J|A|C|S COMMUNICATIONS

Published on Web 10/27/2009

Inhibiting Lateral Domain Formation in Lipid Bilayers: Simulations of Alternative Steroid Headgroup Chemistries

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There is a growing amount of evidence that laterally segregated domains of lipids are an integral part of biological membrane structure and function. These domains, referred to as lipid rafts, are now appreciated as a vitally important functional component of signaling and organization in the cell.[1−3] Experimental models of rafts have been studied extensively through synthetic lipid bilayers, with an emphasis on the biophysical interactions between low- and high-melting lipids and cholesterol.[4−7] The role of steroids in promoting domain formation in these model systems has received a great deal of attention. Cholesterol decreases chain entropy in neighboring lipids due to its rigid ring structure and associates more readily with ordered, saturated chains than with disordered, unsaturated chains. Chemical alterations to the steroid rings and hydrocarbon tail of cholesterol can lead to a decrease in domain formation.[8−15]

The role of the polar headgroup is a less well understood aspect of steroid-induced domain formation. Removing the hydroxyl headgroup altogether or substituting it with more bulky groups has a detrimental effect on phase separation,[8−11] while replacing it with a charged group has a variety of effects.[8,15] Of great interest is the difference between cholesterol and its oxidation product, cholestenone (4-cholesten-3β-one). This reaction is catalyzed in certain bacteria, and its affect on the host membrane is postulated as necessary for pathogenesis.[16] Though cholesterol and cholestenone differ only in the replacement of the hydroxyl group with a keto group and in the shift in position of one double bond (from the B ring Δ^5 to the A ring Δ^4 position), cholestenone's propensity to form ordered domains is substantially reduced.[8−13] In fact, it has been demonstrated that cholestenone can *inhibit* domain formation, making it one of the few identified domain inhibiting steroids[8,9] and worthy of deeper study. It is unknown why this small chemical change leads to such a substantial difference in behavior or if there is a common mechanism that operates among these domain-inhibiting steroids.

Atomistic computational molecular dynamics (MD) simulations have been used extensively to characterize the effect of steroids on lipid bilayers.[17−19] More recently, coarse-grained MD simulations have been developed to extend the access of computational techniques, allowing larger systems and simulations on the microsecond time scale. For example, using the Martini coarse grain force field,[20] cholesterol flip-flop[21,22] and lateral phase separation[23] have been studied. By their very nature, however, coarse-grained simulations are limited as a strategy for studying the effects of small changes in chemistry, as is the case in comparing cholesterol and cholestenone. In this current work, we have overcome this by systematically changing the properties of the steroid headgroup as a means of modeling the effects of altered chemistry. By isolating single physical variables (e.g., headgroup hydrophobicity), our computational strategy allows us to make fundamental connections between physical phenomena (e.g., domain formation) and basic chemical characteristics. This type of computational experiment has previously been successful in our studies of ion−lipid interactions.[24,25]

In the Martini coarse-grain force field, multiple atoms are represented as single beads, with each falling into one of four categories: nonpolar (further classified from most nonpolar [C1] through least nonpolar [C5]), intermediately polar (including either hydrogen bond donating [Nd] or accepting [Na]), polar (from most polar [P5] through least polar [P1]), and charged. Standard parametrization of cholesterol represents the hydroxyl group as type P1.[20] Here, we have run a series of simulations in which we have changed the cholesterol headgroup type to represent a range of hydrophobicities, modeling the physical differences with cholestenone. The geometry of the molecules, namely the bond lengths and angles, was not altered from the cholesterol parametrization presented previously.[20] The bilayers we use here are similar to those previously shown to exhibit lateral phase separation,[23] consisting of 256 di-16:0PC lipids, 256 di-18:2PC lipids, and 128 steroids at full hydration. Each of the eight systems was simulated for 3.5 μs,

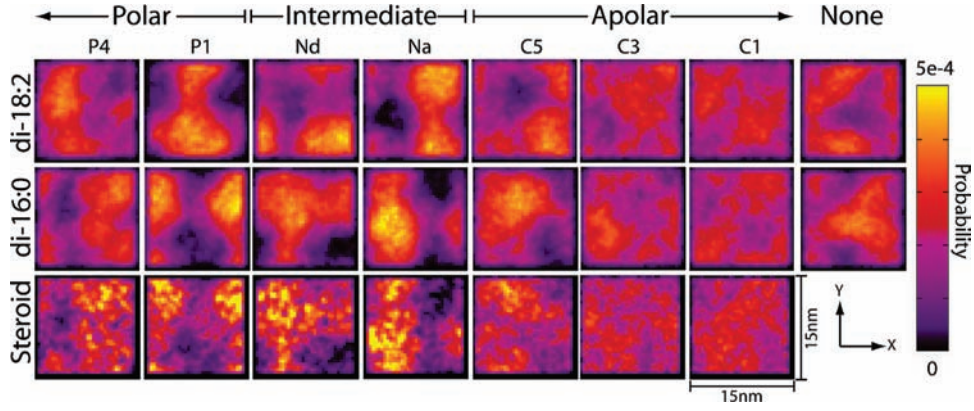

Figure 1. Lateral density of bilayer components, showing varying degrees of lateral separation and colocalization of steroid and saturated lipid. Each column represents results from a single simulation, where the steroid headgroup is represented by the indicated bead type. Probability distributions for each of the bilayer components are given as projections onto the bilayer plane.

10.1021/ja9079258 CCC: $40.75

and the final 1.5 μs were used for analysis. Additional details are provided as Supporting Information.

Our simulations show that headgroup hydrophobicity dramatically affects a steroid's propensity to enhance lateral domain formation, as shown in Figure 1. Those bilayers which contain steroids with polar or intermediately polar headgroups display a distinct separation between the saturated and diunsaturated lipid. These bilayers also show strong colocalization of the steroid with the saturated lipid. The bilayer containing steroids with type C5 headgroups shows separation between lipids, but weaker colocalization of the steroid and saturated lipid. The bilayers containing the steroids with type C3 or C1 headgroups show considerably less separation between the saturated and diunsaturated lipids and far weaker colocalization of the steroid with the saturated lipid. In fact, these bilayers show a lesser extent of separation than a bilayer without any steroid, suggesting that the steroids with nonpolar headgroups function as domain inhibitors. We note that, while we would expect the Na bead to most closely model cholestenone's headgroup, it does not reproduce the experimental behavior (domain inhibition). However, this most likely reflects subtleties in force-field parametrization and lipid composition and does not change our overall conclusion regarding headgroup hydrophobicity and domain formation. The size of the domains was determined by the number of diunsaturated lipids in contiguous contact, and quantitative details are given in the Supporting Information.

These differences in domain formation result from differences in the steroid molecule orientation. Until recently, it was thought that the upright orientation of cholesterol, where the molecule is parallel to the lipid tails and the headgroup is at the lipid−water interface, was the only favorable steroid conformation. However, cholesterol has been shown to favor an orientation perpendicular to the bilayer normal axis, buried in the hydrophobic core while in polyunsaturated lipid bilayers, using neutron diffraction[26] and coarse-grain MD.[21,22] Additionally, in a non-phase separated simulation, a keto-sterone (closely related to cholestenone) was also shown to adopt a perpendicular orientation.[27] As shown in Figure 2, our results demonstrate that, in bilayers containing a saturated and diunsaturated lipid, an increase in headgroup hydrophobicity triggers a switch to this buried, perpendicular orientation, as

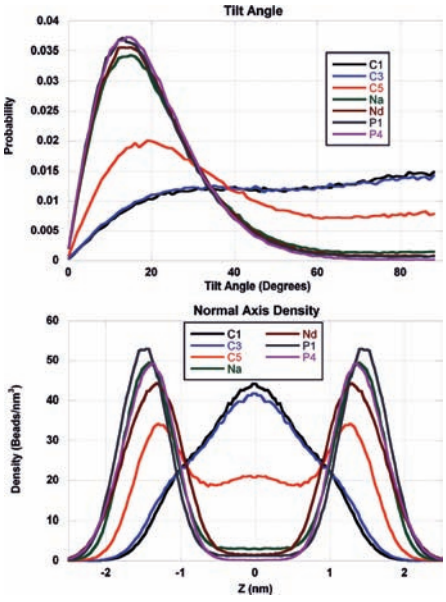

Figure 2. Increasing headgroup hydrophobicity shifts the steroid's tilt relative to the bilayer normal (top) and headgroup depth (bottom), from the upright orientation to a buried, perpendicular orientation.

observed through large tilt values and maximal headgroup density in the bilayer center for the steroids containing type C1 and C3 headgroups. Those steroids with polar and intermediately polar headgroups have lower tilt values, with an average of 21°, similar to that of cholesterol in di-16:0PC found through experiment[28] and computation.[29] These steroids also each have maximal headgroup density at the lipid−water interface, though the steroid with the intermediately polar, hydrogen bond donating headgroup may favor slightly deeper bilayer penetration. Steroids with the C5 headgroup display a trimodal headgroup distribution, thus maintaining an equilibrium between the upright and perpendicular orientations.

Our results, which take advantage of a reductionist, computational strategy, demonstrate that the role of cholesterol's headgroup is to anchor the steroid in the upright orientation, allowing the stiff, steroid rings to order the neighboring lipid tails, thus promoting formation of lateral domains. Changing the physicochemical properties of the headgroup, mimicking the difference between cholesterol and cholestenone, causes the steroids to favor a fully inserted, perpendicular orientation. Our results demonstrate that steroids in this conformation inhibit lateral domain formation, likely by disrupting the interactions between neighboring lipid chains that would otherwise favor domain formation. We suggest that the destabilization of the upright orientation is a common mechanism of domain inhibiting steroids.

Acknowledgment. We thank Christopher Valley for valuable contributions and Dr. Erwin London and for valuable discussions, as well as the Minnesota Supercomputing Institute for computational resources.

Supporting Information Available: Details of the methods and supplemental figures. This material is available free of charge via the Internet at http://pubs.acs.org.

References

(1) Maxfield, F. R.; Tabas, I. *Nature* **2005**, *438*, 612–21.
(2) Munro, S. *Cell* **2003**, *115*, 377–88.
(3) Simons, K.; Toomre, D. *Nat. Rev. Mol. Cell Biol.* **2000**, *1*, 31–9.
(4) London, E. *Curr. Opin. Struct. Biol.* **2002**, *12*, 480–6.
(5) Edidin, M. *Annu. Rev. Biophys. Biomol. Struct.* **2003**, *32*, 257–83.
(6) Silvius, J. R. *Biochim. Biophys. Acta* **2003**, *1610*, 174–83.
(7) Zhao, J.; Wu, J.; Heberle, F. A.; Mills, T. T.; Klawitter, P.; Huang, G.; Costanza, G.; Feigenson, G. W. *Biochim. Biophys. Acta* **2007**, *1768*, 2764–76.
(8) Xu, X.; London, E. *Biochemistry* **2000**, *39*, 843–9.
(9) Beattie, M. E.; Veatch, S. L.; Stottrup, B. L.; Keller, S. L. *Biophys. J.* **2005**, *89*, 1760–8.
(10) Stottrup, B. L.; Keller, S. L. *Biophys. J.* **2006**, *90*, 3176–83.
(11) Wang, J.; Megha; London, E. *Biochemistry* **2004**, *43*, 1010–8.
(12) Ben-Yashar, V.; Barenholz, Y. *Biochim. Biophys. Acta* **1989**, *985*, 271–8.
(13) Slotte, J. P. *Biochim. Biophys. Acta* **1995**, *1237*, 127–34.
(14) Bloch, K. E. *CRC Crit. Rev. Biochem.* **1983**, *14*, 47–92.
(15) Bacia, K.; Schwille, P.; Kurzchalia, T. *Proc. Natl. Acad. Sci. U.S.A.* **2005**, *102*, 3272–7.
(16) Caldinelli, L.; Iametti, S.; Barbiroli, A.; Bonomi, F.; Fessas, D.; Molla, G.; Pilone, M. S.; Pollegioni, L. *J. Biol. Chem.* **2005**, *280*, 22572–81.
(17) Rog, T.; Pasenkiewicz-Gierula, M.; Vattulainen, I.; Karttunen, M. *Biochim. Biophys. Acta* **2009**, *1788*, 97–121.
(18) Kucerka, N.; Perlmutter, J. D.; Pan, J.; Tristram-Nagle, S.; Katsaras, J.; Sachs, J. N. *Biophys. J.* **2008**, *95*, 2792–805.
(19) Olsen, B. N.; Schlesinger, P. H.; Baker, N. A. *J. Am. Chem. Soc.* **2009**, *131*, 4854–65.
(20) Marrink, S. J.; Risselada, H. J.; Yefimov, S.; Tieleman, D. P.; de Vries, A. H. *J. Phys. Chem. B* **2007**, *111*, 7812–24.
(21) Bennett, W. F.; MacCallum, J. L.; Hinner, M. J.; Marrink, S. J.; Tieleman, D. P. *J. Am. Chem. Soc.* **2009**, *131*, 12714–20.
(22) Marrink, S. J.; de Vries, A. H.; Harroun, T. A.; Katsaras, J.; Wassall, S. R. *J. Am. Chem. Soc.* **2008**, *130*, 10–1.
(23) Risselada, H. J.; Marrink, S. J. *Proc. Natl. Acad. Sci. U.S.A.* **2008**, *105*, 17367–72.
(24) Sachs, J. N.; Woolf, T. B. *J. Am. Chem. Soc.* **2003**, *125*, 8742–3.
(25) Sachs, J. N.; Nanda, H.; Petrache, H. I.; Woolf, T. B. *Biophys. J.* **2004**, *86*, 3772–82.
(26) Harroun, T. A.; Katsaras, J.; Wassall, S. R. *Biochemistry* **2008**, *47*, 7090–6.
(27) Rog, T.; Stimson, L. M.; Pasenkiewicz-Gierula, M.; Vattulainen, I.; Karttunen, M. *J. Phys. Chem. B* **2008**, *112*, 1946–52.
(28) Murari, R.; Murari, M. P.; Baumann, W. J. *Biochemistry* **1986**, *25*, 1062–7.
(29) Aittoniemi, J.; Rog, T.; Niemela, P.; Pasenkiewicz-Gierula, M.; Karttunen, M.; Vattulainen, I. *J. Phys. Chem. B* **2006**, *110*, 25562–4.

JA9079258