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The mechanism of the amidases: Mutating the glutamate adjacent to the catalytic triad inactivates the enzyme

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Abstract content
   (Max 300 words)

All known nitrilase superfamily amidase and carbamoylase structures have a second glutamate, in addition to the Glu, Lys, Cys "catalytic triad", that is hydrogen bonded to the catalytic lysine. Mutating this glutamate (E142) to a leucine or aspartate in the amidase from Geobacillus pallidus renders the enzyme inactive. X-ray crystal structure determination shows that the structural integrity of the enzyme is maintained in spite of the mutation, with the catalytic cysteine (C166), lysine (K134) and glutamate (E59) in identical positions to those of the wild-type enzyme. The structural integrity is maintained in the case of the E142L mutant by a chloride ion that is located in the position occupied by E142 Oε1 in the wild-type enzyme and thus interacts with the active site lysine. This site is occupied by D142 Oδ1 in the case of the E142D mutant. The active site cysteine of the E142L mutant was found to form a Michael adduct with acrylamide, which is a substrate of the wild-type enzyme. The crystal structure of the adduct and quantum mechanical modelling show that the amide moiety interacts with the active site in a different manner than it does in the wild-type enzyme. The result is that the double bond of the acrylamide rather than the amide carbonyl carbon is adjacent to the active site cysteine. In the case of the E142D mutant no reactions occur and an acetate is found in the active site pocket. The D142 Oδ2 atom is located in two alternative locations that are respectively 2.1 Å and 4.3 Å from the location of E142 Oε2 in the wild-type enzyme. This demonstrates the role of the hydrogen bond between E142 Oε2 and the substrate amino group in positioning the substrate with the correct stereoelectronic alignment to enable the nucleophilic attack of the carbonyl carbon by the catalytic cysteine.

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