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AFM Study of Model Membrane Made of Mouse Lens Phospholipids

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Abstract

The lipid composition of the eye lens membrane differs between the species. In the lower life span animals, eye lenses have phosphatidylcholine as a major component, while lenses have sphingomyelins as a major phospholipid in higher life span animals. Here, we study the mechanical properties of model membrane made of mouse phospholipid composition with Chol/PL mixing ratio of 0 and 1, respectively using Atomic force microscopy (AFM). The model membrane's height image with 0 mol% Chol shows two phases: solid ordered (s_o) and liquid disordered (l_d). The height difference between the two phases is ~ 1 nm. The model membrane with Chol/PL mixing ratio of 1 exhibit only a single-phase known as liquid-ordered phase (lo). Thus, the membrane roughness of Chol/PL mixing ratio of 1 is 152±8 pm which is significantly lower than the roughness of membrane with Chol/PL mixing ratio of 1, which is 241±36pm. Similarly, the average breakthrough force for the Chol/PL mixing ratio of 0 is 5.4±0.41 nN, while for Chol/PL mixing ratio of 1, the average breakthrough force is 4.88±0.58 nN. We speculate that this slight difference in the membrane breakthrough force may be due to two phases in the 0 mol% Chol-containing membrane.

AFM study of model membrane made of mouse lens phospholipids

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Introduction

The lipid composition of the eye lens membrane varies among the species, age, and condition of the eye. The mouse's lens membrane comprises four major phospholipids (PLs): phosphatidylcholine, phosphatidylserine, phosphatidylethanolamine, and sphingomyelin, with phosphatidylcholine being the dominant phospholipid. Along with that, the cholesterol (Chol) in the lens membrane is significantly high. In a human eye lens membrane, the Chol/PL ratio reaches as high as 4[1]. At this high Chol in the human lens, a separate domain of Chol called cholesterol bilayer domain (CBD) is formed. There is no clear explanation for the presence of high Chol in the lens membrane. Here, we study the properties of the model membrane, resembling mouse lens phospholipid composition with Chol/PL mixing ratios 0 and 1, respectively. Four different lipids used in the PL mixture to model mouse lens membrane are; 1-palmitoyl-2-oleoyl-*sn*-glycero-3-phosphatidylcholine (POPC),1-palmitoyl-2-oleoyl-*sn*-glycero-3-phosphatidylserine (POPS), 1-palmitoyl-2-oleoyl-*sn*-glycero-3-phosphoethanolamine (POPE) and sphingomyelin (SM).

We use atomic force microscopy (AFM) to study a model membrane's properties made of mouse lens phospholipid. We used supported lipid bilayer (SLB) prepared on top of a flat mica disk to obtain the mouse model membrane's images and other mechanical properties. A rapid solvent exchange method followed by probe-tip sonication was employed to prepare small unilamellar vesicles (SUVs). These SUVs in solution were fused in a mica disk for 30 minutes under the AFM head for SLB preparation. All the experiments were performed at room temperature.

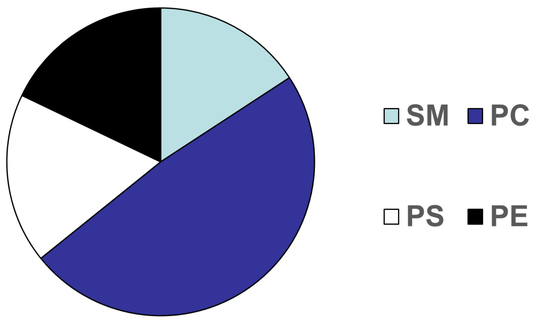

Figure 1. Distribution of mouse lens lipid membrane. Mouse lens membrane consists of phosphatidylcholine as a prominent lipid[2].

Atomic Force Microscopy Methods

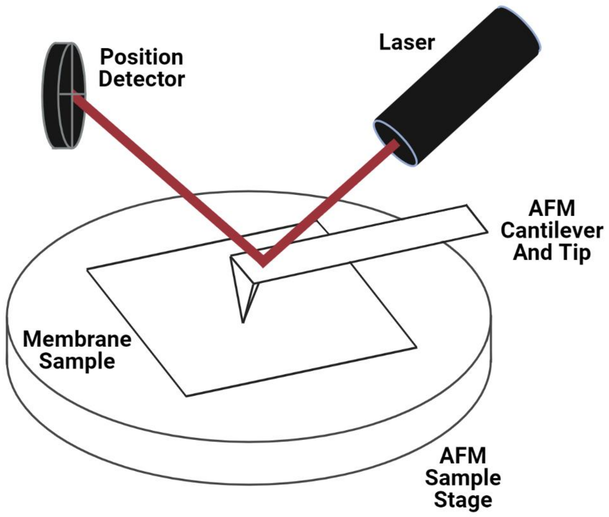

Figure 2. Schematic drawing showing the atomic force microscopy (AFM) method. The membrane sample structure is captured by the reflection of the laser received by the detector. AFM Cantilever and top are repositioned to record multiple regions for the sample by AFM stage movement in x, y, and z-direction. Bruker multimode viii AFM equipped with nanoscope v controller was used to take the images and force curves in this experiment.

Results

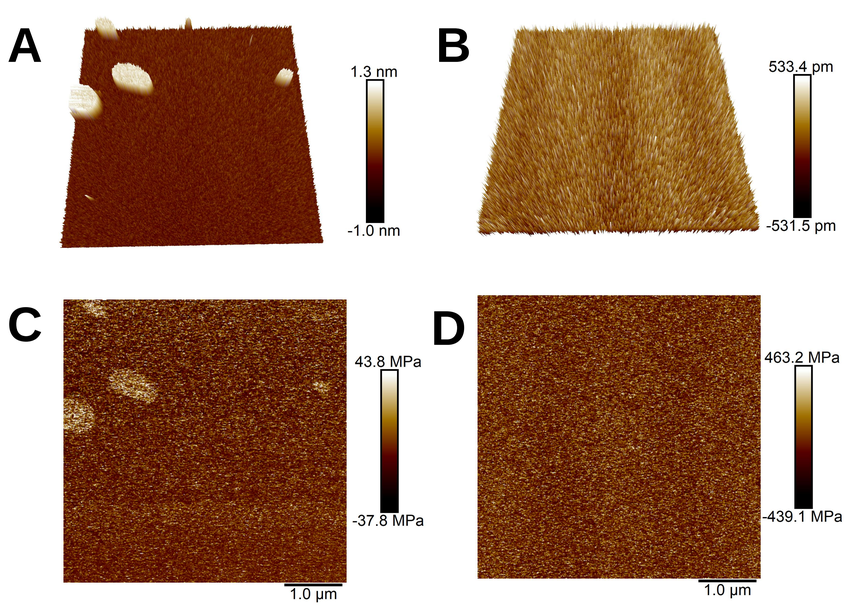

Figure 3. 3D Images of mouse lipid membrane containing a Chol/PL mixing ratio of 0 (A) and a Chol/PL mixing ratio of 1 (B) in the top panel. Phase separated domains seen in 0 mol% Chol containing membrane are transformed to single-phase membrane with the addition of 50 mol% Chol in the model mouse lipid membrane. The height difference between the domains as seen in Chol/PL mixing ratio of 0 is ~ 1nm. The modulus of the same membranes is shown in the lower panel for a Chol/PL mixing ratio of 0 (C) and a Chol/PL mixing ratio of 1 (D).

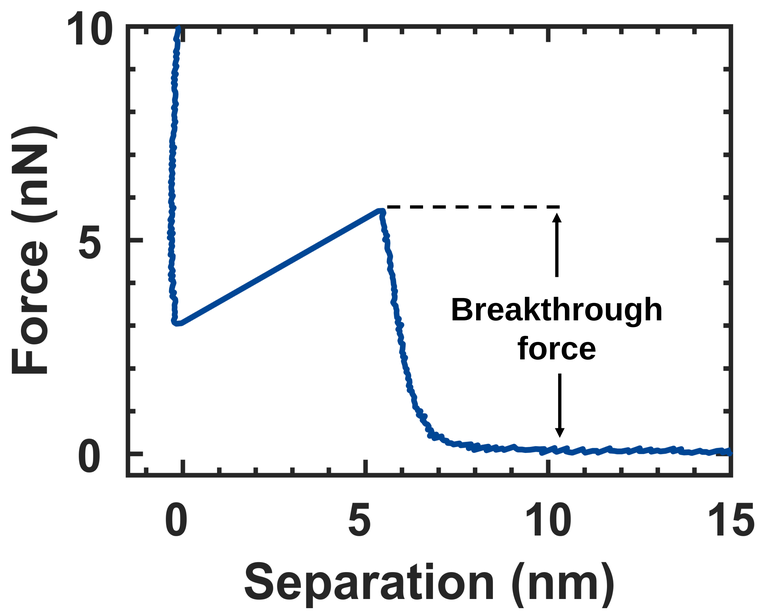

Figure 4. An example of force curve obtained from AFM in a model membrane of mouse lens phospholipids containing a Chol/PL mixing ratio of 0. This curve represents the typical interaction of the AFM tip with the supported membrane and plots the force experienced by AFM tip as a function of tip-substrate separation. The sharp point in the force curve represents the puncture of the membrane and the force associated is called the breakthrough force (F_B).

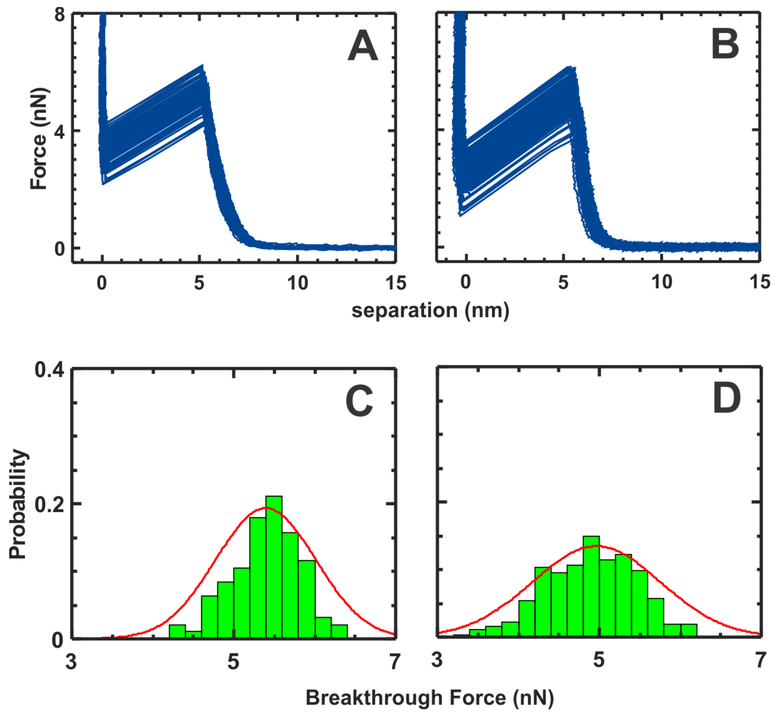

Figure 5. Collection of the force curves from the model membrane of mouse lens phospholipid with a Chol/PL mixing ratio of 0 (A) and a Chol/PL mixing ratio of 1 (B). Y-axis of the data shows the force experienced by the AFM tip, and the x-axis shows the separation of the tip from the mica surface. More than 100 force curves obtained from the membrane of each mixing ratio type are shown here. The distribution of the breakthrough forces obtained from the same force curves for membranes containing a Chol/PL mixing ratio of 0 (C) and a Chol/PL mixing ratio of 1 (D) are shown in the lower panel. The red line is the Gaussian fit for the breakthrough forces.

Table 1. Roughness and the average breakthrough of the model membrane of mouse lens. Data are shown as average ± SD

	Roughness (Rq) in pm	Breakthrough Force (nN)
Chol/PL (0/1)	241±36	5.4±0.41
Chol/PL (1/1)	152±8	4.88±0.58

Conclusions

- The height image of the model membrane of mouse lens phospholipid without Chol has two phases; solid ordered (s_o) and liquid disordered (l_d) phases. The height difference between these phases is ~ 1nm. However, the membrane with a Chol/PL mixing ratio of 1 shows only a single-phase known as the liquid-ordered phase (l_o).
- The model membranes containing a Chol/PL mixing ratio of 1 are smoother than the membranes with no Chol as indicated by the decreased average R_q from 241 pm for Chol/PL mixing ratio 0 to 152 pm.
- The average breakthrough force for membrane without Chol is 5.44 ± 0.41 nN, while for Chol/PL mixing ratio of 1 is 4.88 ± 0.58 nN. We speculate the slight difference between breakthrough forces for the membrane with 0 and 50 mol% Chol may be due to different phases in the membranes.

References

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