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

H. Kristian Ijäs, Max Lönnfors, Thomas K.M. Nyholm *

Biochemistry, Department of Bioscience, Åbo Akademi University, Tykistökatu 6A, FIN-20520 Turku, Finland

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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

* Corresponding author. Tel.: +358 2 2154 272; fax: +358 2 215 4748.
E-mail address: tnyholm@abo.fi (T.K.M. Nyholm).

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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