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Title of Doctoral Dissertation

Molecular basis of lipid presenting and loading mechanisms of CD1b
(CD1b の脂質抗原提示と脂質積込み機構の分子基盤)

CD1b is one of glycoproteins expressed on surfaces of various antigen-presenting cells. CD1b is related to Major Histocompatibility Complex (MHC) class I molecules and is involved in the presentation of lipid and lipid-based molecules to activate T cells. CD1b recognizes glycolipids derived from drug-resistant bacteria such as *Mycobacterium tuberculosis*. CD1 is initially expressed in endoplasmic reticulum (ER) with endogenous lipids and transported to cell surface through secretion pathway. CD1 on the cell surface is recycled by endocytosis and antigen is replaced to exogenous lipids facilitated by saposin proteins. Saposin is endosome/lysosome resident protein and is responsible for lipid transportation to various proteins. One purpose of this research is to elucidate the lipid recognition mechanism of CD1b, and another one is to elucidate the mechanism of lipid transportation from saposin to CD1b.

For the preparation of CD1b protein, CD1b and β2m were expressed as a single peptide with a flexible linker, called as a single chain CD1b (scCD1b) by using silkworm-baculovirus expression system. scCD1b protein was purified by Ni-affinity chromatography, size exclusion chromatography and followed by anion exchange chromatography. The purified scCD1b protein was successfully crystallized using commercially available crystallization screening kit. The X-ray diffraction dataset up to 2.4 Å was collected and the structure was solved by molecular replacement method. The electron density map of the scCD1b showed the presence of endogenous ligand in the binding pocket. Therefore, the lipid was extracted from the scCD1b protein and analyzed by thin layer chromatography. The result showed that the protein binds cholesteryl, phosphatidylethanolamine, and phosphatidylcholine when CD1b was expressed by the silkworm-baculovirus expression system. The endogenous lipids were observed in the A' and T' pockets, the ligand in the T' pocket may act a chaperon-like function to stabilize the structure.

Glycerol monomycolate (GroMM) is one representative lipid from the *mycobacterium tuberculosis*. It has been proved that the GroMM can be presented by CD1b and recognized by the CD1b-restricted T cells. To reveal the GroMM recognition mechanism of CD1b, synthetic

glycerolipid was used for the crystallization. Glycerolipid has only one acyl chain but has the same head group compared to GroMM. The structure of CD1b-glycerolipid complex was determined at 2.9 Å. The structure showed that the acyl chain of glycerolipid inserted into the A' pocket of CD1b, and the head group protruded out of the ligand binding pocket to interact with TCR. CD1b-glycerolipid was superimposed with TCR-CD1b-GMM structure, the head group protruded out of the ligand binding pocket to interact with TCR. The different binding positions of this two ligands may reflect the different immune responses triggered by CD1b-restricted T cells.

Saposin A, B, C and D proteins were expressed and purified from *E.coli*. To reveal the mechanism how saposin proteins transfer lipids to CD1b, pull down assay experiment was conducted. The result showed no saposin proteins had ability to bind CD1b at pH 8.0, whereas only saposin C bound to CD1b at pH 4.8. In addition, the binding analysis was conducted at pH ranged from 4.5 to 8, and the result showed that saposin C binding to CD1b is pH-dependent and is optimal at acidic pH, which is equivalent to that of the endolysosomal compartments.

Saposin proteins form oligomer in the presence of detergents or lipids. To reveal oligomer states of saposin C, saposin and detergent/lipid complexes were prepared. In this study, oligomerization of saposin C was induced by excess amount of LDAO. The phase determination by molecular replacement was failed, this is probably due to considerable conformational change between crystallized saposin C and search models. The structure of saposin C and LDAO complex was determined by ab initio phasing method. The initial model showed lipid binding core of saposin C was more open compared with the published dimeric saposin C (PDB ID: 2QYP). In addition, DMPC was used to form saposin C-DMPC nanoparticle. With the increasing amount of DMPC, the size of the nanoparticle became larger observed by transmission electron microscope.

We succeeded in preparing scCD1b in which CD1b and β2m were single-stranded by using silkworm-baculovirus expression system. The crystal structure of CD1b in complex with silkworm lipids was determined at 2.4 Å. CD1b bound cholesterol, phosphatidylethanolamine, and phosphatidylcholine when expressed in silkworms. The silkworm endogenous lipids inserted into A' and T' pockets. On the other hand, the crystal structure of CD1b-glycerolipid was determined at 2.9 Å, the acyl chain of glycerolipid inserted into A' pocket and the head group were outside the ligand binding pocket to interact with TCR. The GMM glucose head group and glycerolipid glycerol head group have different recognition position to TCR. CD1b specifically bound to saposin C, and the interaction is pH-dependent and optimal at low pH. The acidic pH promotes saposin C conformational changes from close to open, and detergent or lipids can induce the formation of oligomeric saposin C.