Modalities of Cholesterol Binding and Modulation of the NPC Proteins and Scap

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Low density lipoproteins (LDL) and related plasma lipoproteins deliver cholesterol to cells by receptor-mediated endocytosis. The lipoprotein is degraded in late endosomes and lysosomes, allowing cholesterol to be released. Export of cholesterol from late endosomes and lysosomes (hereafter referred to as lysosomes) requires two lysosomal proteins: Niemann-Pick C2 (NPC2), a soluble protein of 132 amino acids; and NPC1, a membrane protein with 13 putative membrane-spanning helices. Recessive loss-of-function mutations in either NPC2 or NPC1 produce NPC disease, which causes death owing to lipid accumulation in lysosomes of liver, brain, and lung.

Consistent with their cholesterol export role, NPC2 and NPC1 both bind to cholesterol. The cholesterol binding site on NPC1 is located in the NH2-terminal domain (NTD), which projects into the lysosomal lumen. This domain, designated NPC1 (NTD), can be expressed in vitro as a soluble protein of 240 amino acids that maintains cholesterol binding activity. This thesis studies NPC2 in detail as summarized below.

Despite a shared role as cholesterol binding proteins, NPC2 and NPC1 (NTD) bind to cholesterol in opposite orientations. The crystal structures of NPC2 and NPC1 (NTD) have been solved, and NPC2 binds cholesterol with the iso-octyl chain facing the interior of the protein, whereas, NPC1(NTD) binds cholesterol with the 3ß-hydroxyl facing the interior of the protein. Another striking difference is the kinetics of this cholesterol binding. NPC2 binds and releases cholesterol rapidly (half-time < 2 min at 4oC), while NPC1 (NTD) binds cholesterol very slowly (half-time > 2 hr at 4oC). However, NPC2 can stimulate the rate of cholesterol binding to NPC1 (NTD) (>15-fold in vitro). This stimulation of cholesterol binding to NPC1 (NTD) by NPC2 is believed to occur through a direct transfer of cholesterol from NPC2 to NPC1(NTD).

Amino acid residues important for binding or transfer of cholesterol on NPC2 were identified through alanine scan mutagenesis. Residues that decreased binding thermodynamics and/or kinetics mapped to areas surrounding the binding pockets on the crystal structures; residues that decreased transfer, but not binding, mapped to discrete surface patches near the exposed residues of the binding pockets. These surface patches may be sites where the two proteins interact to transfer cholesterol. The most deleterious binding mutant was P120S, a residue in the cholesterol binding pocket; the most deleterious transfer mutant was V81D, a residue on the hydrophobic patch extending outward from the cholesterol binding pocket. The above mutants of NPC2 were unable to rescue LDL-stimulated cholesteryl ester synthesis in NPC2-deficient cells, in contrast to wild-type NPC2.

Once LDL-derived cholesterol leaves the lysosomes, it is transported to the endoplasmic reticulum (ER), where it serves a regulatory role in cholesterol homeostasis. In the ER, these regulatory functions include activation of acetyl-coenzyme A acetyltransferase (ACAT), allowing for esterification of cholesterol for storage, and regulation of sterol regulatory element–binding protein (SREBP) localization, a transcription factor that regulates key enzymes for cholesterol synthesis.

SREBP cleavage-activating protein (Scap) is the switch that controls SREBP, and therefore cholesterol synthesis. Scap senses cholesterol abundance in the ER and acts as an escort protein. In sterol depleted cells, Scap escorts SREBP to the Golgi complex, where two proteases cleave SREBP, thereby releasing its transcriptionally active domain so that it can go to the nucleus and activate transcription of genes involved in cholesterol synthesis and uptake. When cholesterol in abundant, the sterol binds to Scap and triggers a conformational change in the protein that prevents it from escorting SREBPs to the Golgi for proteolytic cleavage.

Scap is a 1276 amino acid protein that consists of two domains: an N-terminal domain with 8 transmembrane spanning regions and a C-terminal domain that projects into the cytosol and associates with SREBPs. Previous studies have localized the cholesterol-binding activity of Scap to its membrane domain. Studies described in this thesis identify the cholesterol binding pocket in Scap and identify key residues that play an important role in the protein's responsiveness to cholesterol binding.

The first loop region of Scap (hereafter referred to as Scap(Loop1)) was purified as a recombinant protein and found to have cholesterol binding activity. The specificity of this sterol binding was determined through competition studies and shown to be physiologically relevant. Additionally, this binding affinity and specificity was similar to that of the membrane domain of Scap. Subsequently, alanine scan mutagenesis was performed on Scap(Loop1). Through this approach, several mutations of Scap were identified that constitutively adopt the cholesterol-bound state. This data demonstrates that Scap(Loop1) binds to cholesterol and that the binding then helps induce the conformational change required for Scap to anchor SREBP in ER membranes.