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Physical Properties of High-Cholesterol Containing Membranes

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Introduction

Eye lens fiber cell plasma membranes are loaded with extremely high cholesterol (Chol) content. In human lens fiber cell membrane, the Chol to phospholipid (Chol/PL) molar ratio ranges from 1:1 to 2:1 in the cortex, as high as 3:1 to 4:1 in the lens nucleus. There are four major lipids that builds fiber-cell plasma membrane of eye lens i.e., phosphatidylcholine (PC), sphingomyelin (SM), phosphatidylethanolamine (PE) and phosphatidylserine (PS). The PC is more dominant in younger animals like porcine and bovine whereas SM is more dominant in human eye lens membrane. In the proposed research the lipid membrane chosen for the study is PC.

Electron paramagnetic resonance spin-labeling methods were used to investigate the physical properties of Chol/POPC (1-palmitoyl-2-oleoylphosphatidylcholine) membranes. The Chol/POPC mixing ratio was changed from 0 to 3. The membrane samples were prepared using the rapid solvent exchange method to preserve the compositional homogeneity throughout the membrane suspension. The high Chol content not only saturates the PL bilayer forming phospholipid cholesterol domain (PCD) but also leads to the formation of cholesterol bilayer domains (CBDs) as shown in Fig. 1. Recently, it was shown that CBDs start to form at Chol/POPC mixing ratio of 1 [1]. Here we have investigated the physical properties of the membranes with increasing Chol content using cholesterol analogue spin labels CSL and ASL. Physical properties include maximum splitting, mobility parameter, and hydrophobicity.

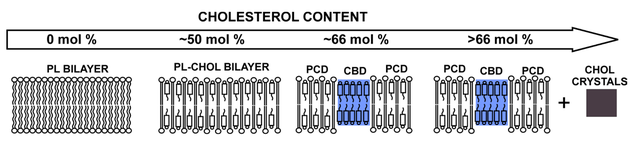

Figure 1. The schematic drawings of membrane structures at increasing Chol/POPC mixing ratios; significant changes within the membrane are presented. Saturation of cholesterol in the membrane leads to the formation of cholesterol bilayer domain (CBD) and later cholesterol crystals.

Results

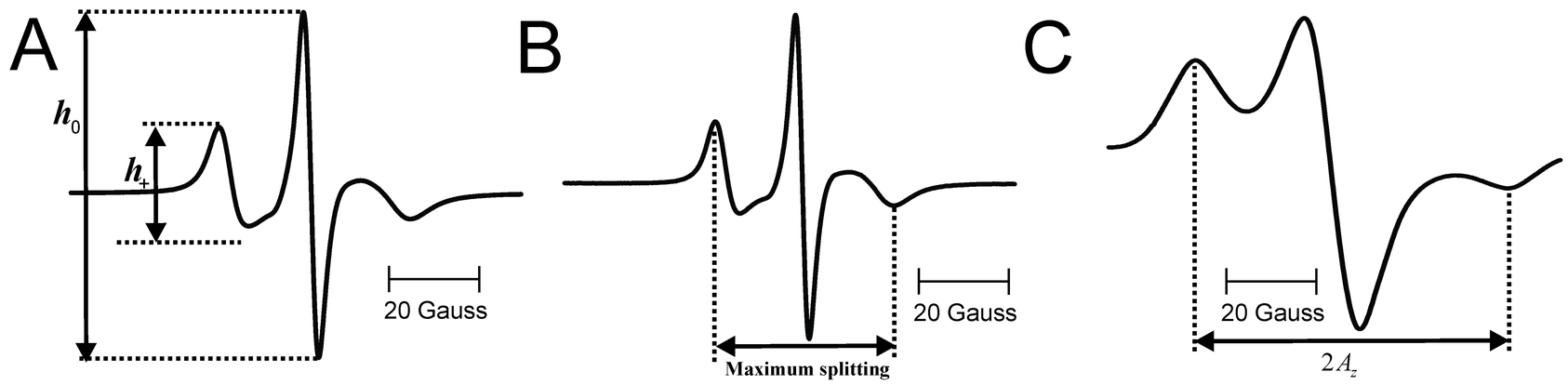

Figure 4. Representative EPR spectra of CSL (A,C) and ASL (B) in the Chol/POPC membranes with Chol/POPC mixing ratio of 1. A). EPR spectra of CSL at 37 ℃ showing the parameters for mobility (h_+/h_0) measurement. B) EPR Spectra of ASL at 37 ℃ showing the measurement of maximum splitting. C) EPR spectra of CSL at 110K showing $2A_z$ measurement. $2A_z$ is the measure of hydrophobicity.

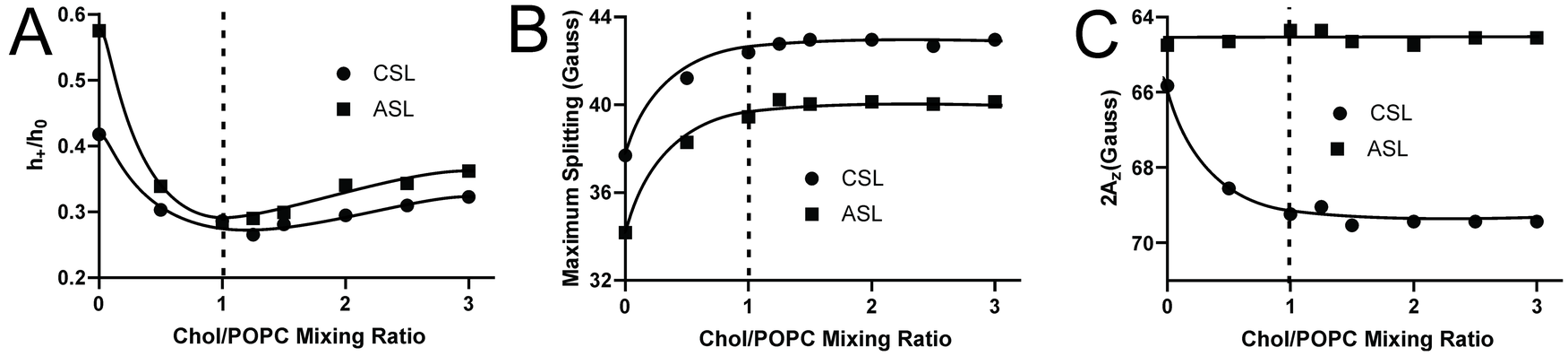

Figure 5. A) Mobility parameter (h_+/h_0) as a function of Chol content. With increasing Chol content, the mobility parameter decreases and then starts to increase at about Chol/POPC mixing ratio of 1 where CBDs are expected to form. This indicates that CBDs have higher dynamics. B) Maximum splitting, a parameter related with the order of the membrane, specifically the amplitude of wobbling motion of the long axes of both spin labels. This parameter increase with increase of Chol content and saturates at about Chol/POPC mixing ratio of 1. C) Hydrophobicity around the nitroxide moiety of ASL and CSL in POPC membranes. The nitroxide moiety of ASL is always located in the hydrophobic membrane center independently of the cholesterol content. With CSl spin label, hydrophobicity decreases with increase of Chol content. Decrease in hydrophobicity means increase in polarity. CBDs begins to form at about Chol/POPC mixing ratio of 1 (designated with the vertical, dashed line in Fig. 5)

EPR Spin-Labeling Methods

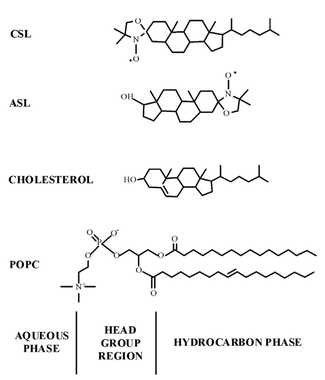

Figure 2. Chemical structure of cholesterol analogue spin labels (CSL and ASL) used in this work. Chemical structures of POPC and cholesterol (CHOL) are also included to indicate approximate locations of these molecules across the phospholipid bilayer.

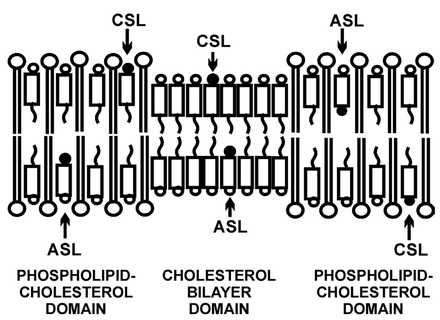

Figure 3. Schematic drawing of both spin labels CSL and ASL in their approximate locations throughout the membrane, including the cholesterol bilayer domain.

Conclusions

- The profiles of maximum splitting increase with an increase of Chol content and saturate at about 50 mol% Chol, showing that CBDs and phospholipid cholesterol domains (PCDs) have similar order.
- The profiles of mobility parameter decrease with an increase of Chol content and start to increase again after the formation of CBDs demonstrating that CBDs have higher dynamics in comparison to PCDs.
- The hydrophobicity in the interior of the membrane does not change with an increase of cholesterol content whereas near the membrane surface, hydrophobicity decreases with an increase of Chol content. This indicates the increase of water accessibility near the polar headgroup region of phospholipids.

References

[1] L. Mainali, W. J. O'Brien, and W. K. Subczynski, Formation of cholesterol Bilayer Domains Precedes Formation of Cholesterol Cyrstals in Membranes Made of the Major Phospholipids of Human Eye Lens Fiber Cell Plasma Membranes (2020), Curr. Eye Res., 45:162-172.

Acknowledgements

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