Static/Dynamic Structural Investigation of Lipid Nanodiscs

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Phospholipid Nanodiscs are lipid-protein complexes in which amphipathic helices, such as apolipoprotein A-I (apoA-I) and its truncated proteins surround the edge of the bilayer. Nanodiscs can be formed by simply mixing apoA-I and lipids (such as dimyristoylphosphatidylcholine (DMPC)) at the gel-liquid crystalline phase transition temperature of the lipid, or by solubilizing these compounds into a micellar solution of cholate with subsequent removal of the surfactant. The reported size of Nanodiscs constituted by apoA-I ranges from 7 to 12 nm, depending on the lipid used or lipid/protein composition. However, their detailed structure and characteristics, especially the dynamic properties of lipids in Nanodiscs, are not well understood.

We previously succeeded in determining the rates of interbilayer exchange and flip-flop of dimyristoylphosphatidylcholine (DMPC) in large unilamellar vesicles (LUVs) by small-angle neutron scattering (SANS) technique [1]. In the present study, we elucidated the static and dynamic properties of apoA-I/DMPC Nanodiscs with SANS.

ApoA-I was isolated from pig plasma. DMPC was mixed with buffer containing apoA-I (DMPC:apoA-I = 80:1 (mol/mol)) and incubated at 25 °C for 8h. Nanodiscs formed were separated from coexisting vesicles and lipid-free proteins by density gradient ultracentrifugation. Nanodiscs consisting of either d54-DMPC (D-disc) or DMPC (H-disc) were prepared.

SANS profiles of D- and H-disc with different scattering contrasts were fitted simultaneously, and whole diameter was estimated at 9.4 nm. From the core volume, the molecular area of DMPC was calculated as 0.50 nm2, which is much smaller than the molecular area in vesicles (0.66 nm2), suggesting closer lipid packing.

Time-resolved SANS measurement was started immediately after mixing an equivalent volume of D-disc and H-disc. Time-course of the normalized contrast was calculated from the scattering intensity. The decay curves at four different temperatures were reproduced well by single-exponential function to provide the rate constant (kex). Noteworthy, the decays were more than 20-fold faster than the theoretical decay with the exchange rate for LUVs. Arrhenius plot for kex suggested that DMPC desorbs from Nanodiscs via enthalpically unfavorable but entropically favorable process. These distinct dynamic properties of lipids in Nanodiscs, including a decreased activation energy compared with LUVs, could be ascribed to the entropically more unstable state in Nanodiscs as suggested by the static SANS measurements. That is, the lower entropy state derived from the closer lipid packing countervails the decremental entropy on the lipid desorption.

In conclusion, the static and time-resolved SANS study clarified the fast lipid dynamics in Nanodiscs, which is connected with the static properties of bilayers altered by the envelopment with the proteins.

References

[1] M. Nakano, M. Fukuda, T. Kudo, H. Endo, T. Handa, Phys. Rev. Lett. 98 (2007) 238101.