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

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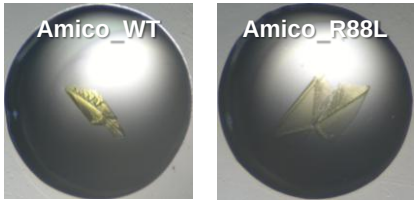
D-amino acid transaminase (DAAT) from *Aminobacterium colombiense* (Amico) is pyridoxal-5'-phosphate-(PLP)-dependent enzyme that catalyze reversible and stereoselective transfer of the amino group between D-amino acids and α-keto acids. The amino acid sequences determining the substrate specificity of Amico share similarities with those of canonical DAAT from *Bacillus suptilis* (bsDAAT) as well as noncanonical DAATs from *Curtobacterium pusillum* (CpuTA) and *Haliscomenobacter hydrossis* (Halhy). Structure of holo-forms of the Amico wild-type (Amico_WT) and the R88L variant of Amico (Amico_R88L) were determined by the RSA. The resulting structures were compared with those of bsDAAT, CpuTA and Halhy.

Crystallization



Method: hanging-drop vapor diffusion

Crystallization conditions:

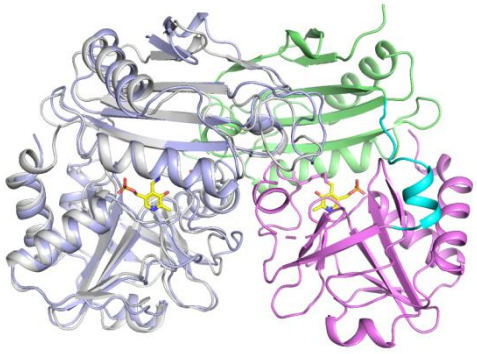
WT **- 0.2 M NaNitrate, 0.1 M Bis-tris propane pH 6.5 + 20% PEG3350, 15 °C**

R88L **– 0.2 M NaNitrate, 0.1 M Bis-tris propane pH 7.8 + 1% organic acid (aminobenzoic acid, salicylic acid, trymesic acid) + 10% PEG3350, 15 °C**

The X-ray diffraction data were collected at the ID23-1 beamline of the ESRF (Grenoble, France)

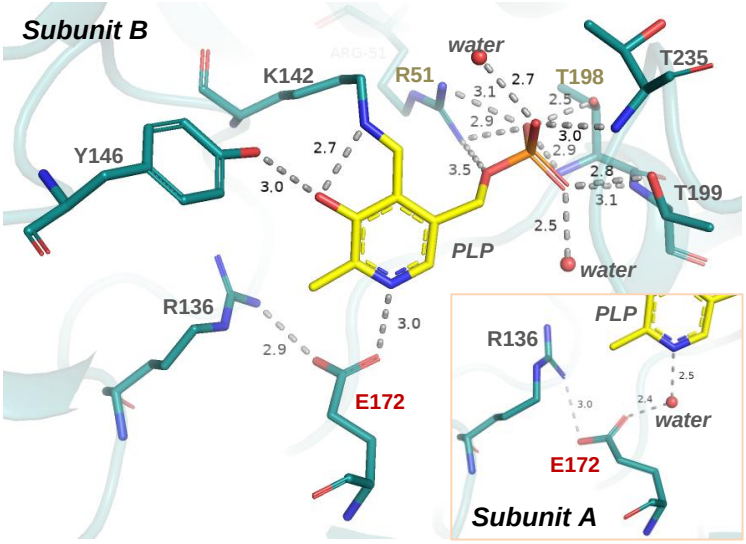
Resolutions: WT – 1.9 Å, R88L – 1.85 Å

3D-Structure of Amico



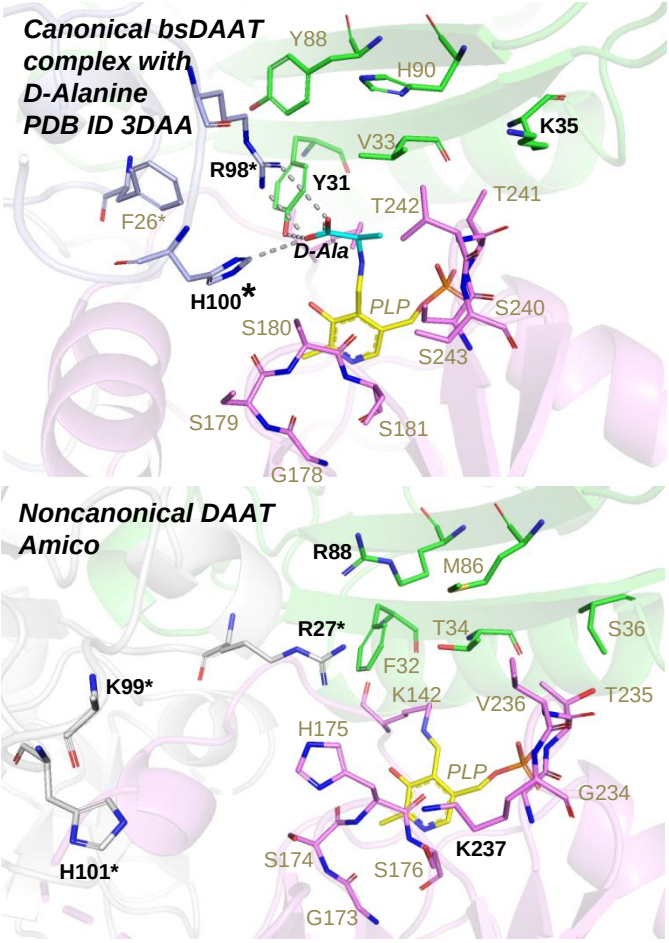
The active dimer of Amico. In the right subunit, the small domain is green, the large domain is pink, and the interdomain loop is cyan. The left subunit (gray) is aligned to the bsDAAT subunit (blue, PDB ID 1DAA). The PLP molecules are yellow.

Binding of the PLP cofactor in the active site of Amico



Residue E172, which forms a hydrogen bond with the N1 atom of the pyridine ring of PLP, has two different orientations.

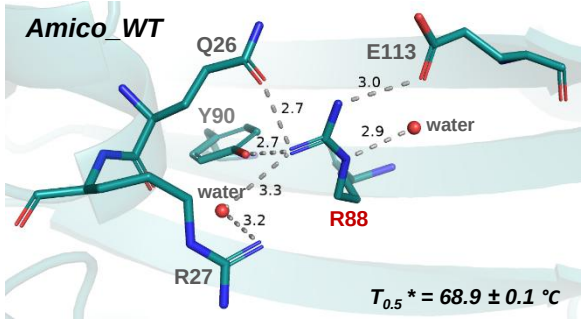
Active sites of Amico and bsDAAT

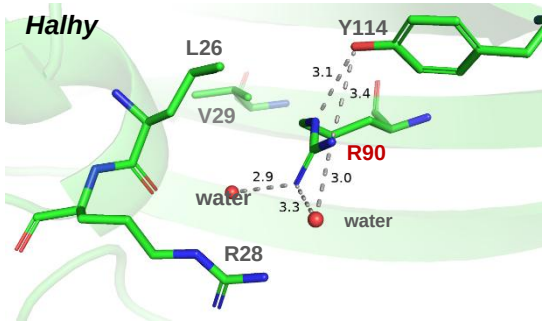


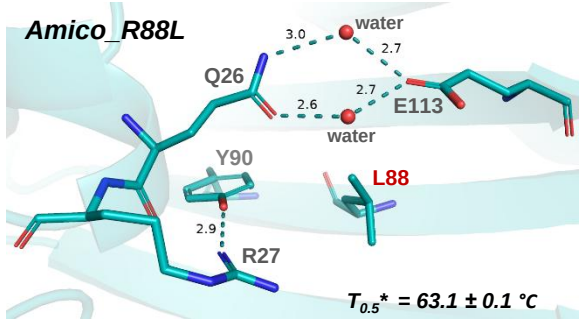
Substrate	k_{cat} / K_D, $M^{-1} s^{-1}$				
	WT	R27L	R88L	K99A, H101A	K237A
D-Glutamic acid	360 ± 140	0.064 ± 0.003	42 ± 2	180 ± 7	4.7 ± 0.1
D-Alanine	5 ± 1	Not detected	1.0 ± 0.4	5 ± 1	1.7 ± 0.5

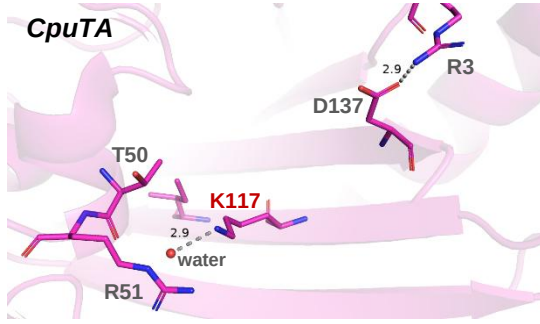
Specificity constant (k_{cat}/K_D) of Amico to the D-Glutamic acid and D-Alanine in half-reactions.

Structural role of the Arg88 residue





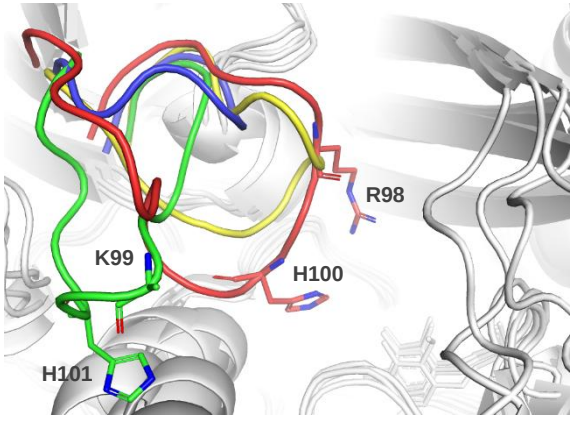




*$T_{0,5}$ is the half-transition temperature between native and denaturated states during thermal unfolding

Arg90 in Halhy and Lys117 in CpuTA correspond to Arg88 in Amico.

O-loop of Amico



Alignment of Amico, bsDAAT, Halhy and CpuTA structures. Amico O-loop is shown in green, bsDAAT in red, Halhy in yellow and CpuTA in blue.

Conclusions

- **Amico_WT and Amico_R88L structures were obtained with resolutions of 1.9 and 1.8 Å, respectively. The asymmetric unit of enzyme contains two subunits and organization of a dimer is typical of TAs of PLP fold type IV.**
- **R27, R88 and K237 residues are important for Amico catalysis of D-amino acids transamination.**
- **Residue E172, which forms a hydrogen bond with the N1 atom of the pyridine ring of PLP, has two different orientations.**
- **In the Amico dimer the O-loop is located outside the O-pocket, otherways then in bsDAAT, CpuTA and Halhy.**
- **Residue R88 forms a rigid network of hydrogen bonds with neighboring residues and contributes to the stability of the active dimer structure.**