DOI 10.18699/SBB-2022-180

Structure features of novel D-amino acid transaminase from *Aminobacterium colombiense*

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Key words: biocatalysts, transaminases, structure biology

Motivation and Aim: D-amino acid transaminases (DAATs) are pyridoxal-5'-phosphate-(PLP)-dependent enzymes that catalyze reversible and stereoselective transfer of the amino group between D-amino acids and α-keto acids. DAATs belong to fold type IV of the PLP-dependent enzymes in which the PLP forms a covalent bond with the catalytic Lys residue as well as a number of noncovalent conservative interactions among TAs of fold type IV. In the active site of known DAATs, two modes of the organization of residues responsible for the substrate binding are observed. The first type is observed in the DAAT from *Bacillus suptilis* (bsDAAT, PDB ID: 3DAA) [1], in which α-carboxylic group is bound by a "carboxylate trap" formed by three residues – Y31 from the βX-strand and R98*, H100* from the O-loop. The second type is observed in TAs from *Curtobacterium pusillum* (CpuTA, PDB ID: 5K3W) [2] and *Haliscomenobacter hydrossis* (Halhy, PDB ID: 7P7X) [3]: here the α-carboxylic group of substrate is bound by two residues, R51*, K117 in CpuTA and R28*, R90 in Halhy. The R51* and R28* residues are directed to the active site from the adjacent subunit, and the K117 and R90 residues are located on the βY-strand. DAAT from *Aminobacterium colombiense* (Amico) in the active site possesses residues similar to the bsDAAT (K99* and H101* on the O-loop) and both CpuTA and Halhy (R27* and R88). To understand the Amico's active site organization, structure of holo-form and the R88L variant were determined by the RSA.

Methods and Algorithms: The gene encoding Amico was optimized, cloned, and expressed with His-tag in *E. coli* cells. The recombinant form of the enzyme was isolated by Ni-IMAC chromatography, and then the His-tag was cut off using a TEV-protease. Subsequent purification of the enzyme included size-exclusion and anion exchange chromatography steps. Crystals of Amico and R88L variant were obtained by the "hanging drop" vapor diffusion method. The X-ray diffraction data were collected at the ID30A-3 beamline of the ESRF synchrotron (Grenoble, France). The data were indexed, integrated, and scaled using Dials [4]. The structures were analyzed in the PyMol data visualization program.

Results: Crystal structure of holoform of Amico was obtained at 1.9 Å resolutions, of R88L variant – at 1.8 Å. The asymmetric unit of enzyme contains two subunits and organization of a dimer is typical of TAs of PLP fold type IV (Fig. 1A). The PLP cofactor is covalently bound to K142 and divides the active site into two pockets: O-pocket (phenolic side) and P-pocket (phosphate side). Noncovalent interactions of PLP with the active site residues in Amico are similar known TAs of fold type IV, except for the E172 residue. Residue E172, which forms a hydrogen bond with the N1 atom of the pyridine ring of PLP, has a different orientation in the subunits: in subunit B it forms hydrogen

bond with N1 atom, however, in subunit A it coordinates N1 atom through the water molecule W131 (Fig. 1B). The O-loop is located outside the O-pocket in the Amico dimer (Fig. 1, C), otherways then in bsDAAT, CpuTA and Halhy. Residue R27* is directed to the active site and does not form hydrogen bonds with any neighboring residues, while residue R88 forms a network of hydrogen bonds with three residues – Q26*, Y90 and E113 (Fig. 1, C). In R88L variant the observed changes in the active site residue positions pointed to the disturbance of the active site integrity due to R88L substitution.

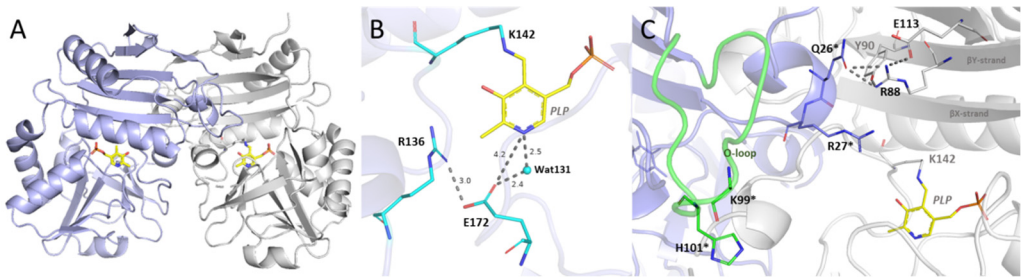

Fig. 1. The structure of Amico. (A) The active dimer of Amico, (B) binding of the N1 atom of PLP and the position of residue E172 in subunit A, (C) organization of the O-pocket. The PLP molecule is shown in yellow, the O-loop in green, the PLP-binding residues in cyan, the A subunit residues in gray, and the B subunit residues in purple

Conclusion: The structure of the Amico holo form and R88L variant were obtained. We found an unusual mobility of the E172 residue that coordinates the N1 atom of the PLP cofactor and a tightly anchored R88 residue in the active site. Thus, the R88 residue may be restricted in mobility during catalysis, unlike similar residues in CpuTA and Halhy, and bear both structural and catalytic function. The removal of the O-loop from the Amico's active site may indicate that K99* and H101* residues are not involved in binding of the α-carboxylic group of substrates.

Acknowledgements: The study is supported by the Russian Science Foundation project 19-14-00164.

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