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Fructan 1-ExoHydrolase IIa from *Cichorium intybus* in complex with ligands

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Fructan 1-Exohydrolase (1-FEH) is involved in fructan degradation. It can remove a terminal fructose from fructans, but not from sucrose. Together with the invertases and fructosyl transferases, the fructan exohydrolases belong to the glycosyl hydrolase family 32 (GH 32). Earlier, we reported the structure of 1-FEH IIa from *Cichorium intybus* [1]. Here, the 1-FEH IIa is described in complex with different inhibitors and substrates. All structures are characterized with one single molecule in the active site, positioned in the β-propeller domain. 1-FEH IIa in complex with sucrose, a strong competitive inhibitor, was resolved with a resolution of 2.50 Å. Also complexes with fructose and 2,5 dideoxy-2,5-imino-D-mannitol, two weaker inhibitors, were elucidated at a resolution of 2.65 Å and 3.25 Å, respectively. In order to create a complex with the best substrate of 1-FEH IIa, 1-kestose, an inactive mutant was produced (E201Q). The complex of 1-FEH IIa and 1-kestose was resolved with a resolution of 3.05 Å. The comparison of the complexes can clarify why sucrose acts as a strong inhibitor whereas 1-kestose acts as an ideal substrate.

[1] Verhaest, M., Van den Ende, W., Le Roy, K., De Ranter, C. J., Van Laere, A., and Rabijns, A. (2005). X-ray diffraction structure of a plant glycosyl hydrolase family 32 protein: fructan 1-exohydrolase IIa of *Cichorium intybus*. Plant J. 41, 400-411.

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Crystal structure of plant asparaginase

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LlA, a protein from *Lupinus luteus*, is a dual-function enzyme highly expressed in developing seeds. On the one hand, it serves as potassium-independent L-asparaginase in the hydrolysis of L-asparagine, the main nitrogen-transport compound, to L-aspartate and ammonia in order to supply nitrogen to developing tissues. On the other hand, it exhibits isoaspartyl peptidase activity and may me involved in the hydrolysis of the aberrant β-peptide bond in isoaspartyl peptides. Isoaspartyl peptides, which accumulate with aging, inhibit germination and specific hydrolytic enzymes are required to support proper plant development. So far, no structural information about plant asparaginases has been available. LlA crystals were obtained from recombinant material. They are triclininic and contain two functional LlA molecules in the asymmetric unit. The structure has been determined at 2.6 Å by molecular replacement using an *E. coli* homolog as the search model. The crystal structure of LlA confirms the classification of potassium-independent plant asparaginases in the family of Ntn-hydrolases, with an N-terminal nucleophile. The α- and gb-subunits that form the mature $(\alpha\beta)_2$ dimer of heterodimers arise from autoproteolytic cleavage of two copies of a precursor protein. In common with other Ntn-hydrolases, the (αβ) heterodimer has a sandwich-like fold with two β-sheets flanked by two layers of α-helices (αββα). The nucleophilic Thr193 residue, which is liberated in the autocatalytic event at the N-terminus of the β-subunit, is part of the active site that is similar to that observed in a homologous bacterial enzyme. A chloride anion complexed in the LlA structure marks the position of the α-carboxylate group of the L-aspartyl substrate/product moiety. The so-called sodium-binding loop of the bacterial enzyme, which is necessary for proper positioning of all components of the active site, shows conserved conformation and metal coordination in the plant enzyme. Unexpectedly, the structure of this plant protein provides interesting clues about the structure and function of the related taspase-1, an enzyme implicated in some human leukemias.