Molecular Mechanism of the Interaction of CERT Protein with Lipid Membrane

Ceramide, which is one component of lipid molecules in cell membrane, is biosynthesized in the endoplasmic reticulum and transferred to the Golgi apparatus by CERT, a cytosolic protein. The START domain of CERT is responsible for the transfer of ceramide. We determined the crystal structures of the CERT START domain in complex with ceramide and specific inhibitors. These crystal structures reveal how CERT recognizes ceramide specifically, and how CERT interacts with membrane.

Ceramide is a member of sphingolipids, which are the major lipid molecules in cell membrane. In mammalian cells, ceramide is synthesized in the endoplasmic reticulum (ER) and transferred to the Golgi apparatus for conversion to sphingomyelin, and further metabolized to other complex glycosphingolipids. Ceramides are not only the structural elements in the lipid bilayer, but act as cell signaling molecules, such as for proliferation, cellular differentiation, programmed cell death, and apoptosis. Ceramide transport from the ER to the Golgi occurs in a nonvesicular manner and is mediated by CERT, a cytosolic 68-kDa protein [1]. CERT contains a lipid-binding START (steroidogenic acute regulatory protein-related lipid transfer) domain at the C-terminus which specifically recognizes and transfers natural D-*erythro*-ceramide efficiently, while not transferring other lipids such as sphingosine, sphingomyelin, cholesterol and phosphatidylcholine [2].

We have determined the crystal structures of the CERT START domain in the apo-form (2.2 Å resolution), and C_6-, C_{16}-, and C_{18}-ceramides bound forms (1.4–2.1 Å resolution) [3]. The overall structure is a compact α/β structure with a large amphiphilic cavity, where one ceramide molecule is buried in the cavity (Fig. 1). Two acyl chains of ceramide are arranged along the cavity lined with hydrophobic amino acids. At the far end of the cavity, the amide and hydroxyl groups of ceramide interact with specific amino acid residues via hydrogen bond networks. Mutational experiments showed the importance of these residues for the transfer activity of CERT. By comparing the structures of the apo- and the ceramide-bound forms of the CERT START domain, we speculated that the α3 and Ω1 loop might act as a gate to the entry and exit of ceramide in the cavity.

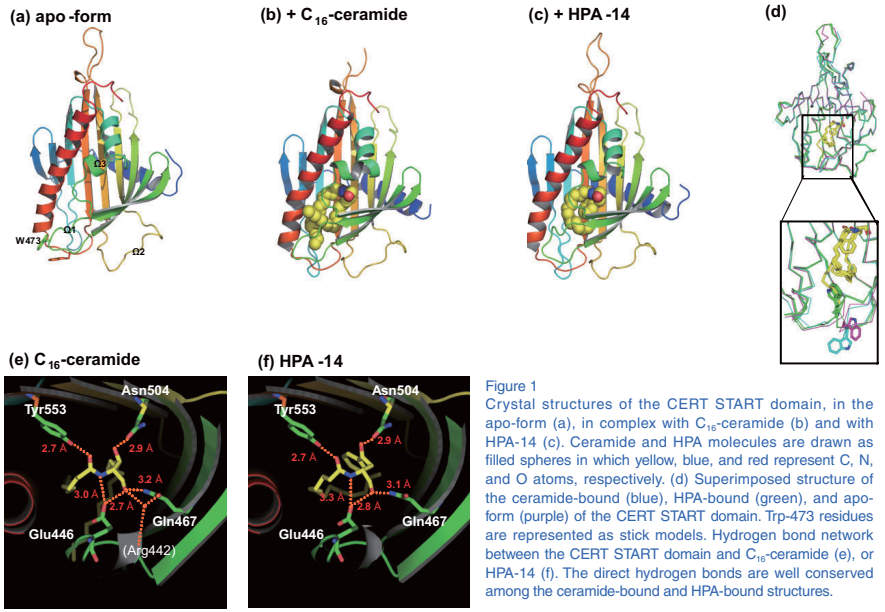

Figure 1
Crystal structures of the CERT START domain, in the apo-form (a), in complex with C_{16}-ceramide (b) and with HPA-14 (c). Ceramide and HPA molecules are drawn as filled spheres in which yellow, blue, and red represent C, N, and O atoms, respectively. (d) Superimposed structure of the ceramide-bound (blue), HPA-bound (green), and apo-form (purple) of the CERT START domain. Trp-473 residues are represented as stick models. Hydrogen bond network between the CERT START domain and C_{16}-ceramide (e), or HPA-14 (f). The direct hydrogen bonds are well conserved among the ceramide-bound and HPA-bound structures.

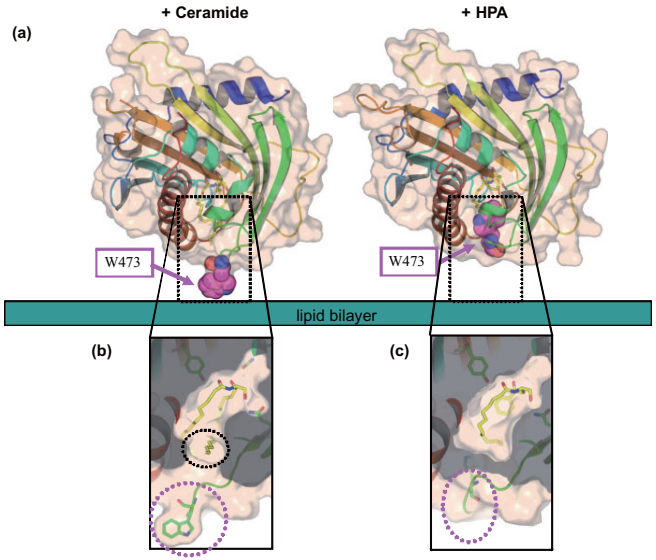

Figure 2
(a) Schematic of the CERT START domain and membrane interaction via Trp-473. Molecular surfaces are drawn in light brown. C_{16}-ceramide bound (left) and HPA-14 bound (right) structures of the CERT START domain are presented. In the case of the HPA-14-bound structure, Trp-473 exists inside the cavity, while it is exposed to the outside of the protein in the ceramide-bound structure. Trp-473 is drawn as filled spheres. Molecular surfaces of the CERT START domain in complex with C_{16}-ceramide (b) and HPA-14 (c) cut at the level of the cavity, are shown. The outer surface and cross section of the CERT START domain are drawn in light brown and gray, respectively. The purple dotted line indicates Trp-473, and the black dotted line indicates a small opening of the cavity in the ceramide-bound CERT START domain.

(1*R*,3*R*)-*N*-(3-hydroxy-1-hydroxymethyl-3-phenylpropyl)alkanamide (HPA), a synthesized analog of ceramide, inhibits ceramide transfer by CERT [2, 4]. We also determined the crystal structures of the CERT START domain in complex with HPAs with different acyl chain lengths (HPA-13, -14, -15 and -16, at 1.8–2.4 Å resolution) [5]. The hydrogen bond networks between HPAs and the protein slightly differ from those between ceramide and the protein, whereas one HPA molecule is also buried in the amphiphilic cavity of the protein (Fig. 1). Interestingly, the Ω1 loop adopts a different conformation when bound to HPA than in ceramide-bound structures. In the Ω1 loop region, Trp-473 shows the largest shift. This residue exists inside the cavity in HPAs-bound structures, while it is exposed to the outside of the protein in the apo-form and ceramide-bound complex structures. Surface plasmon resonance experiments showed that Trp-473 is important for the interaction with membranes. The conformational change of Trp-473 suggests that the Ω1 loop moves during the capture of ceramide or ceramide analogues, and these changes imply that the Ω1 loop is important for the transfer of ceramides by the CERT START domain. These structures provide insights into not only the molecular mechanism of inhibition by HPAs, but also the possible mechanisms by which CERT interacts with ceramide when it binds to the membrane (Fig. 2).

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