Poster

Unusual helical crystal packing of potassium-independent L-asparaginase from *Phaseolus vulgaris.*

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Plant-type (Class 2 in new classification) L-asparaginases are a group of the Ntn-hydrolases, which mature via autoproteolytic cleavage into subunits α and β, and are further divided into potassium-dependent and potassium-independent enzymes. Ntn-hydrolases utilize an N-terminal nucleophile (Thr in this case) in their catalytic mechanism and share the same αββα sandwich fold. Potassium-dependent L-asparaginase from *Phaseolus vulgaris*, PvAIIIK(+), was characterized previously, including the elucidation of the mechanism of its activation by K^+ ions [1]. However, the potassium independent enzyme PvAIIIK(-), which has a lower affinity for L-Asn, is poorly studied. Here, we present the unique crystal structure of the mature PvAIIIK(-) enzyme, fully cleaved into subunits α and β, with eight independent $(\alpha\beta)_2$ dimers in the asymmetric unit. The structure is intriguing from the crystallographic point of view. The PvAIIIK(-) crystals show a complicated fourfold twining in combination with structural pseudosymmetry. The crystals have the rare *P*2 symmetry, with pseudosymmetric 4_1-like helical packing. The eight dimers are segregated into two helical arrangements. Within each helical assembly, the huge 18-stranded central molecular β-sheet of each dimer is extended in both directions by similar β-sheets of its neighbors. In this fashion, an infinite β-sheet helix is generated along [001] throughout the entire crystal. Each "protein helix" (parallel to [001]) is then complemented by its antiparallel copy generated by the crystallographic twofold axis. In almost all PvAIIIK(-) molecules, the flexible linker, dangling at the C-terminus of subunit α after autoproteolytic cleavage, is not visible, exception for two chains, where nine and three linker residues can be modeled. A linker from one chain forms a molecular bridge to the complementary chain in the protein "double helix", thus additionally stabilizing the crystal packing.

[1] Bejger M. et al. (2014). Acta Cryst. D**70**, 1854-1872.

Supported by National Science Centre (NCN, Poland) grants 2020/38/E/NZ1/00035 and 2020/37/B/NZ1/03250.