Title

Human Niemann-Pick Type C2 Disease Protein Expression, Purification and Crystallization

Authors

Yurie T. Kim, *University of Massachusetts Amherst* Follow

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Abstract

Niemann-Pick type C2 (NPC2) protein is a soluble protein that binds unesterified cholesterol. The protein helps transport unesterified cholesterol in tandem with the membrane protein Niemann-Pick type C1 (NPC1). Defects in either of proteins can cause Niemann-Pick type C disease (NPC), which results in the accumulation of unesterified cholesterol and lipids in the late endosome and lysosome. NPC is an autosomal recessive lysosomal storage disease affecting 0.35~2.20 per 100,000 people. Here we present the structural analysis of the human NPC2 glycoprotein, including expression, purification, functional analysis, homology modeling, and crystallographic studies. Human NPC2 was expressed from baculovirus-infected Trichoplusia ni (Tn5) insect cells. The construct contained a hexahistidine purification epitope tag, and the protein was purified using Nickel affinity column chromatography. The purified protein was used in binding studies with dehydroergosterol (DHE), showing that human NPC2 was functional. Using the structure of bovine NPC2, we made a homology model and mapped the human mutations onto the model. Some modeled proteins, such as the V30M and S67P variants, are unclear as to how they lead to disease, thus a structure of the human protein would be informative. Crystallization screens of human NPC2 were performed and led to crystals with a needle-like morphology, which diffracted to 4Å resolution. The structure of human NPC2 will be useful for understanding the mechanism of cholesterol binding and trafficking in cells, and to better understand the human metabolic disease NPC.

First Advisor

Scott C Garman

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