

The Structures of Aspartyl Dipeptidase and Tripeptidase

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Beamline(s): X4A, X12C

Introduction: Aspartyl dipeptidase (peptidase E) and tripeptidase (peptidase T) from *Salmonella typhimurium* are intracellular peptidases with very different specificities and structures. Aspartyl dipeptidase is a serine hydrolase that cleaves dipeptides with an N-terminal aspartate, and tripeptidase is a metallopeptidase that releases the N-terminal, preferably hydrophobic amino acid from tripeptides. While tripeptidase appears to be mainly a prokaryotic enzyme, aspartyl dipeptidase is present in both prokaryotes and eukaryotes.

Methods and Materials: Both peptidases were expressed in methionine auxotrophs and crystallized in PEG 35000 (aspartyl dipeptidase) or ammonium sulphate (tripeptidase). Data were collected at beam station X4A at four different wavelengths around the absorption edge of selenium, processed and reduced with DENZO and SCALEPACK. Initial phases were calculated by MAD methodology with the CNS program package. The structure of aspartyl dipeptidase was refined with both CNS and SHELX using a 1.2Å native dataset collected at beam station X12C. The tripeptidase structure was refined by CNS to 2.4 Å using one of the selenium derivative data sets.

Results: The structure of aspartyl dipeptidase consist of a single domain with two mixed beta-sheets forming a 'V', flanked by six alpha-helices. The active site contains a Ser-His-Glu catalytic triad, and is the first example of a serine peptidase/protease with a glutamate in the catalytic triad. The active site serine is located on a strand-helix motif reminiscent of that found in serine carboxypeptidase, but the polypeptide fold and the organization of the catalytic triad differ from the known serine proteases. The structure of tripeptidase consists of two domains and has the same basic fold as carboxypeptidase G2 from *Pseudomonas* sp. strain RS-16. Two molecules related by crystallographic symmetry make essentially the same contacts as was found for the carboxypeptidase G2 dimer. The active site, despite the presence of two divalent metal ions, is negatively charged.

Conclusions: Aspartyl dipeptidase is the first member of a new family of serine hydrolases and appears to represent a new example of convergent evolution of peptidase activity. Tripeptidase belong to the M20 family of peptidases, as was previously suspected on the basis of a conserved sequence motif. A motif unique for tripeptidase from various prokaryotes is located at a distance from the catalytic center and is probably involved in recognizing the carboxyterminal end of the substrate.

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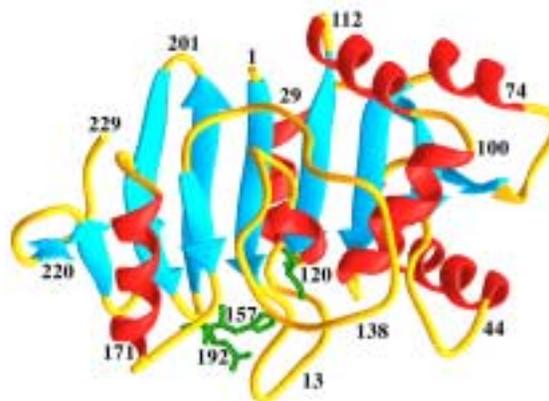


Figure 1. Ribbon representation of the structure of aspartyl dipeptidase. Helices are shown in red, beta-sheets in blue and catalytic triad residues in green.