ELUCIDATION OF LIPID COMPLEX FORMATION MECHANISMS BY STATIC/DYNAMIC STRUCTURAL EVALUATION

Minoru NAKANO
Graduate School of Pharmaceutical Sciences, Kyoto University
E-mail: mnakano@pharm.kyoto-u.ac.jp

A variety of lipid assemblies are fabricated in animal bodies, among which lipids exchange to maintain the homeostasis. Cellular membranes are the stage of signal transduction, where membrane–protein interactions could be controlled by microstructural modification of the membranes. Apprehension of the membrane microstructure and the interaction with proteins from dynamic point of view, as well as static one, is therefore required to explicate the biological functions of the biomembranes. The author has performed a series of experiments regarding biologically-related lipid-lipid and lipid-protein interactions using model membrane systems and X-ray, neutron, and fluorescence techniques, and analyzed the static/dynamic properties of the membranes. The findings are summarized as follows:

1) Evaluation of Lipid Dynamics by Small-Angle Neutron Scattering

The author has designed a method to determine the rate of lipid transfer between liposomes by modifying a so-called "contrast variation method", which is conventional in small-angle neutron scattering (SANS). This technique takes advantage of the large difference in the scattering length density between hydrogenated and deuterated lipids, and the exchange of these lipids between liposomes results in a decrease in the scattering intensity, which can be detected in situ by time-resolved SANS. The observed kinetics of the scattering decays could be explicitly represented by a simple model that includes two independent kinetic parameters, i.e., the rates of transbilayer exchange (flip-flop) and interbilayer exchange. These lipid dynamics were shown to be strongly dependent on the acyl-chain length and headgroup size of phospholipids, and to be affected by the presence of cholesterol. This powerful technique can be applied to the evaluation of enzymatic activities relevant to lipid migration, such as translocases and lipid transfer proteins.

2) Membrane–Protein Interaction Related to HDL Biogenesis

In a process of HDL biogenesis, disc-like lipid-protein complexes are formed by interaction of apolipoprotein A-I (apoA-I) with a membrane protein, ABCA1. This process plays a crucial role in cholesterol homeostasis, however, its detailed mechanism remains unrevealed. The author has investigated the interaction of apoA-I with model membranes by phsico-chemical approach. Nonlamellar phase-forming lipids such as phosphatidylethanolamine have been shown to increase both the acyl-chain lateral pressure and headgroup hydration in phospholipid membranes, and consequently enhance the membrane binding of amphipathic alpha helices, which is an entropy-driven process. Sphingomyelin had a contrary effect on the helices' binding, however, membranes containing this lipid were destabilized and transformed into the disc-like complexes by the binding of apoA-I, due to the coexistence of gel/liquid crystalline phases. The author also revealed that the increased helicity and hydrophobicity of apoA-I at lower pH regions trigger the formation of discs and that an acidic lipid reinforces the disc formation by reducing pH at membrane surface. These results put forward a possibility that ABCA1 capacitates apoA-I for its binding and disc formation by modifying the local lipid composition and pH or inducing the local phase separation.

3) Fabrication of the Lipid Dispersion System of Nonlamellar Liquid Crystalline Phases and Its Application to the Dynamic Property Evaluation of Membrane

The author has succeeded to prepare lipid nanoparticles that include nonlamellar phases, such as bicontinuous cubic and inverted hexagonal phases. This was achieved by the use of amphiphilic polymers as an emulsifier, because the polymers were found not to perturb the internal structures due to their low compatibility with lipids. Fabrication of the dispersion of liquid crystalline phases enabled us to apply spectroscopic analysis to the nonlamellar phases. The author succeeded to extract dynamic properties of the nonlamellar phases, such as the order parameter and wobbling diffusion coefficient, from the fluorescent analysis of the dispersions, and revealed that the increased membrane packing stress by lipids with negative curvature was released during lamellar-nonlamellar phase transition.

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