

# Crystal structures of the CERT START domain in complex with ceramides and specific inhibitors reveal recognition and transport mechanisms of ceramides

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Ceramide is synthesized at the endoplasmic reticulum (ER), and transferred to the Golgi apparatus where ceramide is converted to sphingomyelin. The major pathway of this transport is mediated by CERT, a cytosolic 68-kDa protein, in a nonvesicular manner. The C-terminal ~250 amino acid residues of CERT form a START domain catalyzing intermembrane transfer of ceramide (1).

Here we present crystal structures of the CERT START domain in the apo-, C<sub>6</sub>-, C<sub>16</sub>-, and C<sub>18</sub>-ceramides bound forms at 1.4-2.2 Å resolution. The overall structure is a compact  $\alpha/\beta$  structure with a long tunnel, where one ceramide molecule is buried in the complex structures. Two long hydrophobic chains of ceramide are arranged along the tunnel lined with hydrophobic amino acids, while the amide and hydroxyl groups of ceramide interact with specific amino acid residues via a hydrogen bond network. Mutations of these residues impaired the ceramide transfer activity of the CERT START domain.

We also determined the crystal structures of CERT START domain in complex with antagonists of CERT, HPA-12, 13, 14, 15 and 16, at 1.7 -2.3 Å resolution (2). Interestingly, the hydrogen bond network between HPA and the protein is slightly different from that between ceramide and the protein. There are no drastic conformational changes between the apo and the ceramide or antagonists bound structures. We will discuss how the CERT START domain can specifically recognize ceramide and its antagonists.

(1) Hanada. *et al.*, (2003) *Nature* **426**, 803-

(2) Kumagai *et al.*, (2004) *J. B. C.* **280**, 6488-HPA-12: (1*R*,3*R*)-*N*-(3-hydroxy-1-hydroxymethyl-3-phenylpropyl)dodecamide