Catalysis in the nitrilase superfamily amidases; implications from active site structure

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The nitrilase superfamily amidases catalyze the conversion of various amides to their corresponding acid and ammonia using a highly conserved Glu, Lys, Cys catalytic triad in an acid-base catalysis mechanism. Some of these enzymes are potential biocatalysts in the fine chemical industry, while others like the amidase domain of the NAD⁺ synthetase from Mycobacterium tuberculosis (MTB) are attractive drug targets.

We have recently solved the crystal structure of the amidase from Geobacillus pallidus RAPc8. The most interesting observation in this structure arises from the size and the geometry of the active site pocket, which is arranged in such a way that the reaction intermediate restricts access to the glutamic acid (Glu59) previously thought to be the general base catalyst for the hydrolysis of the acyl intermediate. An alternative choice for a general base catalyst is another glutamic acid residue (Glu142), which has not been characterized before, and which we found to be highly conserved in other structures from the nitrilase superfamily. We have also very recently solved the structure of another amidase from Nesterenkonia sp. The position and coordination of the second glutamic acid residue (Glu139) is also conserved in this amidase. We have proposed a catalytic mechanism that postulates the involvement of this additional glutamic acid as a fourth catalytic residue in the amidases of the nitrilase superfamily. We are presently investigating the role of this residue using both biophysical and structural methods.

Mass spectra from the Geobacillus and Nesterenkonia sp. amidase mutants where the proposed general base catalyst glutamic acid residue has been changed to a leucine and a glutamine respectively, indicate that tetrahedral intermediates of various substrates are being trapped in the active site. This confirms that this residue is indeed involved in catalysis. To further confirm these findings, crystals of the E139Q Nesterenkonia amidase mutant reacted with various substrates have been prepared. The progress on this work will be presented.