Crystal structure of isoaspartyl dipeptidase from E. coli


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In 1995, Gary and Clarke identified a gene in E.coli (iadA) encoding a 41 kDa polypeptide that catalyzes the hydrolytic cleavage of L-isoaspartyl, or L-β-aspartyl, dipeptides [1]. Examination of the deduced amino acid sequence revealed no similarities to other peptidases or proteases. However, a marked similarity to dihydro-orotases and imidases was found, which are involved in the synthesis and the degradation of pyrimidines, reflecting the similarity in the structures of the substrates of these enzymes.

L-aspartyl and asparaginyl residues are two of the most prominent sites for the spontaneous decomposition of proteins. These residues can undergo nonenzymatic intramolecular reactions resulting in the formation of deamidated, racemized, and isomerized derivatives. The major product is the L-isoaspartyl derivative in which the peptide bond from the aspartyl residue is made via the β-carboxyl of the side chain rather than through the α-carboxyl. This kink in the polypeptide chain, as well as the effect of the deamidation of the asparaginyl residue, can be detrimental to protein function.

E. coli can convert L-isoaspartyl residues in proteins to normal L-aspartyl residues by the action of protein-L-isoaspartate O-methyltransferase [2]. E.coli lacking this transferase survive poorly in stationary phase and at elevated temperatures [3]. Additionally, the repair mechanism is limited by the accessibility of the modified residue on the protein surface and the affinity of the enzyme for different subsets of L-isoaspartyl containing proteins. These limitations suggest that additional mechanisms exist for the removal of isoaspartyl residues including catabolic pathways. Isoaspartyl dipeptides can arise from the degradation of damaged proteins because most proteases and peptidases don’t recognize the β-peptide linkage connecting the isoaspartyl residue to its neighbor on the carboxyl side [4]. Without a specific dipeptidase, these dipeptides could accumulate inside the cell in stationary phase.

Isoaspartyl dipeptidase helps to prevent the accumulation of these dipeptides, which may be toxic to the bacteria or may result in the depletion of the pool of amino acids necessary for survival in stationary phase. Today, isoaspartyl dipeptidase is the only member of the metalloprotease family M38 and there is no protease or peptidase sequence similar to the E.coli isoaspartyl dipeptidase to be found in the database. However, an isoaspartyl dipeptidase activity has also been found in rat liver and kidney, but its mechanistic class has never been determined [5].

We have used the zinc ion of isoaspartyl dipeptidase for an MAD measurement at the zinc edge at 90 K on the BW6 wiggler beam line of DORIS. The crystals diffracted to 2.2 Å and the structure of isoaspartyl dipeptidase is currently refined.

References