Establishing Structure-Activity Relationship of an Enzyme with Unknown Function

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One of the most important challenges in biology is to identify the function of a protein that a gene encodes. As a result of structural genomics project a large number of protein structures are deposited in protein data bank (PDB) with no functional information. Deciphering the exact function of the protein is still a daunting task. Some of the challenges faced in the field are that many enzymes may either catalyze many types of reactions with varying catalytic efficiency or may possess several potential substrates if more than one analogue with same functional group can be accommodated in the active site.

In this study we identified the function of NE0047 from *Nitrosomonas Europaea* an enzyme with known structure but unknown function. A combination of bioinformatics, biochemical and X-ray crystallographic studies were employed to determine the function. Sequence similarity network and structural complementarily studies gave a clue that NE0047 is a probable base deaminase. Subsequent experimental studies revealed that the enzyme under study is a guanine deaminase with a catalytic efficiency of 1.2×10^5 M^{-1} sec^{-1}, which is comparable with other guanine deaminases reported in literature. Along with guanine this enzyme can also accept 8-azaguanine, atrazine as substrates. In order to understand the structural basis of substrate specificity, we have solved the crystal structure of the enzyme with various substrates and substrate analogs. In addition, a systematic mutational study of the active site was performed to decipher the role of individual residues in catalysis and to decipher the mechanism of deamination.

We have successfully identified the function of NE0047 and conclude that it primarily serves as a guanine deaminase. Additionally, it also exhibits a moonlighting activity towards ammeline deamination, which is an intermediate involved in the melamine pathway. A structural analysis of the member belonging to this superfamily reveals that although the core fold is conserved among all family members, the substrate binding site is diverse and each deaminase has evolved to accommodate its specific ligand. In addition, X-ray crystallographic and mutational studies show that optimal positioning of the deaminating amine group of the substrate is vital in conferring activity. Furthermore, we also demonstrate that along with zinc, two glutamic acid residues in the active site are involved in proton transfer reactions for the removal of ammonia.