Neutral ceramidases are key enzymes of sphingolipid metabolism that hydrolyze the fatty acyl/sphingosine amide linkage of ceramide at neutral pH. In this issue of *Structure*, Airola et al. (2015) present the first crystal structure of human nCDase and show how complexation with phosphate supports a new catalytic mechanism for Zn-dependent amidases while providing a structurally based explanation for ceramide specificity.

In eukaryotic membranes, sphingolipids play vital structural and signaling roles (Hannun and Obeid, 2008; Maceyka and Spiegel, 2014). Ceramide is a relatively simple sphingolipid (Figure 1) usually found at low concentrations in biomembranes, yet it is an essential metabolic building block for complex sphingolipids, i.e., sphingomyelin and glycosphingolipids, which are key components of lipid raft microdomains (Frisz et al., 2013; Simons and Sampaio, 2011). Ceramide also functions as a signaling molecule that can regulate various processes such as differentiation, proliferation, and programmed cell death, either directly or by serving as source of signaling metabolites. When cleaved by ceramidases (CDases), ceramide generates fatty acid and sphingosine (Figure 1). The latter can be phosphorylated to yield sphingosine-1-phosphate, a potent regulator of cell growth, survival, migration and inflammation (Maceyka and Spiegel, 2014). Thus, CDases are central regulators of both sphingolipid and cellular homeostasis.

CDases consist of three families distinguished by their pH optima (acid, neutral, alkaline), subcellular localization, amino acid sequence, mechanism, and function (Mao and Obeid, 2008). While acidic CDase functions in the lysosome during normal turnover of sphingolipids, neutral CDase and the three alkaline CDases carry out ceramide catabolism in the gut and regulate signal transduction and cell adhesion (Mao and Obeid, 2008; Duan and Nilsson, 2009). Despite their importance, structural insights into CDases have been sparse. Previously, the only crystal structures for CDase were those of the bacterial neutral ceramidase (bCDase) from *Pseudomonas aeruginosa* determined in apo-form and in complex with ceramide containing a short, nonphysiologic N-acetyl group (Inoue et al., 2009). The paucity of structural information has hampered understanding of the molecular workings of ceramidases.

In this issue of *Structure*, Airola et al. (2015) present the crystal structure of human neutral ceramidase, nCDase, determined by molecular replacement using bCDase as a model. The N-glycosylated extracellular region of nCDase was crystallized in complex with phosphate and its structure was determined at 2.6 Å resolution. The overall architecture of nCDase is similar to that of bCDase and composed of a catalytic domain connected to an immunoglobulin (IG)-like domain by a linker region. The authors, however, observe important differences in the catalytic domain structure.

In the human protein, the catalytic domain contains an extra 30-residue subdomain inserted within the loop between β14 and α7 (Figure 2; orange). This insert replaces a 6-residue span forming a shorter but analogous connecting loop in bCDase. In the respective apo- and holo-bCDase structures, the 6-residue span displays different conformations, facing outward (apo) or inward (holo) with regards to the Zn-containing active site. In the bCDase holo-structure, the active site is covered by another long and flexible loop containing the α8-helix (Figure 2; gray), which is more disordered.
Figure 2. Important Novel Features Found in the Crystal Structure of Human Neutral Ceramidase

The overall structures of human nCDase (PDB ID 4WGK) and bCDase (PDB ID 2ZXC), are similarly composed of IG-like domain (yellow) and catalytic domain (cyan) shown in surface representation. The differently positioned n2-loop–s8 element in nCDase (magenta) and loop–s8 region in bCDase (gray) are portrayed in cartoon representation, with the black arrows indicating the beginning and the end of divergence. The subdomain insert (orange) of nCDase is shown in ribbon representation. Bound phosphate atoms are in space-filling representation, with Zn obscured and not visible behind the phosphate. The upper blowup shows the suggested mobility of the nCDase subdomain determined by superposition of the two protein molecules of the asymmetric unit. Two disulfide bonds are shown as sticks. The lower blowup shows the suggested position of bound ceramide (yellow stick representation with oxygen in red and nitrogen in blue) in the transition state within the catalytic center (not shown) derived from the position of tetrahedral phosphate (orange stick representation with oxygen in red) in the crystal structure of nCDase.

in apo-bCDase, and could function as a movable lid for the pocket. The position of the lid is probably stabilized by interaction with bound ceramide, as well as with the adjacent 6-residue span in the “inward” conformation.

Interestingly, in human nCDase, the novel 30-residue subdomain insert (Figure 2; orange) replacing the 6-residue span of bCDase (Figure 2; gray), displays specific mobility (Figure 2; upper blowup) revealed upon superposition of two protein molecules of the asymmetric unit. Stabilization of the subdomain conformation is aided by two internal disulfide bridges, formed by four cysteines that are conserved in eukaryotes. While the role of this movable but conformationally rigid subdomain is not absolutely clear, it probably stabilizes the adjacent region of the catalytic domain, a loop-like connector of the n2- and s8-helices. The entire n2- “loop”–s8 element of human nCDase (Figure 2; magenta) differs both conformationally and positionally from the analogous part of the bCDase holo-structure (Figure 2; gray), despite the rest of both structures displaying close similarity (Figure 2; surface representation). The positioning of the nCDase n2-“loop”–s8 element ‘along the edge’ of the lipid hydrophobic pocket enables access by phosphate that localizes over the deeply embedded Zn-ion (Figure 2). The authors propose that the deeply embedded catalytic Zn\(^{2+}\) ion at the bottom of the hydrophobic pocket sterically excludes sphingolipids with larger headgroups while facilitating specific recognition of the small hydroxyl headgroup of ceramide. They also suggest that their crystal structure can serve as a model for the transition state of ceramide hydrolysis, in which the position of the phosphate atoms coincides with the amide group of ceramide (Figure 2; lower blowup). A general acid-base catalysis mechanism is proposed for amide bond hydrolysis by nCDase. In this mechanism, the Zn\(^{2+}\) ion functions to activate a water molecule for nucleophilic attack of the amide carbon. His196 serves as a general base for proton extraction from water and subsequently, a general acid to shuttle this proton to the nitrogen of ceramide during amide bond cleavage.

The authors convincingly develop their logic and support their ideas by simulations that involve docking computations. What emerges is a proposed catalytic mechanism for ceramidase action that is unique among amidases by involving stabilization of the transition state oxygen through protein side-chain interactions rather than by direct Zn-coordination. Notably, all conserved residues of the nCDase active site participate in the proposed mechanism, making for an especially intriguing, persuasive, and powerful reaction scheme.

The study represents a significant contribution to our current knowledge of ceramidase while providing the very first crystal structure of a eukaryotic ceramidase. The new structural information for human nCDase in complex with the transition state analog phosphate provides valuable insights for redefining the catalytic mechanism of this novel lipid amidase. The question still remaining for future studies is the degree to which the modeled, substrate-bound structural state portrays the conformational and positional behavior of human neutral ceramidase during hydrolysis of native ceramide substrates.

REFERENCES


Previews

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