Poster Presentation

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Structure based protein engineering to confer selectivity of guanine deaminase

A. Bitra[1], R. Anand[1]

[1]*Indian Institute of Technology Bombay,Department of Chemistry, Mumbai, India*

Guanine deaminases (GDs) are important enzymes involved in both purine metabolism and nucleotide anabolism pathways. Here we present the molecular and catalytic mechanism of NE0047 and use the information obtained to engineer specific enzyme activities. NE0047 from Nitrosomonas europaea was found to be a high fidelity guanine deaminase (catalytic efficiency of 1.2 × 105 M−1 s−1). However; it exhibited secondary activity towards the structurally non-analogous triazine based compound ammeline. The X-ray structure of NE0047 in the presence of the substrate analogue 8-azaguanine help establish that the enzyme exists as a biological dimer and both the proper closure of the C-terminal loop and cross talk via the dimeric interface is crucial for conferring catalytic activity. It was further ascertained that the highly conserved active site residues Glu79 and Glu143 facilitate the deamination reaction by serving as proton shuttles. Moreover, to understand the structural basis of dual substrate specificity, X-ray structures of NE0047 in complex with a series of nucleobase analogs, nucleosides and substrate ammeline were determined. The crystal structures demonstrated that any substitutions in the parent substrates results in the rearrangement of the ligand in a catalytically unfavorable orientation and also impede the closure of catalytically important loop, thereby abrogating activity. However, ammeline was able to adopt a catalytically favorable orientation which, also allowed for proper loop closure. Based on the above knowledge of the crystal structures and the catalytic mechanism, the active site was subsequently engineered to fine-tune NE0047 activity. The mutated versions of the enzyme were designed so that they can function either exclusively as a GD or serve as specific ammeline deaminases. For example, mutations in the active site E143D and N66A confer the enzyme to be an unambiguous GD with no secondary activity towards ammeline. On the other hand, the N66Q mutant of NE0047 only deaminates ammeline. Additionally, a series of crystal structures of the mutant versions were solved that shed light on the structural basis of this differential selectivity.

[1] *A.Bitra, H.Bhukya, A.S.Tanwar, et al, Biochemistry, 2013, 52, 3512−3522,* ***[2]*** *A.Bitra, A.Biswas, R.Anand, Biochemistry, 2013, 52, 8106-8114*

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