Biosynthesis Of Deuterated Lipids for Structural and Biophysical Characterization of Biomembranes And Membrane Proteins

Qiu Zhang[1], Honghai Zhang[1], Matthew Keller[2], Wellington Leite[1], Shuo Qian[1], Robert Hettich[1], Hugh O'Neill[1]
[1]Oak Ridge National Laboratory, [2]University of Tennessee
zhangq@ornl.gov

Membrane proteins play crucial roles in many cellular processes, however, studying membrane proteins is challenging because of their complex structure and fragility when isolated from their native environment. One solution is to embed membrane proteins in a membrane-mimic to provide a more native environment to facilitate their characterization. Small-angle neutron scattering (SANS) is an ideal technique to obtain structural information on biomacromolecules under physiologically relevant conditions. With this technique, deuterated phospholipids need be used to suppress their 1H signal in SANS measurements. Currently, there are three ways to obtain deuterated phospholipids; extraction of native lipids from cells produced in deuterated media, chemical synthesis, or semi-synthetic approaches that combine both routes. In this study, we report on producing deuterated phosphatidylethanolamine (PE) by extraction and fractionation from native Escherichia coli extracts, and phosphatidylcholine (PC) from an engineered E. coli strain. The PC synthase (PCs) pathway was introduced into E. coli to produce partially deuterated and perdeuterated PC by feeding deuterated E. coli cultures with hydrogenated or deuterated choline chloride. The isolated PC product was confirmed by 1 H Nuclear Magnetic Resonance (NMR) and Liquid Chromatography - Mass Spectrometry (LC-MS) was used to determine the deuteration level of PC produced under different growth conditions. These materials can be used for neutron scattering studies with micelles, bicelles, liposomes, styrene-maleic acid lipid particles (SMALPs), and Membrane Scaffold Protein (MSP)-based lipid nanodiscs to produce a membrane-mimicking environment for studying membrane proteins, and can be used for deuterated lipids for NMR studies as well.