Development of an assay for the intermembrane transfer of cholesterol by Niemann-Pick C2 Protein: A model for drug delivery

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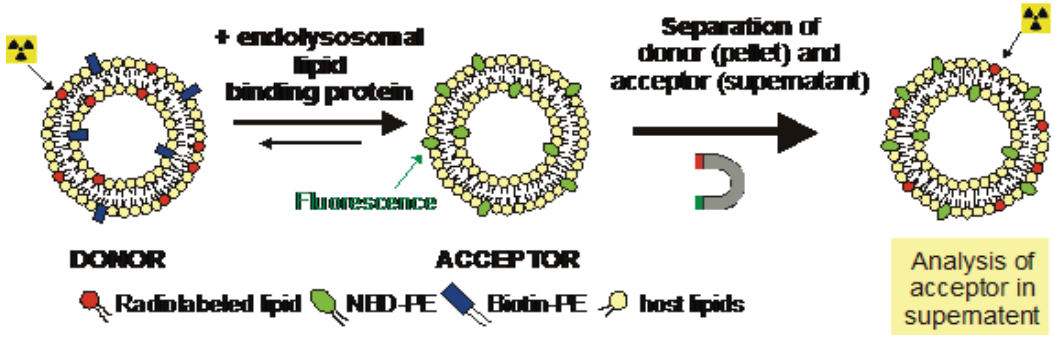
Cholesterol is an essential component of mammalian cellular membranes with crucial structural and regulatory functions. Its amount and intracellular distribution is tightly regulated by *de novo* synthesis, esterification and intracellular transport; otherwise severe metabolic disorders such as Niemann-pick type C (NPC), Wolman syndrome and hypercholesterolemia may result. Previous cholesterol transfer studies were hampered because cholesterol lacks a functional group that could readily be tagged without affecting the physical and chemical properties. Transfer studies of fluorescent cholesterol esters or cholesterol analogs do not correctly reflect the properties of unmodified cholesterol. We therefore developed a convenient, liposomal assay system that allows for probing intermembrane transfer of radioactively labelled cholesterol from donor to acceptor vesicles.

Bovine NPC2 (bNPC2) was isolated from milk and purified as previously described[1]. Donor and acceptor liposomes were designed to mimic the composition of inner lysosomal membranes. Donor liposomes in addition contain 1 mol % ^{14}C-cholesterol and 4 mol % of Biotin-derivative of phosphatidylethanolamine (Biotin-X-DHPE) while the acceptor liposomes also contain fluorescent lipid, NBD-PE, to facilitate the monitoring of structural integrity of liposomes. Each type of liposome was prepared by mixing appropriate amount of lipids, drying and hydrating in appropriate buffer solution. The dispersion was subjected to 8 freeze-thaw cycles, sonication and extrusion through polycarbonate filters in a mini-extruder.

The final amount of donor and acceptor liposomes in the assay mixture was 4 and 20 nmol of lipid respectively in a total volume of 200 µl. The transfer was started by the addition of bNPC2 and the samples were incubated at the appropriate temperature and time. Cholesterol transfer was stopped by adding 66 µl of 1 M Tris buffer (pH 8). Streptavidin-coated paramagnetic particles were added to separate donor and acceptor liposomes. The mixture was incubated for at least 15 minutes to allow complete binding of Biotin-X-DHPE-containing donor liposomes by *BioMag* streptavidin. The tubes were then placed in a magnetic separation stand to pull the donor-streptavidin complex to one side of the tube wall. Acceptor vesicles were then quantitatively transferred into other reaction tubes for radioactive and florescence measurements. The amount of cholesterol transferred from donor to acceptor was calculated[2] from the readings taking care of the various control experiment readings.

NPC2 catalyzes transfer to acceptor vesicles in a dose- and time-dependent manner. This also depends on temperature, pH, ionic strength, lipid composition of the model membranes, and the ratio of donor to acceptor vesicles. This assay system promises to be a valuable tool for quantitative and mechanistic studies of protein-mediated lipid transfer and drug delivery.



References

1. Friedland, N., Liou, H.L., Lobel, P., and Stock, A.M. *Proc. Natl. Acad. Sci USA* 2003, **100**: 2512-2517.
2. Babalola J.O., Wendeler, M., Breiden, B., Arenz, C., Schwarzmann, G., Locatelli-Hoops, S., and Sandhoff, K. *Biol Chem*. 2007, **388:** 617-626.