MS7-P10 Optimizing expressed cyclic peptide library generation: a quantitative and structural study

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In recent years peptides have been rediscovered as potential drugs, due to the increasing demand for the discovery of new therapeutic molecules. Particularly cyclic peptides (CPs) belong to the most effective, high-affinity drug-like agents, which exhibit a wide range of biological activities. CPs are extremely variable in the structures and conformations they adopt and, owing to the cyclisation they are exceptional stable. In the last decade, methods have been developed to genetically encode and express libraries of CPs, taking advantage of the protein-splice reaction carried out by split-inteins. In combination with an in vivo screening assay this provides a powerful tool for the straightforward identification of the active members against a chosen target. In this split-intein mediated ligation of proteins and peptides (SICLOPPS).[1] However the efficiency and velocity of the cyclization reaction strongly depends on the split-intein used and target sequence to be cyclized.[2] This results in great variation of the amount of CP produced and bias of the library towards certain sequences.

Here we compare efficiency as well as sequence bias of CP generation of natural split-inteins DnaE from *Synechocystis sp PCC6803* and *Nostoc punctiforme*. Using synthetic ^{13}C-labeled reference peptides and liquid chromatography mass spectrometry we quantitatively determined the cyclization efficiency in vivo of different hexameric target sequences. In order to elucidate the molecular mechanisms for the observed target sequence preferences, we determined the crystal structures of the pre-splice complexes of the split-inteins with different CP sequences. These data will help to assess the potential quality and bias of the genetically encoded CP screening library generated using split inteins.

Literature

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MS7-P11 Structural features of NPC1L1 and NPC1 proteins in complex with cholesterol: comparison between X-ray crystal structure and docking study

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Cholesterol homeostasis in the human body is maintained by *de novo* synthesis, intestinal absorption, and biliary and fecal excretion. The absorption of dietary cholesterol in intestine is mediated with NPC1L1 (Niemann-Pick type C1 like 1), a polytopic transmembrane protein of 1332 amino acids, which shares sequence homology with NPC1 (Niemann-Pick type C1). Like its homolog, NPC1L1 was predicted to have a typical signal peptide and 13 membrane-spanning domains, which is in consistent with recent experimental data. The X-ray crystallography structure of this domain without cholesterol (PDB id: 3QNT) shows it has a conserved *N*-terminal "NPC1" domain, and extensive *N*-linked glycosylation sites.

Since its discovery, several studies demonstrated the importance of NPC1L1 as one of it the key players in both dietary cholesterol absorption and biliary cholesterol re-absorption. On the other hands, NPC1 and NPC2 are main players of cholesterol control in lysosome and it is known that mutation of one of these proteins leads to disease, called Niemann-Pick disease type C (NPC) disease. The crystal structures of *N*-terminal domain (NTD) of NPC1 were determined with and without cholesterol (PDB id: 3GKI and 3GKH).

The structure of NPC1L1 in complex with cholesterol can provides insights into the mechanism of the cholesterol mediation by NPC1L1 and we attempted to get the NPC1L1-cholesterol complex structure with molecular docking study. Comparison of the resulting complex structure with that of NPC1-cholesterol complex can give better understand of the role of conserved "NPC1" domain absorption of cholesterol in small intestine. In this study, we have re-determined the crystal structure for NPC1L1 protein. We will propose the structure of NPC1L1 in complex with cholesterol and the role of NPC1L1-cholesterol complex in bulk cholesterol endocytosis of enterocyte membrane. We believe the current study can contribute the development of cholesterol absorption inhibitor for hypercholesterolemic patient ultimately.

Keywords: NPC1L1, cholesterol