

Abbreviations: CTL, cholesta-5,7,9 (11)-trien-3-beta-ol; DPH-PC, 2-(3-(diphenylhexatrienyl)propanoyl)-1-hexadecanoyl-sn-glycero-3-phosphocholine; K_X, the molar fraction partitioning coefficient; K_R, the relative partitioning coefficient; DLPC, 1,2-dilauroyl-sn-glycero-3-phosphocholine; DMPC, 1,2-dimyristoyl-sn-glycero-3-phosphocholine; DPPC, 1,2-dipalmitoyl-sn-glycero-3-phosphocholine; mβCD, methyl-β-cyclodextrin; LUV, large unilamellar vesicle

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so-called membrane rafts, which have been implied to be important in membrane trafficking [9,10]. Currently, it is thought that nanoscale lateral domains, or membrane rafts, composed of plasma membrane lipids and proteins could form in Golgi membranes, and that these would later merge into larger units through the action of a clustering agent. Finally, plasma membrane aimed transport vesicles would be released, as has been observed in studies in yeast [11]. The nanoscale lateral domains could form when a critical concentration of plasma membrane components is reached. The coexistence of liquid-disordered and liquid-ordered phases was observed in yeast whole cell lipid extracts, and a dependence on sphingolipid sterol interactions was suggested [12].

Recent work has shown that the partitioning of transmembrane peptides into cholesterol enriched phospholipid bilayers is dependent on the hydrophobic matching between the lipid bilayer and the hydrophobic length of the peptides [13]. This suggests that proteins with suitably long transmembrane helices could partition into membrane raft-like domains, and thereby be inserted into plasma membrane aimed transport vesicles. Alternatively, the transmembrane proteins could also act as a driving force for the formation of nanoscopic domains by collecting select lipids around them, as predicted in the lipid shell model [14]. Evidence for the shell model has been obtained with at least transmembrane peptides that mimic the transmembrane helices of proteins [15,16].

Recently, the sterol affinity for phospholipid bilayers containing two different peptides, KALP23 or WALP23, was determined. In the study WALP23 increased, whereas KALP decreased, the affinity of the sterol for the bilayer [15]. Since KALP23 is known to have a shorter effective hydrophobic length than WALP23, the authors concluded that the effects of the two peptides on the sterol's affinity for the bilayers were linked to hydrophobic matching, and that a positive mismatch has a positive effect, and a negative mismatch a negative effect on sterol affinity. To verify this we conducted a series of new experiments, where both the length of the transmembrane peptides and the bilayer thickness were varied to obtain detailed information on the role of hydrophobic matching in sterol partitioning. The results show that both peptide partitioning into phospholipid bilayers (in agreement with previous reports [3,4]), and the sterol affinity for the bilayers depended on hydrophobic matching between transmembrane helices and lipid bilayers. This suggests that proteins too can contribute to the formation of lateral sterol-enriched domains in cell membranes.

2. Materials and methods

2.1. Materials

All phospholipids were purchased from Avanti Polar lipids (Alabaster, AL) and cholesterol and methyl-β-cyclodextrin (mβCD) from Sigma/Aldrich (St. Louis, MO, USA). Peptides were obtained from Genscript Corp. (Piscataway, NJ). The peptides used in this work have this composition Ac-GWW(LA)$_n$LWWA-NH2, in which n was 6 in WALP19, 8 in WALP23 and 10 in WALP27. Cholesta-5,7,9-trien-3 beta-ol (CTL) was synthesized and purified as described in [17]. Stock solutions of phospholipids were made by dissolving the lipids in hexane:2-propanol (3:2 v:v). The concentrations of the phospholipid stock solutions were determined according to [18]. Peptide stock solutions were made by dissolving the peptides in trifluoroethanol, and the concentration were determined from the absorption at 280 nm. The stock solution were stored at −20 °C and warmed to ambient temperature prior to use. The water used was purified by reverse osmosis through a Millipore UF Plus water purification system. The resistivity of the water was 18.2 MΩcm.

2.2. CTL partitioning between bilayers and methyl-β-cyclodextrin

CTL partitioning between large unilamellar vesicles (LUVs) and methyl-β-cyclodextrin was measured as described previously [15,19]. In short, multilamellar vesicles of DLPC, DMPC or DPPC with 2 mol% CTL with and without peptides (WALP19, WALP23 and WALP27) were prepared. LUVs were prepared from these by extruding the lipid suspension through membranes with 200 nm pores. The LUVs were mixed with cyclodextrin (0–1 mM) and the samples were incubated until the equilibrium distribution of CTL between LUVs and cyclodextrin was reached. The steady-state anisotropy of CTL was measured in samples with different mβCD concentrations and the molar concentration of CTL, C_{CTL}^{LUV}, in the LUVs in each sample was calculated from the measured anisotropies according to

$$C_{CTL}^{LUV} = C_{CTL}\frac{(r_i - r_{CD})}{(r_{LUV} - r_{CD})} \quad (1)$$

where C_{CTL} is the total concentration of CTL in the samples, r_{LUV} is the anisotropy of CTL in the specific phospholipid bilayer, r_i is the CTL anisotropy in the sample and r_{CD} is the anisotropy of CTL in the CTL–mβCD complex. To determine the sterol's affinity for the phospholipid bilayers the molar fraction partition coefficient K_X was calculated. This partitioning coefficient describes the equilibrium partitioning of CTL between LUVs and mβCD (larger K_X equals stronger bilayer affinity). The molar fraction partition coefficient K_X was determined by plotting the calculated molar concentrations of CTL in the phospholipid bilayers against the mβCD concentration and fitting the obtained curves with the following equation

$$C_{CTL}^{LUV} = \frac{C_L - C_{CTL} + (C_{CD})^n/K_X}{2} \times \left(\sqrt{1 + 4\frac{C_L C_{CTL}}{[C_L - C_{CTL} + (C_{CD})^n/K_X]^2}} - 1\right) \quad (2)$$

where C_L is the phospholipid concentration, C_{CD} is the cyclodextrin concentration, C_{CHOL}^{LUV} is the cholesterol concentration in lipid bilayers and C_{CHOL}^{CD} is the concentration of cholesterol in complex with mβCD. The phospholipid concentration was determined after anisotropy measurements in all samples so that the correct concentration was used in the calculations. For this the samples were freeze dried and re-dissolved in methanol, after which the peptide concentration in the samples were determined by measuring absorbance at 280 nm, and the phospholipid concentration was determined according to Rouser et al. [18].

The relative partitioning coefficient K_R was calculated by dividing the K_X obtained from peptide containing samples with the K_X obtained from samples with only PC. To be able to compare results from samples with different peptide concentrations the change in K_R (ΔK_R) per mol% peptide in the samples was used when different WALP peptides were compared.

3. Results

3.1. Equilibrium partitioning of cholestatrienol between mβCD and phospholipid bilayers of different hydrophobic thickness

To vary the hydrophobic bilayer thickness in the experiments vesicles were prepared from PCs with different acyl chain lengths: DLPC, DMPC and DPPC. All experiments were performed at temperatures at which the bilayers were in the liquid disordered state. Before the peptides were added to the samples, the CTL affinity for the bilayers composed of the three different PCs was determined. Fig. 1A shows the measured CTL anisotropy in the samples as a function of mβCD content. As can be seen the CTL anisotropy in samples without mβCD increased with the lipid acyl chain length, but with an increasing cyclodextrin concentration the anisotropy in the samples decreased towards the anisotropy of CTL–mβCD complexes. Using Eq. (1) the amount of vesicle-bound CTL in the samples was calculated, and by fitting the data with Eq. (2), molar fraction partitioning coefficients (K_X) were obtained. Fig. 1B shows the amount of membrane bound CTL and the obtained

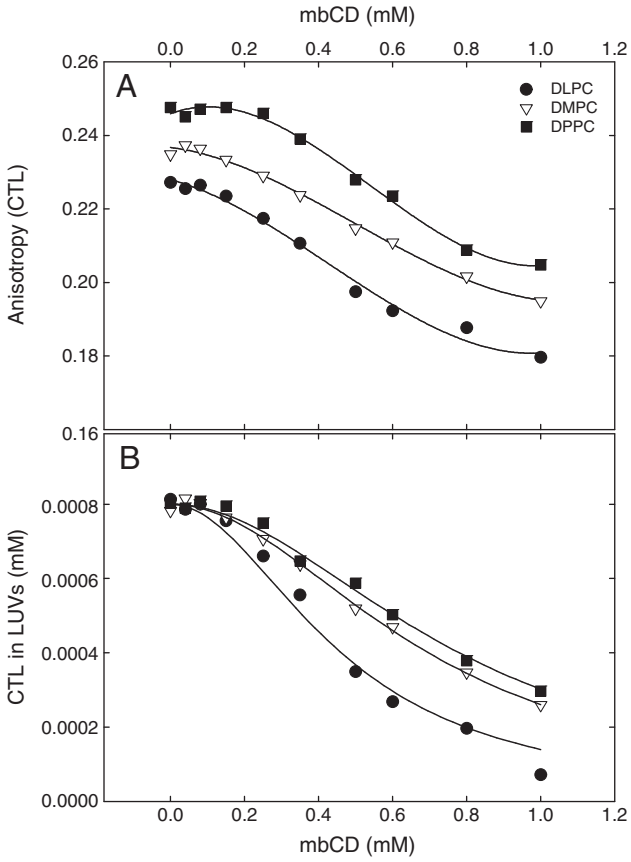

Fig. 1. Cholestatrienol partitioning between mβCD and phospholipid bilayers with different thickness. Panel A shows representative data from one experiment with each phospholipid lipid. The measurements were made at 23 (DLPC), 37 (DMPC) and 50 °C (DPPC). Panel B shows the CTL concentrations in the bilayers as function of cyclodextrin concentration and the curves obtained from fitting the data with Eq. (2).

fits, and the obtained partitioning coefficients are shown in Fig. 2. As expected, CTL partitioned more into bilayers composed of PCs with longer chains. In the current range used the acyl chain length dependence of K_X was linear, and this dependence was used as a ruler to evaluate how the effect of peptides on sterol partitioning corresponded to change in bilayer thickness.

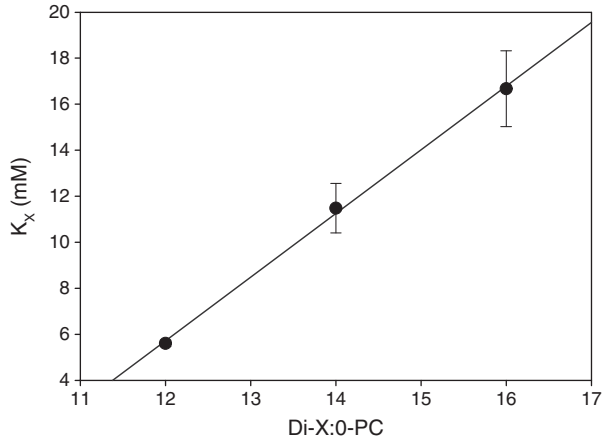

Fig. 2. Cholestatrienol partitioning between mβCD and phospholipid bilayers with different thickness. Molar fraction partitioning coefficients (K_X) determined with fluid DLPC, DMPC and DPPC bilayers at 23, 37 and 50 °C, respectively.

3.2. The effect of peptides with different hydrophobic lengths on cholestatrienol binding to DMPC bilayers

Having characterized the CTL binding to bilayers of different thicknesses we proceeded by studying how three different transmembrane peptides (WALP19, WALP23 and WALP27) influenced the sterol's partitioning between DMPC LUVs and mβCD. All three peptides had the same amino acid composition except that their hydrophobic length was varied by varying the length of the central LA segment. For these experiments samples with 2 or 4 mol% of the different peptides in DMPC were prepared. As it was possible that the actual peptide amounts in the final samples would vary due to different affinity of the peptides for the bilayers, both phospholipid and peptide concentrations in the samples were determined after the partitioning experiments had been performed. This showed that the two shorter peptides inserted much more efficiently into DMPC bilayers than the longer WALP27. This suggests that a too large positive mismatch is unfavorable for peptide insertion into phospholipid bilayers.

The partitioning coefficients that were obtained using the same approach as with peptide free vesicles (see above) are shown in Fig. 3. As can be seen the K_X was increased by all three peptides, but the effect was larger with the longer peptides, and apparently the effect of peptide length increased with peptide concentration.

Having seen how the peptide length affected the sterol partitioning we wanted to relate the effects to the hydrophobic matching situation in the different peptide–lipid systems. Hence, information on the hydrophobic thickness of the bilayer and the hydrophobic lengths of the peptides were needed. For the DMPC bilayer a hydrophobic bilayer thickness of 25.4 Å has been measured at 30 °C [20]. However, as our experiments were performed at 37 °C we needed to correct for the effect of this temperature change. Deuterium NMR has been used to determine the bilayer thickness of DMPC bilayers at temperatures between 30 and 65 °C [21], and as these results were in good agreement with the X-ray results at 30 °C we used the NMR results to approximate the bilayer thickness at 37 °C, and obtained a bilayer thickness of 25.0 Å. The hydrophobic length of WALP peptides has commonly been calculated as 1.5 Å per residue in the hydrophobic LA core, which would mean that the hydrophobic lengths of WALP19, WALP23 and WALP27 would be 19.5, 25.5 and 31.5 Å, respectively. However, these calculations don't take into account the possible contributions of the flanking tryptophan residues. Recently, Strandberg and co-workers approximated the effective hydrophobic lengths of WALP peptides showing that also the tryptophan residues contributed to the hydrophobic length of the peptides [22]. Taking the tryptophan contribution

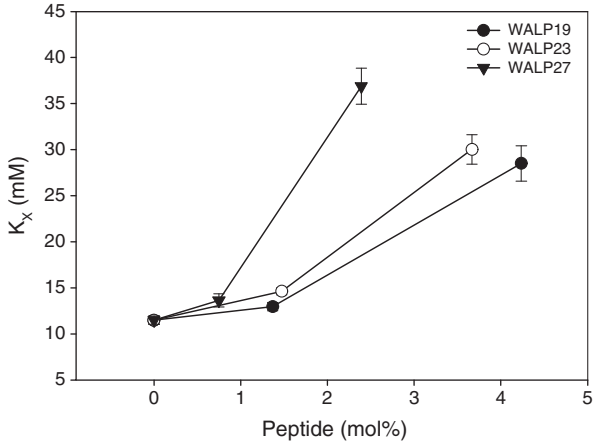

Fig. 3. Effect of peptide length on cholestatrienol partitioning between mβCD and DMPC vesicles at 37 °C. Samples were prepared with 2 or 4 mol% WALP19, WALP23 or WALP27. After the fluorescence measurements the real lipid and peptide concentration in the samples were determined as described in the Materials and methods section. The partitioning coefficients were calculated using the determined concentrations.

into account the effective hydrophobic length of WALP19, WALP23 and WALP27 would be 21.4, 27.4 and 33.4 Å, respectively. As is seems reasonable that the tryptophan residues would contribute to the hydrophobic length, we used these hydrophobic lengths to validate our partitioning results. The bilayer thicknesses of all bilayers and the effective hydrophobic lengths of all peptides used in this study are summarized in Table 1 together with the calculated hydrophobic mismatch for all peptide–lipid combinations.

Based on the approximated hydrophobic thickness of DMPC bilayers and the effective hydrophobic lengths of the peptides, WALP19 would be negatively mismatched, while WALP23 would be slightly positively mismatched and WALP27 clearly positively mismatched with the DMPC bilayers. As all peptides still increased CTL binding to the bilayer it seems that the amount of negative mismatch that WALP19 induces is not enough to lead to a decrease in the affinity of the sterol for the bilayer.

3.3. The effect of hydrophobic matching on sterol partitioning between mβCD and phospholipid bilayers

To gain more insight into how hydrophobic matching affects sterol affinity for phospholipid bilayers, we carried out equilibrium partitioning experiments with the same peptides in DLPC and DPPC bilayers. Samples were prepared with 2 mol% WALP19, WALP23 or WALP27 in DLPC and DPPC bilayers, and the actual lipid and peptide content in the samples was determined after the equilibrium partitioning experiments were performed. As the estimated hydrophobic mismatch between the lipid bilayers and peptide transmembrane helices in these samples was relatively large, sucrose gradient centrifugation was carried out to insure that the lipids and peptides formed only one type of lipid–peptide aggregate (results not shown), and that there were e.g. no non-lamellar aggregates, which have previously been reported to form in situations of large hydrophobic mismatch [3].

The results of the CTL partitioning experiments with peptides containing DLPC and DPPC LUVs are shown together with the DMPC results in Fig. 4. As the amount of inserted peptide in the vesicles varied with the hydrophobic matching in the samples the results are shown as the change in the relative partitioning coefficient per mol% peptide in the bilayers. The relative partitioning coefficients were obtained by dividing the partitioning coefficients (K_X) obtained with peptide containing LUVs with the K_X determined in peptide free LUVs composed of the same PC. The peptide concentrations in all samples were between 1 and 2 mol%, except for some of the samples containing WALP27 and DLPC or DMPC in which the peptide concentration was below 1 mol% peptide.

From the results shown in Fig. 4 it is clear that the influence of peptides on CTL binding to the PC bilayers is influenced by hydrophobic matching. The longest peptide (WALP27) has the largest effect in all of the bilayers except in the DLPC bilayers, likely due to the positive hydrophobic mismatch being too large. For all of the peptides the smallest effect on sterol partitioning was observed in DPPC bilayers, probably because the affinity was high in these bilayers already without peptide. In DPPC bilayers the shortest peptide (WALP19) had a negative effect on CTL bilayer binding, i.e. the presence of the negatively mismatched peptide in the bilayers expelled the sterol from the bilayer.

Table 1
Hydrophobic lengths of peptides, hydrophobic bilayer thicknesses and hydrophobic mismatch situations in the used lipid–peptide systems.

Lipid		Peptide		Hydrophobic mismatch (Å)		
Name	Hydrophobic thickness (Å)	Name	Hydrophobic length (Å)	DLPC	DMPC	DPPC
DLPC	21.6	WALP19	21.4	−0.2	−3.6	−6.5
DMPC	25.0	WALP23	27.4	5.8	2.4	−0.5
DPPC	27.9	WALP27	33.4	11.8	8.4	5.5

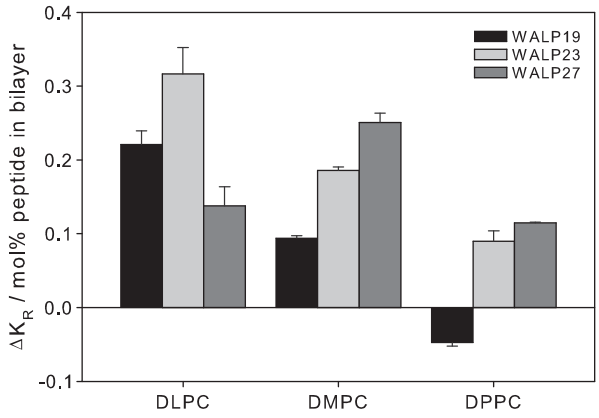

Fig. 4. Hydrophobic matching effects on cholestatrienol partitioning between mβCD and phospholipid bilayers. The results are shown as the change in the relative partitioning coefficient (ΔK_R) per mol% peptide in the membranes. The measurements were made at 23 (DLPC), 37 (DMPC) and 50 °C (DPPC).

3.4. The effect of hydrophobic matching on peptide insertion into phospholipid bilayers

The partitioning experiments showed that the insertion efficiency of the three peptides in different PC bilayers varied. By measuring the peptide and lipid content in the final samples the partitioning efficiency in all lipid–peptide combinations was determined. The results are shown in Fig. 5. Based on the results it seems that WALP19 and WALP23 inserted equally well into all PC bilayers, suggesting that the negative mismatch did not affect peptide insertion. WALP27 on the other hand inserted poorly into DLPC and DMPC but as well as WALP23 and WALP19 into DPPC bilayers. Hence, it seems that the insertion of peptides into bilayers composed of saturated PCs is less effective if the peptide's hydrophobic length is much longer than the bilayer thickness.

4. Discussion

The importance of hydrophobic matching between lipid bilayers and protein transmembrane segments in membrane trafficking was recognized decades ago [8]. Cholesterol's thickening effect on phospholipid bilayers, and the increase in membrane cholesterol content from the ER towards the plasma membrane are thought to guide

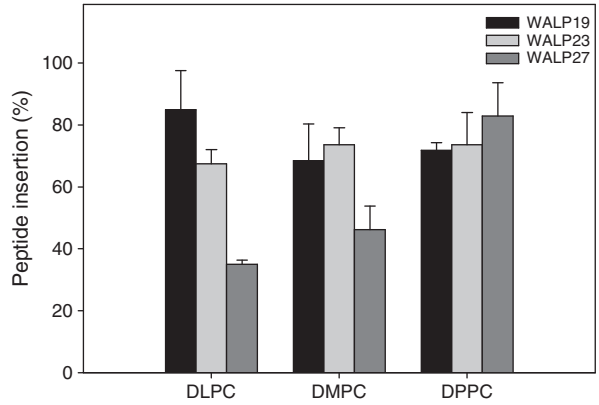

Fig. 5. Peptide insertion into phospholipid bilayers with different hydrophobic thickness. The insertion efficiency of WALP19, WALP23 and WALP27 into DLPC, DMPC and DPPC bilayers was obtained by determining the lipid and peptide content in the samples used in the partitioning experiments.

membrane protein trafficking. Lundbeak and co-workers estimated that the changes in a bilayer's physical properties are sufficient to allow for effective sorting of membrane proteins [23]. Recently, it was reported that the insertion of transmembrane peptides into cholesterol enriched phospholipid bilayers depends on the hydrophobic length of the transmembrane peptides [13]. This suggests that if domains enriched in cholesterol and other plasma membrane lipids form in Golgi membranes, proteins with longer transmembrane helices (i.e. plasma membrane proteins) would partition into these domains, while non-plasma membrane proteins would not.

Should membrane proteins be considered as passive participants that just move along with their surrounding lipids? Based on our current knowledge this seems unlikely. From studies on peptide interactions with lipids it is known that transmembrane peptides influence lipid structure [2], and for example the MAL protein has been reported to induce the formation of platforms enriched in specific lipids [24]. Studies using peptides and MAL suggest that hydrophobic matching is a key factor regulating protein interactions with their surrounding lipids.

Results of a previous study suggested that hydrophobic matching could determine whether sterols are attracted to or expelled from the vicinity of membrane proteins [15]. Hence, the current work was aimed at clarifying, in more detail, how hydrophobic matching affects sterol interactions with protein surrounding phospholipids.

The equilibrium partitioning experiments were done using the fluorescent cholesterol analog cholestatrienol, which in an earlier test partitioned in a relatively similar way to cholesterol in phospholipid bilayers [15]. Compared to cholesterol based approaches cholestatrienol allows the use of low sterol concentrations, which simplifies the interpretation of the results as one can be sure that no sterol enriched phases are formed in the samples.

To evaluate the effect of hydrophobic matching on the affinity of sterols for the bilayers the hydrophobic thickness of the phospholipid bilayers and the effective hydrophobic lengths of the peptides should be known. The hydrophobic thickness of the bilayers that are used in this evaluation is based on the work of Petrache and co-workers [21]. To estimate the hydrophobic lengths of the peptides is less straight forward. In the discussion below we use the effective hydrophobic lengths determined by Strandberg and co-workers [22]. Theoretically, however, the effective hydrophobic length of the peptides could be both longer or shorter than their estimations.

The results from the equilibrium partitioning experiments (summarized in Fig. 4) showed that hydrophobic matching between transmembrane peptides and PC bilayers affected the sterol–PC interactions. Positive mismatch, i.e. when the hydrophobic length of the peptide exceeds the hydrophobic thickness of the bilayer, increased the affinity of the sterol for the bilayer. However, when the positive mismatch was extreme (WALP27 in DLPC) the effect was smaller than when the positive mismatch was moderate. In situations of positive mismatch the peptide surrounding PC bilayers have been shown to become thicker [25], which would explain the higher sterol binding affinity, as the sterol has a higher affinity for thicker bilayers (Figs. 1 and 2). In situations of extreme positive mismatch it is likely that other adaptations to the mismatch decrease in the thickening effect and thereby the peptides do not increase the sterol affinity to the same degree. For example, the peptides may be more tilted or aggregate to a larger extent. In situations with hydrophobic match (WALP19 in DLPC and WALP23 in DPPC bilayers) and in situations of moderate negative mismatch (WALP19 in DMPC) the presence of peptides in the bilayers also increased the sterols' affinity for the bilayer, but not as much as upon positive mismatch. This seems illogical considering that these peptides should not increase the thickness of the bilayer (at least in theory). However, based on deuterium NMR experiments the acyl chain order in PC bilayers is increased also in situations of moderate negative mismatch [26]. According to the NMR data WALP19 significantly affects the acyl chain order in DMPC bilayers, but not in DPPC bilayers. This could explain why the peptide effect on sterol binding was positive in DMPC bilayers but negative in DPPC bilayers, despite the negative mismatch in both bilayers. Hence, it seems that a sufficiently large negative mismatch was needed before the peptides decreased the affinity of the sterol for the bilayer. Alternatively, the effective hydrophobic length of WALP19 is longer than estimated. The hydrophobic length of the KALP23 peptide, which previously was reported to decrease CTL's affinity for DMPC bilayers [15], has been predicted to resemble that of a WALP16 [27]. Hence, the mismatch situation in the KALP23–DMPC system would be a slightly larger negative hydrophobic mismatch than in the WALP19–DPPC bilayers, i.e. the hydrophobic mismatch would be sufficient to decrease the sterol's affinity for the bilayer according to the results in the present study.

When the peptide concentration in the PC vesicles was increased the effect of the peptides on the sterol affinity for the bilayers became larger (Fig. 3). This is in agreement with previously reported data [15]. With WALP27 the effect of an increase in concentration was larger than with WALP23 and WALP19. Possibly, the relatively large positive mismatch in the WALP27–DMPC system may lead to peptide clustering, as this has been reported to occur in such mismatch situations at peptide concentrations higher than 2 mol% [28]. The phospholipids surrounding peptides that are in a cluster should be more affected by the peptides than are lipids next to a single protein. Therefore, sterols would also be expected to interact more strongly with the phospholipids in and around peptide clusters. This is in agreement with molecular dynamics simulation results showing that upon positive mismatch more cholesterol was associated with clustered proteins than with non-clustered proteins [16].

To evaluate the extent of proteins' ability to influence lipid organization in membranes the effect of peptides on CTL partitioning was compared to how the sterol interacted with PC bilayers of different thickness. In experiments with pure PC bilayers increasing the hydrophobic bilayer thickness from 21.6 to 27.9 Å linearly increased the sterols affinity for the bilayer. Using this linear function as a ruler we could evaluate how the effect of the peptides on sterol partitioning compared to an increase in bilayer thickness. With <2 mol% peptide in the bilayers the effect on CTL partitioning resembled a change in bilayer thickness between −0.7 Å (WALP19 in DPPC) and 2.0 Å (WALP27 in DPPC). The largest effect per mol% peptide, 1.6 Å, was observed for WALP27 in DMPC bilayers. With more than 2 mol% peptide in the bilayers the peptide effects on CTL partitioning behavior resembled a change in bilayer thickness of between about 9 and 14 Å. From deuterium NMR experiments the effect of 3.33 mol% WALP23 on the thickness of a DMPC bilayer has been calculated to be about 1 Å [25]. This can be compared to the effect of a similar WALP23 concentration on sterol affinity in DMPC bilayers resembling an increase in bilayer thickness of about 10 Å. Already with about 1.5 mol% peptide the effect resembled ~1.2 Å. This suggests that it is not merely the peptides' effect on bilayer thickness that increases the sterol's affinity for the bilayers. A possible problem in the setup could be that the presence of peptides in the bilayers limits the access of mβCD to the bilayers, which could appear as an increase in the bilayer affinity of the sterol. However, as similar results were obtained in an experimental setup where there was TopFlour-cholesterol partitioning between donor and acceptor LUVs, without cyclodextrin or other sterol transporters (Supplementary data), this does not seem to be the case. Therefore, other explanations have to be found for the large effects of the peptides on the sterol's affinity for the bilayers. One possibility is that CTL is located close to WALP peptides in the bilayers, where the ordering effect of the peptides on the lipid bilayer is largest, and the effect on bilayer thickness is larger than the bulk lipid bilayer thickness, which was measured in the deuterium NMR experiments [25]. However, as the effect on sterol partitioning at higher peptide concentrations resembled an excessive thickening of the bilayer, which could only be explained by closeness to the peptides, we think that the sterol and the peptides may form clusters, at least at higher peptide concentrations. Such

clustering could explain the observed high sterol affinity for the bilayers, based on published molecular dynamic data [16].

When the concentrations of lipids and peptides were determined in the samples it was observed that the insertion efficiency of WALP27 became lower with decreased bilayer thickness (Fig. 5). WALP19 and WALP23 inserted with similar efficiencies into all PC bilayers. Hence, it seems that the insertion of WALP peptides into saturated PC bilayers was hampered by an extreme positive mismatch, while a negative mismatch did not affect peptide insertion, at least in the tested range. In unsaturated PC bilayers negative hydrophobic mismatch has also been reported to decrease the insertion efficacy of WALP peptides [25].

In conclusion, our results showed that hydrophobic matching between lipid bilayers and transmembrane peptides affects the affinity of sterols, like cholesterol, for the bilayer. This suggests that proteins may have an active role in the formation of lateral domains in cell membranes, and together with lipids drive this segregation, that is believed to facilitate the transport of lipids and membrane proteins from the Golgi to the plasma membrane. Membrane proteins could promote the lateral segregation of membrane components both by attracting and repelling cholesterol. Since higher local concentrations of peptides had an enhanced effect on sterol partitioning it is expected that protein clustering, e.g. through interactions with galectines [29], also should increase the impact of the proteins on the lateral membrane structure. Besides influencing the lateral membrane structure hydrophobic mismatch between the transmembrane segments of proteins and lipid bilayers could also influence the distribution of cholesterol in the cell by affecting non-vesicular transport, which is also considered important for sterol distribution within the cell [30].

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Appendix A. Supplementary data

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.bbamem.2012.11.034.

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