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The Origin of Cholesterol's Condensing Effect

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Abstract

The condensing effect of cholesterol on fluid bilayers of 1,2-dipalmitoyl-*sn*-glycero-3-phosphocholine has been compared with that of dihydrocholesterol and coprostanol by means of nearest-neighbor recognition measurements. Whereas dihydrocholesterol exhibits a condensing power that is equivalent to that of cholesterol, the action of coprostanol is significantly weaker. These results provide strong support for a template mechanism of condensation and argue against an umbrella mechanism.

Cholesterol's ability to condense fluid phospholipid membranes has been known for almost a century.¹⁻⁴ Because mammalian membranes are rich in cholesterol and low-melting phospholipids, this condensing effect is presumed to play a major role in the structure and function of such membranes.⁵ Despite numerous experimental and theoretical investigations, the means by which cholesterol induces phospholipid condensation is poorly understood. According to a popular umbrella mechanism, "acyl chains and cholesterol become more tightly packed as cholesterol content increases, because they share limited space under phospholipid headgroups".⁶ In an alternate template mechanism, the flexible acyl chains of the phospholipid complement, perfectly, cholesterol's planar nucleus to produce a high number of close hydrophobic contacts and tight packing.⁷ In this paper, we present experimental evidence that distinguishes between these two mechanisms and provides insight into the origin of cholesterol's condensing effect.

To differentiate between an umbrella versus a template mechanism, we have compared the condensing power of cholesterol with that of dihydrocholesterol and coprostanol (Chart 1). Because cholesterol and dihydrocholesterol are very similar in structure, they have a similar cross-sectional area in the monolayer state.⁸ In addition, since they both have a planar nucleus, these two sterols are capable of producing a high number of close hydrophobic contacts with neighboring acyl chains. Thus, based on an umbrella and a template mechanism, cholesterol and dihydrocholesterol are expected to have a similar condensing power. In the case of coprostanol, however, the umbrella and template mechanisms lead to predictions that are diametrically opposed to one another. Due to the *cis*-fusion of its A and B rings, coprostanol occupies a larger cross-sectional area in the monolayer state.⁸ Thus, if the umbrella mechanism were operating, coprostanol should have a stronger condensing effect due to increased crowding beneath the phospholipid's head group; that is, further condensation of the acyl chains would be necessary to prevent contact of both lipophilic moieties with the bulk aqueous phase (Figure 1). In contrast, the template mechanism leads to the prediction that coprostanol should have a weaker condensing effect since fewer close hydrophobic contacts with neighboring acyl chains are possible because of its curvature. In principle, therefore, a quantitative comparison of the condensing power of these three sterols

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SUPPORTING INFORMATION. Experimental procedures and tables of raw data. This material is available free of charge via the Internet at <http://pubs.acs.org>.

should confirm a similarity between cholesterol and dihydrocholesterol, and allow one to distinguish between an umbrella versus a template mechanism for sterol-induced acyl chain condensation.

To quantify the condensing power of these sterols, we measured their effects on fluid bilayers made from 1,2-dipalmitoyl-*sn*-glycero-3-phosphocholine (DPPC) using the nearest-neighbor recognition (NNR) method and exchangeable monomers **A** and **B** (Chart 2).^{9,12} As discussed elsewhere, three major advantages that NNR measurements have over classic monolayer analyses are: (i) they provide quantitative insight into differences in lipid packing, (ii) they are highly sensitive and can detect differences in membrane compactness that correspond to tens of calories per mole of lipid, and (iii) they probe the physiologically-relevant, fluid bilayer state.¹²

In brief, NNR measurements take molecular-level snapshots of bilayer organization by detecting and quantifying the thermodynamic tendency of exchangeable monomers to become nearest-neighbors of one another. Typically, two lipids of interest (**A** and **B**) are converted into exchangeable dimers (homodimers **AA** and **BB**, and heterodimer **AB**), which are then allowed to undergo monomer interchange via thiolate-disulfide exchange. The resulting equilibrium that is established is governed by an equilibrium constant, $K = [\text{AB}]^2 / ([\text{AA}][\text{BB}])$. When monomers **A** and **B** mix ideally, this is reflected by an equilibrium constant that equals 4.0. When hetero-associations are favored, the equilibrium constant is greater than 4.0; favored homo-associations are indicated by a value that is less than 4.0. As shown previously, NNR measurements that use low concentrations of exchangeable lipids **A** and **B** in host membranes made from DPPC and cholesterol reflect changes in the compactness of the host membrane; that is, the exchangeable lipids act as chemical sensors.¹⁰ Thus, the transition from compact liquid-ordered (l_o) bilayers to ones that are less compact in the liquid-disordered (l_d) phase, is reflected by a steady decrease in K . In addition, values of K yield nearest-neighbor interaction free energies, ω_{AB} , between **A** and **B**, where $\omega_{\text{AB}} = -1/2 RT \ln(K/4)$.¹³

Before carrying out NNR measurements, we first examined whether the phase properties of DPPC-based liposomes would be significantly affected by replacement of cholesterol with dihydrocholesterol or coprostanol. With this aim in mind, we measured the fluorescence of the phase-sensitive probe, Laurdan, in liposomal dispersions and determined generalized polarization (GP) values as a function of temperature.¹⁴ Here, $\text{GP} = (I_{440} - I_{490}) / (I_{440} + I_{490})$, and I_{440} and I_{490} are the emission intensities at these wavelengths ($\lambda_{\text{ex}} = 350 \text{ nm}$). Such GP values reflect the polarity surrounding the Laurdan moiety and are very sensitive to changes in phase.¹⁴

At low cholesterol concentrations (2.5 mol%), a well-defined gel to fluid phase transition was evident with a melting temperature of ca. 41°C (Figure 2). However, when 40 mol% cholesterol was included in the membrane, which is known to maintain the l_o phase from 30°C to 55°C, the GP values decreased, modestly, and almost linearly with increasing temperatures.¹⁵ Bilayers that were rich in dihydrocholesterol gave a temperature profile that was nearly identical with that of the cholesterol-rich analog. Similarly, coprostanol-rich bilayers exhibited a “ l_o -like” signature except that the GP values were significantly lower at each temperature, implying that these bilayers are less compact and allow for greater penetration of water. Additionally, the increase in slope that is apparent at the lower temperatures implies that some “softening” of the membrane has occurred.

Thiolate-disulfide equilibration reactions were then carried out at 45°C in liposomes (~200 nm) made from DPPC/cholesterol/*X*/**A**/**B** (here, *X* is coprostanol or dihydrocholesterol) having the following mole percentages: (a) 57.5/37.5/0/2.5/2.5, (b) 57.5/27.5/10/2.5/2.5, (c)

57.5/17.5/20/2.5/2.5, and (d) 57.5/0/37.5/2.5/2.5. As shown in Figure 3, incremental replacement of cholesterol with coprostanol led to a steady decrease in K , reflecting a steady decrease in the compactness of the bilayer. In sharp contrast, incremental replacement of cholesterol with dihydrocholesterol did not significantly alter the value of K . Based on the values of K in liposomes containing 0, 10, 20 and 37.5 mol% coprostanol, the nearest-neighbor interaction free energies between **A** and **B**, ω_{AB} , are calculated to be -283.30 ± 29.70 , -241.70 ± 27.17 , -201.42 ± 30.87 , and -144.43 ± 25.98 cal/mol of lipid, respectively. Taken together, these results show that cholesterol and dihydrocholesterol are very similar in condensing power and that both sterols are significantly stronger condensing agents than coprostanol.^{16,17} Thus, they provide strong support for a template mechanism and strong evidence against an umbrella mechanism.

Recently, we have obtained experimental evidence that cholesterol is more effective as a condensing agent when its rigid nucleus occupies space near the surface of the membrane.¹² This finding implies that it is the minimization of hydrocarbon contact with the bulk aqueous phase that drives acyl chain condensation. If one now takes the template effect into account, then a simple and complete picture of cholesterol's condensing action comes into focus; that is, cholesterol acts by (i) maximizing hydrocarbon contact between itself and the acyl chains of a neighboring phospholipid, thereby minimizing contact of both lipophilic moieties with the bulk aqueous phase, and (ii) contributing to the formation of a contiguous hydrophilic surface. It should be noted that this physical picture is in excellent agreement with previous molecular dynamics simulations of bilayers made from DPPC and cholesterol, which have shown that cholesterol's hydroxyl group, but not its hydrocarbon rings, are wetted by water.^{18,19}

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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REFERENCES

1. Leathes JB. *Lancet*. 1925; 208:853–856.
2. Demel RA, van Deenen LLM, Pethica BA. *Biochim. Biophys. Acta*. 1967; 135:11–19.
3. Stockton BW, Smith ICP. *Chem. Phys. Lipids*. 1976; 17:251–261. [PubMed: 1033045]
4. Vist M, Davis JH. *Biochemistry*. 1990; 29:451–464. [PubMed: 2302384]
5. Gennis, RB. *Biomembranes: molecular structure and function*. Springer-Verlag; New York: 1989.
6. Huang J, Feigenson GW. *Biophys. J*. 1999; 76:2142–2157. [PubMed: 10096908]
7. Sugahara M, Uragami M, Yan X, Regen SL. *J. Am. Chem. Soc.* 2001; 123:7939–7940. [PubMed: 11493081]
8. Stottrup BL, Keller SL. *Biophys. J*. 2006; 90:3176–3183. [PubMed: 16461392]
9. Davidson SKM, Regen SL. *Chem. Rev.* 1997; 97:1269–1280. [PubMed: 11851451]
10. Cao H, Zhang J, Jing B, Regen SL. *J. Am. Chem. Soc.* 2005; 127:8813–8816. [PubMed: 15954788]
11. Mitomo H, Chen W-H, Regen SL. *J. Am. Chem. Soc.* 2009; 121:12354–12357. [PubMed: 19658396]
12. Janout V, Turkyilmaz S, Wang M, Manaka Y, Regen SL. *Langmuir*. 2010; 26:5316–5318. [PubMed: 20329715]

13. Almeida PFF. Biochim. Biophys. Acta. 2009; 1788:72–85. [PubMed: 18775410]
14. Parassi T, DiStefano M, Loiero M, Ravagnan G, Gratton E. Biophys. J. 1994; 66:120–132. [PubMed: 8130331]
15. Chiang Y-W, Costa-Filho J, Freed JH. J. Phys. Chem. 2007; 111:11260–112.
16. In a previous NMR study, the ability of a variety of sterols to promote *homoassociation among exchangeable phospholipids* bearing palmitoyl or stearoyl chains was examined. In this case, phospholipid—sterol association was not measured: Cao H, Tokutake N, Regen SL. J. Am. Chem. Soc. 2003; 125:16182–16183. [PubMed: 14692747]
17. Previous monolayer measurements (Demel RA, Bruckdorfer KR, van Deenen LLM. Biochim. Biophys. Acta. 1972; 255:311–320. [PubMed: 5010999]), and related measurements in similar systems (Supporting Information), indicate that cholesterol and dihydrocholesterol are stronger condensing agents than coprostanol.
18. Smondyrev AM, Berkowitz ML. Biophys. J. 1999; 77:2075–2089. [PubMed: 10512828]
19. Berkowitz ML. Biochim. Biophys. Acta. 2009; 1788:86–96. [PubMed: 18930019]

Figure 1.
Stylized illustration of the consequences of cholesterol and coprostanol on acyl chain packing according to the template and umbrella mechanisms.

Figure 2.

Plot of general polarization versus temperature for liposomes made from the following molar percentages of lipids: (○) DPPC/DPPG/cholesterol 95/2.5/2.5; (■) DPPC/DPPG/cholesterol, 57.5/2.5/40; (□) DPPC/DPPG/cholesterol/dihydrocholesterol, 57.5/2.5/2.5/37.5; (▲) DPPC/DPPG/cholesterol/coprostanol, 57.5/2.5/2.5/37.5.

Figure 3.

Bar graph showing K in liposomes containing the following mole percentages of cholesterol/coprostanol: (i) 40/0, (ii) 30/10, (iii) 20/20, (iv) 2.5/37.5, and cholesterol/dihydrocholesterol: (v) 30/10, (vi) 20/20 and (vii) 2.5/37.5. Here, lipid **B** is included in the cholesterol count. Error bars represent one standard deviation.

Chart 1.

Chart 2.