Investigation of Inner Leaflet Lipids Influence on Sphingomyelin Domain

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Keywords: sphingomyelin, phosphatidylserine, liquid ordered domain, DPH anisotropy, fluorescence lifetime

Lipid rafts are functional domains that exist in biological membranes and play an essential role in signal transductions. Recently, the interaction of sphingomyelins (SMs) was revealed to be crucial for forming the lipid rafts by assembling the nanometer-scale SM domains within the liquid-ordered (Lo) phase[1]. The disruption of membrane homeostasis as seen for apoptosis partially exposes inner-leaflet lipids to the outer-leaflet, but its effect on the SM domain formation has not been evaluated. In this study, we examined the order of the membrane and the SM-gel domain in the presence of the inner leaflet lipids, 16:0-18:1 PS (POPS) or 16:0-18:1 PE (POPE) (Fig. 1) using fluorescence anisotropy and fluorescence lifetime with different fluorescence probes.

The DPH anisotropy showed that the stability of the palmitoyl chain of PSM is reduced in the order of PC(low) < PS < PE(high). However, adding cholesterol (Cho) to induce the Lo phase made the difference insignificant. In sharp contrast, a decrease in the average fluorescence lifetime of *trans*-parinaric acid (tPA) indicated that PE and PS disrupt the order of the SM gel phase while the effects of PC are relatively small. We also observed this phenomenon in the Lo phase consisting of SM and Cho. Based on the results, the headgroups of the PX (X=C, S, E) lipids affect the interaction of the saturated/unsaturated lipid chains (PO) with the SM-gel phase or the SM-Cho Lo phase, resulting in the difference in the degree of chain ordering. The decrease in the tPA lifetimes suggests that POPE and POPS, normally localizing in the cytosolic leaflet, destabilize the SM domains in the lipid rafts.

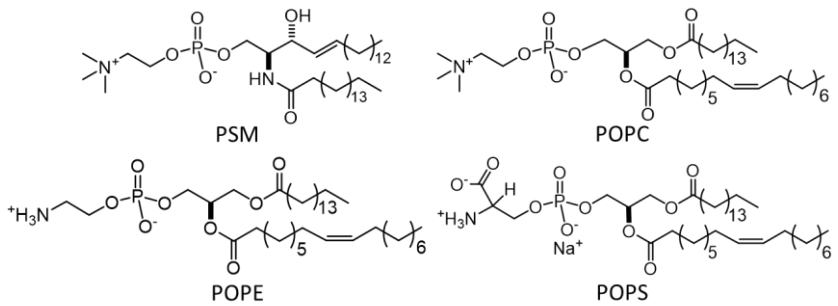

Fig. 1 Chemical structures of the lipids used in ths study

1) Yano, Y.; Hanashima, S.; Tsuchikawa, H.; Yasuda, T.; Slotte, J. P.; London, E.; Murata, M. *Biophys. J.* **2020**, *119* (3), 539–552.