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Family portrait of L-asparaginases taken by crystallography, with an important new member

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The simple hydrolysis of the amide group at the side chain of L-asparagine, leading to L- aspartate and ammonia, has at least three enzymatic solutions in the living organisms, catalyzed by enzymes classified in three structural Classes and five types. Class 1 L-asparaginases, previously known as bacterial-type (a frequent misnomer in this field, as representatives can usually be found in all domains of life), are the most thoroughly studied, as high affinity type II enzymes from this group are used as potent antileukemic drugs. Their catalytic center, originally interpreted as a T-K-D version of the serine protease triad, has been recently re-interpreted as a system of two T residues and a proton relay with an activated Y residue and a conduit of H-bonded water molecules, that works according to a double-displacement mechanism. The origin of the Y residue in these homotetrameric enzymes differentiates type I and II. Class 2 L-asparaginases are Ntn-hydrolases, i.e. they are produced as single-chain precursors that undergo autoproteolytic activation, liberating a T nucleophile at the N terminus of subunit β. The enzymatic activity (which can also hydrolyze L-Asn modifications, such as β-peptides or glycosylation) is based on a pair of T residues, which also work in a ping-pong mode. The mysterious Class 3 enzymes have been found so far in bacteria and in pathogenic fungi. The prototypic type V enzyme ReAV is found in *Rhizobium etli*, the nitrogen-fixing symbiont of common bean. By structural homology to such enzymes as serine β-lactamases or glutaminases, the active site in this Class was identified as consisting of two Ś-K tandems plus a unique and odd zinc coordination site that plays a role in substrate docking but not in catalysis. One of the S residues is the primary nucleophile, with the rest of the tandems serving as activator and proton sink. In some of the high-resolution crystal structures of ReAV[1] and its homologs, the S nucleophile is surrounded by three close electron density peaks arranged in a tetrahedral manner. Originally interpreted as an unusual hydration pattern, this mystery motif is now interpreted as a covalent modification.

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Reference

1. J. I. Loch, B. Imiolczyk, J. Śliwiak, A. Wantuch, M. Bejger, M. Gilski, M. Jaskolski, Nature Commun. 12 (2021) 6717.