Elucidation of inter-leaflet coupling in asymmetric membranes induced by very long chain sphingomyelin based on solid-state NMR

(1 Department of Chemistry, Graduate School of Science, Osaka University, 2Faculty of Medicine, Oita University, 3Department of Materials Chemistry, Graduate School of Engineering, Nagoya University) ○Tanatchphong Keeratisakulsith1, Yuichi Umegawa1, Hiroshi Tsuchikawa2, Shinya Hanashima1, Michio Murata1, Wataru Shinoda3

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Recent molecular dynamic (MD) simulation study on asymmetric membrane has shown that interdigitated acyl chains of N-lignoceryl-sphingomyelin (LSM, Fig. 1) containing an outer leaflet of the system preferentially reduce the partitioning of cholesterol (Chol, Fig. 1) content on the domain of the opposite leaflet, which induces an anti-registration domain distribution. Upon this finding, further investigation on the domain modulation property in each leaflet of biomembrane-like model bilayers is a crucial aspect to deepen our understanding of interleaflet coupling interactions in biological membranes. Thus, to elucidate the interleaflet interactions, we performed local mobility analysis of C labelled 1-palmitoyl-2-oleoyl-sn-glycero-3-phosphocholine (1,13C-POPC, Fig. 1) using solid-state NMR in both LUV symmetric and asymmetric membrane systems.

In this study we adopted the cyclodextrin (CD) catalyzed lipid-exchange method between donor multilamellar vesicle (MLV) and acceptor large unilamellar vesicle (LUV) to prepare asymmetric LUV (aLUV). We have successfully prepared N-palmitoyl-sphingomyelin (PSM, Fig. 1) /1-13C-POPC/Chol aLUV with methyl-alpha-cyclodextrin (Mα-CD) mediated lipid-exchange method. However, to our knowledge, there has been no example of preparation of LSM-containing PC/Chol asymmetric system by using the lipid-exchange method. Therefore, we tried to set up the conditions for the lipid exchange using light scattering measurements. The results showed that LSM-CD complex formation was facilitated at ~65°C at a 40-fold molar ratio of methyl-beta-cyclodextrin (Mβ-CD)/LSM; higher concentration of CD could be essential for efficient incorporation of longer acyl-chain SM species in CD.

We have also performed chemical shift anisotropy (CSA) measurements on symmetric membrane system of both LSM/1-13C-POPC/Chol and PSM/1-13C-POPC/Chol at 23°C, which did not show significant difference in chemical shift anisotropy between the two systems. This result revealed that no significant ordering effect on the local carbonyl position of the 13C-POPC probe. As suggested by the MD simulation study, the CSA measurements with aLUV systems could lead to their significant difference, which is currently underway.


Figure 1. Chemical structures of lipids, 1'-13C-POPC, Chol, PSM, LSM.