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Interaction of D-cycloserine with a D-amino acid transaminase from *Haliscomenobacter hydrossis*

Bakunova A.*, Matyuta I., Nikolaeva A., Boyko K., Popov V., Bezsudnova E.
Bach Institute of Biochemistry, Research Centre of Biotechnology, RAS, Moscow, Russia
** albakunova@mail.ru*

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Motivation and Aim: Studying the structures of enzyme complexes with inhibitors is a great way to explore the substrate recognition mode of the enzyme. Recently, we characterized pyridoxal-5'-phosphate (PLP)-dependent D-amino acid transaminase (DAAT) from bacterium *Haliscomenobacter hydrossis* (Halhy) with a new type of the recognition site for the substrate α-carboxyl group [1]. D-cycloserine (D-CS) is a cyclic analog of serine. Various PLP-dependent enzymes, including transaminases (TA), are able to accommodate D-CS in their active sites. D-CS is proposed to react with TA through the canonical sequence of reaction steps and finally forms irreversibly a stable aromatic compound with PLP or dissociates from the active site [2–5]. Determination of the structure of the Halhy complex with D-CS can shed light on both the mechanism of D-CS interaction with Halhy and the mode of substrate binding in the active site of the new type of DAAT.
Methods and Algorithms: We explored the interaction of D-CS with Halhy by the combination of UV-Vis spectroscopy, enzyme kinetics and X-ray crystallography.
Results: D-CS is a strong inhibitor of Halhy with IC_{50} value of 3 μM. The inhibited enzyme shows an absorbance maximum near 330-340 nm, indicative of a completed catalytic half-reaction. In the crystal structure D-CS is covalently attached to the cofactor and presents in two different states: an intermediate product, external aldimine, and a stable aromatic compound. Contrary to expectations, D-CS is not held by numerous noncovalent interactions in the active site. The only hydrogen bond was found between the oxygen of D-CS carbonyl group and the ε-amino group of the catalytic lysine.
Conclusion: D-CS binds PLP irreversibly; however, the excess of PLP restores the activity of Halhy. In the active site of Halhy D-CS interacts specifically with PLP, however, no specific interaction with the residues of the recognition site is observed. The modes of the natural substrates binding and D-CS coordination are different.
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