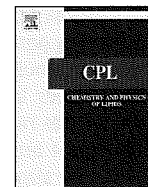




Contents lists available at ScienceDirect

Chemistry and Physics of Lipids

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



On the puzzling distribution of cholesterol in the plasma membrane

H. Giang, M. Schick*

Department of Physics, University of Washington, Seattle, WA 98195, United States

ARTICLE INFO

Article history:

Received 19 November 2015

Received in revised form 4 December 2015

Accepted 9 December 2015

Available online xxx

Keywords:

Cholesterol

Phosphatidylethanolamine

Bending energy

Spontaneous curvature

ABSTRACT

The distribution of cholesterol between the two leaves of the plasma membrane in mammalian cells presents a conundrum; given cholesterol's known affinity for sphingomyelin, which resides predominantly in the exoplasmic leaf, why is it that experiment finds a majority of the cholesterol in the cytoplasmic leaf? This article reviews a recently proposed solution to this puzzle.

© 2015 Elsevier Ireland Ltd. All rights reserved.

1. Introduction

Given the importance of cholesterol in the cell, one would have thought that its distribution between the two leaves of the plasma membrane would not only be well-known, but also well-understood. There is a certain amount of agreement now on what the distribution is, but little understanding on why it is what it appears to be. To clarify the problem, one should recall some basic information. First it is well known that cholesterol can translocate rapidly between the two leaves of the plasma membrane (Lange et al., 1981; Muller and Hermann, 2002; Steck and Lange, 2002). Thus one would assume that it reaches an equilibrium distribution in which its chemical potentials in the two leaves are the same. Second the interaction between cholesterol and sphingomyelin (SM) is known to be particularly favorable (Niu and Litman, 2002) being due to that between the rigid rings of the former and the saturated chains of the latter (Epanand and Epanand, 2004; Zheng et al., 2007). Third, almost all of the sphingomyelin is in the outer, exoplasmic, leaf of the plasma membrane (Devaux, 1991). It would then seem to follow inexorably that the majority of cholesterol would be found in the outer leaf; its chemical potential there would be negative due to the favorable interaction with SM, while its chemical potential in the inner leaf would be equally negative due to its entropy at low concentration. That this conclusion does follow from the chain of reasoning finds support from molecular dynamics simulations (Perlmutter and Sachs, 2011; Polley et al., 2012).

While the reasoning may be impeccable, the conclusion is not borne out by several experiments on human erythrocytes (Muller and Hermann, 2002; Blau and Bittman, 1978; Lange and Slayton, 1982; Lange, 1984; Brasaemle et al., 1988; Schroeder et al., 1991), Chinese hamster ovaries, (Mondal et al., 2009), and synaptic plasma membranes (Wood et al., 1990; Igbavboa et al., 1996). All uniformly agree that cholesterol is *not* found predominantly in the exoplasmic leaf. A similar uniformity does not exist as to what the distribution of cholesterol actually is. Some report that cholesterol is rather evenly divided between the two leaves (Blau and Bittman, 1978; Lange and Slayton, 1982; Lange, 1984; Muller and Hermann, 2002), while others find that it is predominantly found in the inner, cytoplasmic, leaf (Brasaemle et al., 1988; Schroeder et al., 1991; Wood et al., 1990; Igbavboa et al., 1996; Mondal et al., 2009). The experiments do not measure native cholesterol directly, but employ various cholesterol analogs labeled in some way. Thus the difference in measured distribution may be due to the different analogs employed. Nonetheless it is clear that cholesterol is not found predominantly in the outer leaf where the sphingomyelin is, hence something must be drawing it to the inner leaf. But what? That is the puzzle.

We recently proposed a solution to it as follows (Giang and Schick, 2014): Phosphatidylethanolamine is one of the major components of the inner leaf of the plasma membrane comprising some 25% of the phospholipids there (Devaux, 1991). Because of its small head group, phosphatidylethanolamine (PE) has a tendency to form inverted hexagonal phases in water. For palmitoyloleoylphosphatidylethanolamine, that phase becomes stable around a temperature of 70 °C. Thus, when it is confined to a bilayer, there must be some bending energy penalty for this confinement.

* Corresponding author.

We proposed that cholesterol could relieve in two ways this bending energy penalty by going to the cytoplasmic leaf. First of all, its presence would simply dilute that of the PE. Of course that is true of any other lipid as well, but cholesterol is the only one which translocates rapidly between leaves. Secondly it is well known that cholesterol tends to order the tails of lipids. Hence it might well reduce the spontaneous curvature of the PE lipids and thereby further reduce the bending energy penalty of confining PE to a bilayer. If the above reasoning were correct, then one would expect that the addition of cholesterol to a system of PE and water would stabilize the lamellar phase of PE and therefore increase the transition temperature from the lamellar phase to the inverted hexagonal phase. That experiment has been done (Epan and Bottega, 1987). On the initial addition of cholesterol to a PE and water system, the transition temperature decreases; that is, the inverted-hexagonal phase is stabilized. Presumably this is due to the cholesterol filling the energetically expensive interstitial voids between cylinders. But with further addition of cholesterol to concentrations which are comparable to those in the plasma membrane, the transition temperature does indeed increase. Thus sufficient cholesterol does stabilize the lamellar phase of PE in water.

In order to make these qualitative ideas quantitative, we considered a very simple and standard model for the free energy of the system, namely regular solution theory. We take the system to consist of two leaves. There are N_{SM} molecules of SM, N_{C_0} of cholesterol, and N_{PC} molecules of phosphatidylcholine (PC) in the outer layer, and N_{PE} molecules of PE, N_{C_i} molecules of cholesterol, and N_{PS} molecules of PS phosphatidylserine (PS) in the inner layer. The area per lipid of all lipids is taken to be $a = 0.7 \text{ nm}^2$ except for cholesterol which is taken to be $a_c = 0.4 \text{ nm}^2$. We require the areas of each leaf to be the same,

$$(N_{SM} + N_{PC})a + N_{C_0}a_c = (N_{PE} + N_{PS})a + N_{C_i}a_c = A.$$

It is convenient to define the mol fractions of the components in the outer leaf, $y_{SM} = N_{SM}/(N_{SM} + N_{C_0} + N_{PC})$, etc. and similarly for the mol fractions in the inner leaf. In terms of these mol fractions, the regular solution free energy can be written

$$F_{bi} = N_0 f_0(y_{SM}, y_{PC}, y_{C_0}, T) + N_i f_i(y_{PE}, y_{PS}, y_{C_i}, T),$$

$$f_i = 6\epsilon_{PS,PE} y_{PS} y_{PE} + 6\epsilon_{PS,C} y_{PS} y_{C_i} + 6\epsilon_{PE,C} y_{PE} y_{C_i} + k_B T (y_{PS} \ln y_{PS} + y_{PE} \ln y_{PE} + y_{C_i} \ln y_{C_i}),$$

$$f_0 = 6\epsilon_{SM,PC} y_{SM} y_{PC} + 6\epsilon_{SM,C} y_{SM} y_{C_0} + 6\epsilon_{PC,C} y_{PC} y_{C_0} + k_B T (y_{SM} \ln y_{SM} + y_{PC} \ln y_{PC} + y_{C_0} \ln y_{C_0}).$$

where N_0 and N_i are the total numbers of molecules in the outer and inner leaves respectively, and $\epsilon_{PS,PE}$ is the free energy of interaction between the PS and PE. We have assumed an average of six nearest-neighbor interactions per molecule. These interactions are estimated from experiment (Almeida, 2009; Giang and Schick, 2014). From the free energy, the chemical potential of cholesterol in the inner and outer leaves can be obtained. The six unknown mol fractions are then obtained from the following six conditions: the chemical potentials of cholesterol in both leaves are equal to one another, the mol fractions in the outer leaf sum to unity, as do the mol fractions in the inner leaf, the ratios of the mol fractions of SM to PC in the outer leaf are set to be 1.1 and of PS to PE in the inner leaf to be 0.52, values essentially given by experiment. Finally the total mol fraction of cholesterol in the system is taken to be fixed at its experimental value, 0.4 (van Meer, 2011). Once the mol fractions are calculated, it is straightforward to obtain the fraction of the total cholesterol which is in each leaf.

If we follow this program with the above simple model, we find that only 25% of the total cholesterol is in the inner leaf. This is not surprising, and reflects the reasoning we laid out in Section 1: cholesterol interacts favorably with SM, almost all the SM is in the outer leaf, hence that is where most of the cholesterol should be. So the simple model vindicates the impeccable reasoning, and confirms that it is missing something essential. Consequently we add the bending energy to it.

The bending energy can be written as

$$F_b = \int d^2r \frac{\kappa}{2} (H(r) - H_0)^2, \quad (1)$$

where κ is the bending modulus of the bilayer, $H(r)$ is the actual local curvature of the system, and H_0 is the preferred, or spontaneous, curvature of the membrane due to its composition. This expression assumes that there is no intrinsic difference in energy to bend the bilayer toward the cytoplasmic side or the exoplasmic side. Because we take the membrane to be flat, $H(r) = 0$. We assume that the local spontaneous curvature of the bilayer is a weighted sum of the spontaneous curvatures of its components. However the only component in the system which has a spontaneous curvature of significant magnitude is PE (Kollmitzer et al., 2013), for which $H_{PE} = -0.316 \text{ nm}^{-1}$. Thus this energy reduces to

$$F_b = A \frac{1}{2} \kappa y_{PE}^2 H_{PE}^2, \\ = \frac{1}{2} \left[N_i + N_0 - \left(1 - \left(\frac{a_c}{a} \right) \right) (N_{C_0} + N_{C_i}) \right] \frac{1}{2} \kappa y_{PE}^2 H_{PE}^2.$$

Note that this term is quadratic in the mol fraction of PE and is positive; i.e. the bending energy is a cost. In this way, the bending energy acts as a repulsive interaction between the PE molecules or, equivalently, an attractive interaction between PE and all of the other kinds of molecules. The cholesterol, however, is the only lipid in the outer leaf that can readily respond to this attraction by translocating to the inner leaf where it dilutes the PE. Because the majority of experiments have been done on erythrocytes, we take the bending modulus to be $\kappa = 44 k_B T$, that obtained from measurement on red blood cells (Evans, 1983), and repeat our procedure. We find that the fraction of the total cholesterol in the inner leaf has increased from 25% to 38.6%. We now implement the idea that cholesterol reduces the spontaneous curvature of PE, presumably by ordering its tails. In other words, we take the spontaneous curvature of PE to be cholesterol-dependent. To model this dependence, we imitate the effect of cholesterol on the temperature of transition from lamellar to inverted-hexagonal phase of PE in water (Epan and Bottega, 1987). In particular, we will require that the spontaneous curvature begin to decrease when the mol fraction of cholesterol in the inner leaf is approximately 0.3, and to decrease rapidly when the mol fraction is about 0.4. We choose the form

$$H_{PE}(y_{C_i}) = H_{PE}^0 - B \frac{y_{C_i}}{y_{min}} + \frac{B}{\lambda} \left(\frac{y_{C_i}}{y_{min}} \right)^\lambda, \quad (2)$$

with $B = 0.05$, $y_{min} = 0.3$, and $\lambda = 8$. This form is shown in Fig. 1 for several choices of λ .

With this choice of a cholesterol-dependent spontaneous curvature, we find that 58% of the total cholesterol is in the inner leaf. Thus the mechanism we propose does lead to the conclusion that the majority of cholesterol in the plasma membrane should be found in the inner leaf, the reason being that that is where the PE is. The mol fractions of the three components in the outer leaf are $y_{SM} = 0.34$, $y_{PC} = 0.31$, $y_{C_0} = 0.35$ while those of the inner leaf are $y_{PS} = 0.19$, $y_{PE} = 0.36$, and $y_{C_i} = 0.45$. Because our mechanism depends upon the bending energy, and the bending moduli of the bilayers enclosing different vesicles within the cell are presumably different, it is of interest to examine how the fraction of cholesterol

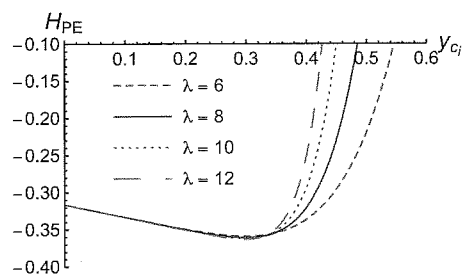


Fig. 1. Spontaneous curvature of PE in the presence of cholesterol as modeled by Eq. (2) for four values of λ .

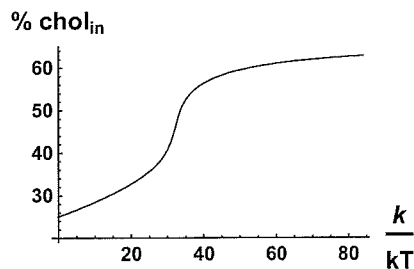


Fig. 2. Percent of cholesterol in the inner leaf as a function of the bending modulus κ .

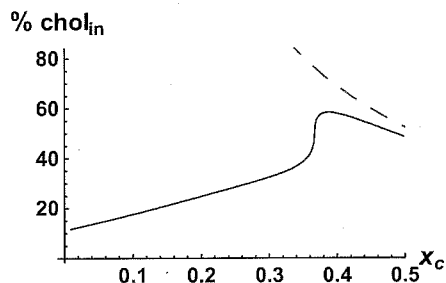


Fig. 3. Percent of total cholesterol in the inner leaf as a function of the total cholesterol mol fraction x_c . The dashed line shows the solubility limit of cholesterol.

depends upon the bending modulus, all other things being equal. This is shown in Fig. 2

Our mechanism also depends upon the total amount of cholesterol in the membrane which is only large in the plasma membrane and late-stage endosomes (van Meer et al., 2008). Thus it is of interest to see how the fraction of cholesterol in the inner leaf depends upon the total amount of cholesterol in the membrane. That is shown in Fig. 3. One sees that if there is not much cholesterol and if there were SM in the outer leaf, little cholesterol would be in the inner leaf. The fraction of cholesterol in the inner leaf increases with the total fraction of cholesterol and must attain the value of 1/2 were the membrane to be completely cholesterol. The interesting result is that the fraction of cholesterol in the inner leaf exceeds 1/2 for values of the total cholesterol content typical of human erythrocytes (van Meer, 2011).

2. Conclusion

We have proposed a way to understand the experimental observations that the majority of the cholesterol in the mammalian plasma membrane is found in the inner, cytoplasmic, leaf; it is because that is where then phosphatidylethanolamine is. Its presence there comes at the cost of a bending energy penalty which the presence of cholesterol can relieve, both by diluting the PE and

by reducing its spontaneous curvature. This idea ties together the cholesterol distribution in the plasma membrane with completely independent experiments which show that sufficient amounts of cholesterol stabilize the lamellar phase of PE in water relative to the inverted-hexagonal phase (Erand and Bottega, 1987; Takahashi et al., 1996). There are additional effects which could either decrease or increase the fraction of cholesterol in the inner leaf. Among the former is the fact that cholesterol is not very soluble in polyunsaturated fatty acids (Brzustowicz et al., 2002), and such chains are found predominantly in the inner leaf. As for the latter, it has been suggested that the presence of SM in the outer leaf with very long chains that reach into the inner leaf could attract the cholesterol there (Courtney et al., 2015). In addition, we have utilized the simple form for the bending energy of Eq. (1) and have assumed that the spontaneous curvature of a mixture of lipids is a weighted sum of their spontaneous curvatures. This is a reasonable assumption, but one which must be checked (Sodt et al., 2015). Further the form of the bending energy assumes that bending toward the cytosol or away from it are energetically equivalent. Because of the cytoskeleton, this may not be the case. Were the bending energy proportional to the first power of the PE concentration rather than to the second as we have assumed, the fraction of cholesterol in the inner leaf would certainly be greater than what we have obtained here. Be that as it may, we believe that the mechanism that we have proposed goes a long way in resolving the puzzling distribution of cholesterol in the plasma membrane.

Acknowledgments

We wish to thank Paulo Almeida, William Clay and Josh Zimmerman, and Richard Erand for stimulating conversations. This work was supported by the National Science Foundation under grant No. DMR-1203282.

References

- Almeida, P., 2009. Thermodynamics of lipid interactions in complex bilayers. *Biochim. Acta* 1788, 72–85.
- Blau, L., Bittman, R., 1978. Cholesterol distribution between the two leaves of the lipid bilayer of the human erythrocyte: ghost membranes. *J. Biol. Chem.* 253, 8366–8368.
- Brasaemle, D., Robertson, A., Attie, A., 1988. Transbilayer movement of cholesterol in the human erythrocyte membrane. *J. Lipid Res.* 29, 481–489.
- Brzustowicz, M., Cherezov, V., Zerouga, M., Caffrey, M., Wassail, S., 2002. Controlling membrane cholesterol content, a role for polyunsaturated (docosahexaenoate) phospholipids. *Biochemistry* 41, 12509–12519.
- Courtney, K., Zhang, C., Zhai, X., 2015. Long acyl chain sphingolipids govern visible microdomains and cholesterol in both model and plasma membranes. *Biophys. J.* 108, 32a.
- Devaux, P., 1991. Static and dynamic lipid asymmetry in cell membranes. *Biochemistry* 30, 1163–1173.
- Erand, R., Bottega, R., 1987. Modulation of the phase transition behavior of phosphatidylethanolamine by cholesterol and oxysterols. *Biochemistry* 26, 1820–1825.
- Erand, R., Epand, R.F., 2004. Non-raft forming sphingomyelin-cholesterol mixtures. *Chem. Phys. Lipids* 132, 37–46.
- Evans, E., 1983. Bending elastic modulus of red blood cell membrane derived from buckling instability in micropipet aspiration tests. *Biophys. J.* 43, 27–30.
- Giang, H., Schick, M., 2014. How cholesterol could be drawn to the cytoplasmic leaf of the plasma membrane by phosphatidylethanolamine. *Biophys. J.* 107, 2337–2344.
- Igbavboa, U., Avdulov, N., Schroeder, F., Wood, W., 1996. Increasing age alters transbilayer fluidity and cholesterol asymmetry in synaptic plasma membranes of mice. *J. Neurochem.* 66.
- Kollmitzer, B., Heftberger, P., Rappolt, M., Pabst, G., 2013. Monolayer spontaneous curvature of raft-forming membrane lipids. *Soft Matter* 9, 10877–10884.
- Lange, Y., Slayton, J.M., 1982. Interaction of cholesterol and lysophosphatidylcholine in determining red cell shape. *J. Lipid Res.* 23, 1121–1127.
- Lange, Y., Dolde, J., Steck, T., 1981. The rate of transmembrane movement of cholesterol in the human erythrocyte. *J. Biol. Chem.* 256, 5321–5323.
- Lange, Y., 1984. The dynamics of erythrocyte membrane cholesterol. *Prog. Clin. Biol. Res.* 159, 137–151.
- Mondal, M., Mesmin, B., Mukherjee, S., Maxfield, F., 2009. Sterols are mainly in the cytoplasmic leaflet of the plasma membrane and the endocytic recycling compartment of CHO cells. *Mol. Biol. Cell* 20, 581–588.

- Muller, P., Hermann, A., 2002. Rapid transbilayer movement of spin-labeled steroids in human erythrocytes and in liposomes. *Biophys. J.* 82, 1418–1428.
- Niu, S.-L., Litman, B., 2002. Determination of membrane cholesterol partition coefficient using a lipid vesicle-cyclodextrin binary system: effect of phospholipid acyl chain unsaturation and headgroup composition. *Biophys. J.* 83, 3408–3415.
- Perlmutter, J.D., Sachs, J.N., 2011. Interleaflet interaction and asymmetry in phase separated lipid bilayers: molecular dynamics simulations. *J. Am. Chem. Soc.* 133, 6563–6577.
- Polley, A., Vemparala, S., Rao, M., 2012. Atomistic simulations of a multicomponent asymmetric lipid bilayer. *J. Phys. Chem. B* 116, 13403–13410.
- Schroeder, F., Nemezc, G., Wood, W.G., Joiner, C., Morrot, G., Ayrault-Jarrier, M., Devaux, P., 1991. Transmembrane distribution of sterol in the human erythrocyte. *Biochim. Biophys. Acta* 1066, 183–192.
- Sodt, A., Venable, R., Lyman, E., Pastor, R., 2015. Lipid–lipid interactions determine the membrane spontaneous curvature. *Biophys. J.* 108, 181a.
- Steck, T., Lange, Y., 2002. Probing red blood cell membrane cholesterol movement with cyclodextrin. *Biophys. J.* 83, 2118–2125.
- Takahashi, H., Sinoda, K., Hatta, I., 1996. Effects of cholesterol on the lamellar and the inverted hexagonal phases of dielaidoylphosphatidylethanolamine. *Biochim. Biophys. Acta* 1289, 209–216.
- van Meer, G., Voelker, D., Feigenson, G.W., 2008. Membrane lipids: where they are and how do they behave? *Nat. Rev. Mol. Cell Biol.* 112, 112–124.
- van Meer, G., 2011. Dynamic transbilayer lipid asymmetry. *Cold Spring Harb. Perspect. Biol.* 3, 1–11.
- Wood, W., Schroeder, F., Hogy, L., Rao, A., Nemezc, G., 1990. Asymmetric distribution of a fluorescent sterol in synaptic plasma membrane: effects of chronic ethanol consumption. *Biochim. Biophys. Acta* 1025, 243–246.
- Zheng, L., McQuaw, C., Ewing, A.G., Winograd, N., 2007. Sphingomyelin/ phosphatidylcholine and cholesterol interactions studied by imaging mass spectroscopy. *J. Am. Chem. Soc.* 129, 15730–15731.